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The Equilibrium Technology and Nature For Civilized Living

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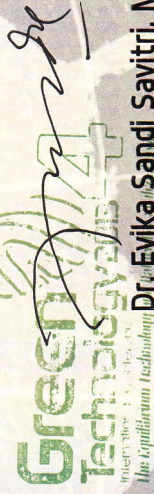
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2014

PREFACE to the proceeding of the 4th International Conference Green Technology

International Conference Green Technology 4th . to be held at the Islamic State of University Maulana Malik Ibrahim, Malang, East Java, Indonesia, on Saturday 9th November 2013. The Conference Green Technology is held annually at the Faculty of Science and Technology, Islamic State of University Maulana Malik Ibrahim Malang. The conference will address a range of critically important themes in the various fields that address the complex and subtle relationships between *biotechnology, environmental and biodiversity, green physic and engineering, green architecture, green ICT and modeling.*

The 103 scientific participants, 63 of whom were presenters, and 50 are participant had many fruitful discussions and exchanges that contributed to the success of the conference. The 20 participants from other countries made the conference truly international in scope. The 57 abstracts that were presented on the one days formed the heart of the conference and provided opportunity for discussion. The 6 abstract will be presented by poster session. Of the total number of presented abstracts, all of these will be publish in the proceedings volume.

There were 5 plenary lectures covering of the conference: Prof. Hitoshi Sawada, Professor and Director of Sugashima Marine Biological Laboratory, Graduate School of Science, Nagoya University, Dr. Akira Kikuchi Associate professor of Hydroecology, Faculty of Civil Engineering Universiti Teknologi Malaysia, Dr. Ardyono Priyono **Green Computing and Technology** Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia, Ir. Muhaimin Iqbal Practice Agroindustry, Indonesia, Dr.drh. Bayyinatul Muchtaromah, MSi Animal Physiology Laboratory. Department of Biology. Faculty of Science and Technology. State Islamic University of Maulana Malik Ibrahim Malang, Indonesia

A conference such as this requires a huge amount of work from many people. In particular, we take this opportunity to thank those on the local organising committee as well as Departement of Biology and Departement of Physic and other comitte in Faculty of Science and Technology Maulana Malik Ibrahim State Islamic University and who supported the conference in a multitude of ways.

We also wish to acknowledge the support and contributions of the many students supporters of the conference from Russian, Afganistan, Philipines, Thailand, and Madagascar. We are also very grateful to the sponsors for their financial support PT New Module Int. Scientific Technical Supplies. We hope that this Supplementary Proceedings, both in hardcopy and on the web, will provide a valuable resource for all participant.

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Biotechnology

No	Presenter	Title	Page
1	Siti Muzaiyanah	Effects Of Siam Weed (<i>Chromolaena Odorata</i> (L) Leaves Extracts On Seedlings Growth Of Purslane (<i>Portulaca Oleraceae</i>)	5
2	Sholihin	Productivity and Stability of Promising Clones For High Tuber and Ethanol Yield Based On Multivariate	9
3	Surya Diantina, Darda Effendi, Ika Mariska	Effect of Medium and Explant source on Cassava (<i>Manihot esculenta</i> Cranz) Callus Induction	13
4	Surya Diantina and Sri Hutami	Rapid Multiplication Procedure for Gadung's (<i>Dioscorea hispida</i>) Tuber-Seeds Propagation	18
5	Pujiati, Muh. Waskito Ardhi, Ani Sulistyarsi	Isolation Of Cellulolytic Mold From Soil Of Teak Forest In Kresek, Madiun	23
6	Ragapadmi Purnamaningsih) and Sri Hutami)	In vitro selection on sugarcane calus conducted from gamma ray irradiation for aluminium tolerance	28
7	Sri Hutami and Ragapadmi Purnamaningsih	Shoot Multiplication of Taro (<i>Colocasia esculenta</i> var. Antiquorum) through in vitro culture	34
8	Sri Hutami and Ragapadmi Purnamaningsih	Preliminary Field Trial Of Bio-6 Patchouli Clone In Three Locations	41
9	Evy Latifah, Eli Korlina, Kuntoro Boga and Joko Maryono	Grafting Technology for Sustainable Improvement of Tomato Production: A Field Study in Kediri, East Java	49
11	Evika Sandi Savitri, Nawafila Februyani	The Effect of Metal Ion Cu ²⁺ for Development and Secondary Metabolites (Stigmasterol and Sitosterol) Callus of Purwoceng (<i>Pimpinella alpine</i> Molk.)	54
12	Kartini Zailanie and Hartati Kartikaningsih	Study on the composition of dietary fiber on the parts of the thallus brown algae (<i>Sargassum duplicatum</i>) and fatty acids on the parts of the thallus brown algae (<i>Sargassum duplicatum</i>)	61
13	Ikhsan Matondang	Use of Compost and Coconut Water as Media Plant Tissue Culture Propagation	69
14	Harini Nurcahya Mariandayani; Dedy Duryadi Solihin; Sri Sulandari; Cece Sumantri	Polymorphisms of Insuline-like growth factor-I (IGF-1) and Pituitary Positive Transcription Factor-1 (Pit-I) Genes and their Effect on Growth Traits in Indonesian Native Chickens	75
15	Ernawati Sinaga, Suprihatin, Ida Wiryanti	Anticancer Activity Of Methanolic Extract Of Bangle Hantu (<i>Zingiber ottensii</i> L.) Rhizomes On Breast Cancer Cell Lines MCF-7	86
16	Ikna Suyatna Jalip, Suprihatin, Ida Wiryanti, Ernawati Sinaga	Antioxidant Activity And Total Flavonoids Content Of Curcuma Rhizome Extract	93

17	Sri Endarti Rahayu, Sri Handayani, Noverita, Ikna Suyatna Jalip	ANTIFUNGAL AND PRELIMINARY PHYTOCHEMICAL SCREENING OF LEAF EXTRACT OF <i>Pandanus odoratissimus</i> L.f	100
----	--	--	-----

Environment and Biodiversity

No	Presenter	Title	Page
18	Nurul Istiqomah dan Amik Krismawati	Application of organic fertilizer and NPK to increase Sweet corn production in malang east java	106
19	Nurul Istiqomah dan Dwita Indrarosa	Study Of Liquid Organic Fertilizer From Urine Of Rabbit And Urea On Growth And Production Of Mustard (<i>Brassica Juncea</i> L)	113
20	Kuntoro Boga Andri and Evy Latifah	Socio-economic Aspect of Vegetable Production System In East Java: The Case of Vegetable Farmers in Kediri and Blitar	121
21	Kuntoro Boga Andri, M. Winarno , P.B. Daroini	Characteristic of Mango diversity in East Java: Case Study in Tiron, Kediri	127
22	Agung Sri Darmayanti, Siti Sofiah and Deden Mudiana	Effect of Trees Architectural model and Total Litter at Three Different Types of Agroforestry Management for The Value of Infiltration and Run Off	132
23	Eko Prasetyo Kuncoro, Mochammad Zakki Fahmib, Handoko Darmokoesoemob	Adsorption Study Of Nickel From Aqueous Solution By Coal Fly Ash	140
24	Marheny Lukitasari, Nurul Kusuma Dewi, Joko Widiyanto	Plankton Communities In Bening Reservoir Madiun	147
25	Nia Yudha Yanti and Dwi Suheriyanto	Macroinvertebrate Diversity At Sengguruh And Sutami Reservoirs Regency Of Malang	152
26	Dewi Ayu Lestari	Characterization of external Morphology on various seeds in Purwodadi botanic garden	159
27	Soejono	Quality of trees diversity growing around water springs at southern part of pasuruan district, east java	165
28	Noverita, Fitra Awaludin, Bimo H. Satrio, Fandi Lastanto , MuhammadTohir, Ratnawati	Biodiversity of Macroscopic Fungi In Three Different Habitat In Paniis Village Ujung Kulon West Java	172
29	Rini Fitri	Sustainable biodiversity conservation based agroforestry in sub-watershed of krueng simpo aceh province	179
30	Akira Kikuchi	Applicability of Easy-to-use Escherichia coli Test Strip for Community Development Program on Drinking Water Safety	183

31	Yudi Widodo, Nila Prasetyaswati and Nasir Saleh	Harmony Induction Of Cassava Under Agro-Forestry: Cease Ecological Reduction And Reach Economic Seduction	191
32	Yudi Widodo	Earthling Imagination Into Implemented Green Innovation For Community Welfare: Praxis Of Root Crops Under Agroforestry	206
33	Abdulkadir Rahardjanto, Haryoto Kusnoputranto, Dwita Sutjningsih, Francisia SSE Seda	Activation Communities On Sustainable River Conservation Based On Macroinvertebrates Knowledge In Brantas Upper Watershed Area	219
34	Zainal Arifin and Indriana Ratna Dewi	Effect Of N-Inorganic And Organic Fertilization On Growth And Yield Of Sweet Corn	227
35	Zainal Arifin and Indriana RD	Study Of N Fertilization On The Growth And Results Of Cucumber Local Sumenep Of Large Fruit And Cucumber Local Sumenep Of Small Fruit	232
36	Bagus Setiawan, Titut Yulistyarini, Liliek Harianie	The Potentialof Lauraceae Family as CO ₂ Absorber and Carbon Storage in Purwodadi Botanic Gardens	240
37	Aniswatul Khamidah and Nurul Istiqomah	Soymilk Ice Cream Processing Technology From Kaba, Argomulyo And Wilis Varieties Of Soybean With Purple Sweet Potato Substitution	247
38	Aniswatul Khamidah	Pumpkin Sauce Processing Technology	256

Green Architecture

No	Presenter	Title	Page
39	Yusfan Adeputera Yusran, Ary Dedy Putranto, Iwan	An Alternatif Acoustic Wall from Gaba-gaba	264
40	Aldrin Yusuf Firmansyah, MT	Urban Green Open Space Conservation of Ijen Sportpark Area in Malang City by Involve User and Ecological Consumption Variables	271
41	Chandra Dwiratna	Planning of green space as effort to control air pollution (a case study at sidoarjo regency)	280
42	Agung Sedayu	Improvement Priority Of Service Quality Of Purwoasri Terminal Kediri	286

Green Chemistry

No	Presenter	Title	Page
43	A.Ghanaim Fasya, Suci Amaliyah, Siti Khairul Bariyyah, Umi Khamidah, A. Hanapi and Romaidi	Toxicity, Antioxidant and Antibacterial Activity Test of Methanol Extract of Chlorella sp. Microalgae Result Cultivation in Tauge Extract Medium	287
44	Nety Kurniaty	Synthesis of Sansalvamide A, a Peptidomimetic as Novel Anti-tumor Agents, Using Olefin Metathesis via Microwave Irradiation	296
45	Anis Artiyani	Aprocessing Heavy Metal In River Water Trough Coagulant – Flocculation Process Using Moringa Seeds (<i>Moringa oleifera</i>) or “ biji kelor” as Biokoagulan	307
46	Roihatul Muti'ah, Elok Kamilah Hayati, Khairunnisak	The Effectivity Of Etanolic Extract Sunflower Leaves (<i>Helianthus Annus</i>) As Anti-Malarial Agent Against <i>Plasmodium Berghei</i>	313

Green ICT and Mathematical Modelling

No	Presenter	Title	Page
47	Rivatul Ridho Elvierayani, Abdussakir	Spectrum of the Laplacian Matrix of Non-commuting Graph of Dihedral Group D_{2n}	321
48	Deri Ismawati, Ari Kusumastuti,	Analysis of Newton Gregory Polinomial for Numerical Solution of String Wave Model	324
49	Fachrul Kurniawan	Completion Camper Reaver Game Scenario In 3d View With Uninformed Search Methode Using Netlogo Software	329
50	Fresy Nugroho, Hani Nurhayati	Educational 3D Games Using Finite State Machine to Increase Arabic Learning Quality	332
51	Irwan Budi Santoso	Comparison Of Pearson Correlation And Conditional Mutual Information To Construct Model Of Tree-Augmented Network (Tan) (Case Study Character Handwriting Recognition)	337
52	NurAzizah and Ari Kusumastuti	Solution Of Van Der Pol Equation Using Adams Bashforth Moulton Fourth Order Methods	344
53	Siska Dwi Oktavia, Sri Susanti and Wahyu Henky Irawan	Domination And Total Domination Contraction Number From Some Simple Connected Graph	349
54	Usman Pagalay, Ernawati Efendi and Ari Kusumastuti	Discertization Reaction-Diffusion Models With Finite Difference Method	362
55	Yunifa Miftachul Arif and Fachrul Kurniawan	Implementation Of Multi Sensor System For Msr-H01 Hexapod Robot	371

Green Physics AND Engineering (GPE)

No	Presenter	Title	Page
56	Amilatul Farida and Erna Hastuti	Characterization And Analysis Of Crystal Structure Mixed Keratin-Magnesium Composite With Rietveld Method	378
57	Eko Marsyahyo, Heru Suryanto and I.G.N Nitya Santhiarsa	Review on Engineering Green-Materials and Applications from Tropical Plants Sources for Sustainable Future in Indonesia	384
58	Firdaus Chairuddin, Wihardi Tjaronge, Muhammad Ramli and Johannes Patanduk	Experimental Study Permeable Asphalt Pavement Used Domato Stone (Quarsite Dolomite) as Course Agregate for Surface Layer of Road Pavement	392
59	Firdaus Chairuddin, Wihardi Tjaronge, Muhammad Ramli and Johannes Patanduk	Laboratorium Testing Gradation And X-Ray Themography To Properties Permeable Asphalt Pavement With Rice Hush Ash As Filler And Domato Stone As Course Agregate	402
60	Khotimatul Munawaroh and Erna Hastuti	Electrical Properties Of CaCO_3 Filled Chitosan-Pva Membranes	414
61	Nelly Budiharti, Elvianto Dwi D, Faidliyah Nilna M and Iftitah Ruwana	Natural Dyestuff Production	420
62	Triwulandari S. Dewayana, Dedy Sugiarto and Dorina Hetharia	Identification Of Selection Criteria For Green Automotive Component Industry	424
63	Nasrul Rofiah Hidayati and Anggit Sasmito	Influence Of Binder Types And Levels On Calorific Value Of "Char Jerbas" Briquettes	430

EFFECTS OF SIAM WEED (*Chromolaena odorata*) (L) LEAVES EXTRACTS ON SEEDLINGS GROWTH OF PURSLANE (*Portulaca oleraceae*)

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ABSTRACT

Siam leaves was reported content a flavonoid 4', 5-Dihydroxy-3', 7-dimethoxyflavone. This compound was reported was having affect on growth inhibition of grass. Purslane is one weed soy and peanuts. The purpose of this study was to determine the effect of Siam weed leaves extract on the growth of Purslane. The research was conducted in the laboratory of Agronomy Balitkabi Malang on December-January, 2013. The experiment was laid in Randomized Complete Design. The threat design of concentration level of the extract Siam weed that made by using maserasi metode, were 10%, 20%, 30%, 40% repeated three times. The crude extract was obtained from the leaves of Siam weed were blended and diluted with water based on the concentration of the treatment. The dilution was allowed to stand for 24 hours and then filtered by using kassa. The results showed that (1) Extract solution of siam extract has potentio to inhibite the growth of purslane (2) Extract Siam weed solution concentration of 40% were able to suppress the growth of purslane highest of other treatments. This solution inhibited the stem length up to 33% and the root length up to 56%. The Siam weed leaves extract has potential to be used for bioherbicide.

Keywords : Siam weed, Purslane, Siam leaves extract

INTRODUCTION

Weed controlled by using herbicides is habitual farmer attitude till now. The advantage of using herbicides is be able to save energy and can be used under any circumstances. Then, the losses of herbicides, are: (1) using the same herbicide continuously can rise the weed development growth, especially annual weed that are difficult controlled by herbicide (Sebayang, 2005) in (Anonymous 2012), (2) may lead to the pollution of water resources, (3) causing soil damage, and (4) left a toxic herbicide residues on agricultural products (Indira and Suwahyono 2008).

Increasing public awareness of the importance of environmental sustainability, make public demand on agricultural products that are environmentally friendly farming was rising. One of alternative weed eradication efforts and plantation agriculture that

environmentally friendly farming is using bioherbisida. Bioherbisida or natural herbicide is a type of herbicide that the active composition is resulted by residue metabolism of microorganisms or its microorganisms (Indira and Suwahyono 2008) such as allelopati (Junaedi *et al.*, 2006).

Siam weeds are herbaceous plant found in many plantation, include the Asteraceae family and has a flavonoid named 4', 5-Dihydroxy-3', 7-dimethoxyflavone (Che Man, 2010). These weeds come from Central America, but it has spreaded across the tropics and sub-tropics. Siam has potential as organic fertilizer and bio-pesticides. Some researches about this still in the laboratory-scale experiments and the results provide good prospects to be developed sustainable farming (Murrinie, 2011). Akinmoladun (2007) showed the result of the leaf Siam that extracted by

distilled with water and methanol solvent. The result reported that using distilled both of water and methanol solvent shows that leaves of Siam contains tannins, steroids, terpenoids, flavonoids and cardiac glycosides. while alkaloids only found in analisis on leaf Siam weed using methanol solvent. Total phenolic contained in Siam weed is $0,01 \pm 0,00$ mg/g. The extraction by using Methanol is able to identify the content of Alkaloids, Tannins, Phlobatannins, Steroids, Terpenoids, Flavonoids. Then the extraction by using distilled water is able to identify the content of Saponins, Tannins, Phlobatannins, Anthraquinones, Steroids, Terpenoids, Flavonoids (Akinmoladun 2007).

Terpenoids are components of plants that have a smell and can be isolated from plant materials by distillation or referred to as an essential oil. Steroids are a group of compounds consisting of sterols, bile acids, sex hormones, hormone adrenokortikoid, cardiac and saponin aglycone. These compounds have a physiological effect that is different between each compound (Lenny 2006^a). Coumarin are phenolic compounds that are generally derived from higher plants and are rarely found in organisms. Flavonoids are the largest group of phenolic compounds in nature that have 15 carbon atoms, in which two benzene rings (C_6) is bound to a chain of propane (C_3). Alkaloids are a class of organic compounds derived from plants. Each class of alkaloid compounds containing at least one nitrogen atom which is usually alkaline. This class of alkaloids are toxic and no part that serves as a drug (Lenny 2006^b). Then Tannin compounds are difficult to separate from the water and may cause brownish yellow color on the surface of the water and can affect the taste and odor (Praveen and Upadhyaya 2012). Saponins are glycosides natural who have various pharmacological properties including cytotoxic activity (Podolak *et al.*, 2010).

Purslane is a broadleaf weeds found on soybean and peanut plants (Rukmana and Sugandi 1999), included weeds season, succulent and grow erect or spreading (depending on light). Stems are round, fleshy, reddish color, length 10-50 cm. These weeds is able to dominate the land up to 2.28% on the density of 20 x 20 cm (Murrinie, 2011).

This paper aims to determine the effect of leaf siam weed extract on the growth of purslane as a broad-leaved weeds.

MATERIALS AND METHODS

The research was conducted at the Agronomy Laboratorium of Balai Penelitian Tanaman Aneka Kacang dan Umbi (Balitkabi) Malang, December 2012 - January 2013. The experiments were each extract of Siam weed conducted of five treatment arranged in randomized block design with three replication. The treatments were each extract of siam weed concentration 0% (control), 10% (0,1 g/ml), 20% (0.2 g/ml), 30 % (0.3 g/ml), 40% (0.4 g/ml). The crude extract was obtained from the leaves of Siam weed were blended and diluted with water based on the concentration of the treatment. The dilution was allowed to stand for 24 hours and then filtered by using kassa.

The purslane was planted on soil that mixed with sand by ratio 3:1 on a pot that has volume 75 ml. 100 seeds purslane was planted in it. Application of the treatment were done once every 10 days starting from planting by 10 ml per pot. Wet weight, dry weight, stem length and root length of purslane were measured at 30 days after sowing.

RESULT AND DISCUSSION

There are differences in the wet weight of purslane in the treatment of siam weed leaf extract application and without the application of siam weed leaf extract (control). At the control treatment, purslane wet weight reached 0.0069 g, then the purslane that treated by siam weed extract application, shows vary value between 0.0024 g to 0.0056 g. Wet weight reduction due to the application of siam weed extract the lower leaves with increasing concentrations of siam weed leaf extract. The siam leaves extract inhibited purslane growth up to 19, 36, 45, 65%. Meanwhile, dryweight of purslane likely similar in value, both in treatment applications siam weed extract at various concentrations and control, that is 0.0006 g to 0.0010 g (**Table 1**).

Table 1. The mean values of wet weight, dry weight and water content of purslane in different concentration of Siam weed extract.

Treatment	Wet weight (g)	Dry weight (g)	water content (g)
Extracts of Siam weed 0%	0.0069 ^a	0.0010 ^a	0.0059 ^a
Extracts of Siam weed 10%	0.0056 ^a	0.0009 ^a	0.0047 ^a
Extracts of Siam weed 20%	0.0044 ^a	0.0010 ^a	0.0034 ^a
Extracts of Siam weed 30%	0.0038 ^a	0.0006 ^a	0.0032 ^a
Extracts of Siam weed 40%	0.0024 ^a	0.0008 ^a	0.0016 ^a

Purslane wet weight value are varied (**Table 1**), from largest to smallest according to the level of concentration of siam weed leaf extract was given, but have almost the same dry weight value. This shows that the potensial water absorption is different in each application siam weed leaf extract. Table 1 shows the power absorption by purslane decreased with increasing concentration of the solution. Decreasing the absorption of water was caused by the content of tannins and flavonoids contained in siam weed leaf extract. Sihombing *et al* (2012) argue that the tannins and flavonoids are compounds that can damage membrane structure cell then make the permeability decreased. Decreased membrane permeability makes the plant water requirement is not fulfilled in an optimal, so that metabolism is abnormal, consequently can inhibit plant growth and development of purslane.

The decreasing of plant cell permeability capacity to absorp water affected the inhibition of purslane growth. The growth of purslane was characterized by shoot length and root length. Table 2 shows shoot length and root length that decreased by the increasing concentration of the extract.

Table 2. The mean values of root and shoot length of purslane (cm) in different concentration of Siam weed extract.

Treatment	Shoot length (cm)	Root length (cm)
Extracts of Siam weed 0%	1.2 ^a	0.6 ^a
Extracts of Siam weed 10%	0.9 ^b	0.5 ^{ab}
Extracts of Siam weed 20%	0.85 ^{bc}	0.3 ^{bc}
Extracts of Siam weed 30%	0.7 ^{bc}	0.3 ^{bc}
Extracts of Siam weed 40%	0.67 ^c	0.2 ^c

The shoot length of purslane at control treatment is 1.2 cm while purslane in the treatment of siam siam leaf extract applications were ranging from 0.9 cm to 0.67 cm. The purslane root length in treatment applications siam weed leaf extract 20% and 30% tend to be similar, while the purslane root length that applied with siam leaf extract 20% and 30% tend to be the same. The shortest root length of purslane is purslane that applied with siam leaf extract 40%, that is 0.67 cm. Application in the treatment of weed leaf extract 40% of this, purslane roots can be pressed up to 67%. This is due to the tannin content can inhibit germination (Widyawati et al 2009), decrease growth rate and decrease energy metabolism (Chung *et. al* 1998).

CONCLUSION

1. Extract solution of siam extract has potention to inhibite the growth of purslane.
2. Extract solution Siam weed concentration of 40% were able to inhibite the growth of Purslane highest of the shoot length up to 33% and root length up to 56%.

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PRODUCTIVITY AND STABILTY OF PROMISING CLONES FOR HIGH TUBER AND ETHANOL YIELD BASED ON MULTIVARIATE

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ABSTRACT

It is expected that demand for cassava in Indonesia will increase markedly in the future. This is because of increases in the human population and in industrial processing using cassava as the raw material. Increasing cassava yields should therefore be our main goal for increasing cassava production. The planting of high-yielding varieties is one of the main components in increasing productivity. Cassava (*Manihot esculenta*) in Indonesia is planted in dry areas with varied environmental conditions. New variety will be released after tested in some environments. The aim of the study was to evaluate the productivity and stability of cassava promising clones. The experiments were done during two years (2007/2008 and 2009) in Lumajang (East Java), and Banyuwangi (East Java), Lampung Selatan (Lampung), Lampung Timur (Lampung) and Lampung Tengah (Lampung). The experiments were done using a RCBD design, three replications. The plot size was a 5 m x 5 m. Plants distance was 100 cm x 80 cm. Doses of fertilizers was 93 kg N+ 36 kg P₂O₅ + 60kg K₂O/ha. The clones used were CMM 99008-3, OMM 9908-4, OMM 9904-70, CMM 99023-12, OMM 9904-111, CMM 99023-4 and MLG 10311 as promising clones, as well as Adira 4 and UJ5 (released varieties). Parameter recorded was fresh tuber yield (t/ha). Research results can be seen that the clones and locations Interaction was significantly difference for tuber yield in 9 months. The mean tuber yield of OMM 9908-4 was the highest. Clone 9 (ADIRA 4), clone 2 (OMM 9908-4), clone 3 (OMM 9904-70), clone 4 (CMM 99023-12) were stable clones. But clone 5 (OMM 9904-111), clone 1 (CMM 99008-3), clone 6 (MLG 10311), clone 7 (MLG 10311), and clone 8 (UJ5) were not stable clones.

Keywords : Productivity, stability, multivariate, cassava

INTRODUCTION

Self sufficient for food and energy is important thing for any country. Cassava is potential crop for program for self sufficient for food and energy. Tuber of cassava can be used as raw material for ethanol industry for energy, and many food product can be made from cassava for food security. Cassava production in Indonesia 24.2 millions ton with productivity 21.4 t/ha. This production should be increased because at the moment, demand is high than supply and It is predicted that demand for cassava in Indonesia will increase markedly in the future. Cassava production can be increased by intensification and extensification. Intensification can be done by using high yielding varieties, and extensification can be done by planting cassava on the potential area for cassava.

Until now, there are eleven released varieties, one of them is UJ5, and this variety has already developed in Lampung. UJ5 has been released in 2000. This variety was introduced from Thailand with name Kassesart 50. Yield trials of this variety mostly were done in Lampung. Adira 4 is the other released variety. This variety is popular in Java. This variety has been released in 1987. This variety is resulted from opened pollination with female parent BIC 528. Mite is important insect in Java. Adira 4 is more tolerant than UJ5 in response to mite attack.

Cassavas are planted in various environments. Sumatera area is relatively wet with rain distribution almost trough a year, West Java is medium, Central Java and East java is relatively dry with clear differences between rainy season and dry season, On the contrary, in Sumatera area, the rain almost through a year.

To analyze the stability of genotype, there were a few techniques of analysis. Parametric approach only give the aspect of stability individually, so this approach cannot provide the picture of response overall. In fact, response of genotype to environment is multivariate. In Parametric approach, response of genotype to environment is transferred to univariate via index of stability. To response this situation, multivariate analysis can be used for analysis of stability of genotypes. Kasno *et al.* (1989) used this analysis for justification of stability of genotypes.

Some promising clones have been identified that resulted from previous cassava breeding activities.

These clones are needed to be tested in various locations/environments conditions before releasing the superior ones as new varieties. The aim of the study was to evaluate the productivity and stability of cassava promising clones.

MATERIALS AND METHODS

The experiments were done during two years (2007/2008 and 2009) in Lumajang (East Java), and Banyuwangi (East Java), Lampung Selatan (Lampung), Lampung Timur (Lampung) and Lampung Tengah (Lampung). The experiments were done using a RCBD design, three replications. The plot size was a 5 m x 5 m. Plants was distance was 100 cm x 80 cm. Doses of fertilizers was 93 kg N+ 36 kg P₂O₅ + 60kg K₂O/ha. Data recorded on each plot were tuber yield in nine months (kg/ha). Tuber yield was analyzed using MSTATC program to obtain the combined analysis of variance. Cluster dendrogram for genotype was constructed using IRRISTAT program.

RESULTS

The analysis of variance of 9 clones, four locations, and two years for fresh tuber yield are given in Table 1. The result can be seen that Interaction of clones and locations was significantly different for the fresh tuber yield in 9 months. The average of tuber yield of OMM 9908-4 was the highest, followed by MLG 10311, OMM 9904-70, CMM 9904-100, Adira 4, and UJ5.

Grouping of clones/varieties based on tuber yield in 9 months is presented in Figure 1. Clones/varieties tested can be grouped into group A (consist of 4 clones/varieties) and group B (consist of 5 clones/varieties). In group A can be grouped into group C (only 1 clone 9 (Adira 4) and group D (consist of 3 clones/varieties: clone 2 (OMM 9908-4), clone 3 (OMM 9904-70), clone 4 (CMM 99023-12). Group B can be grouped into group E (only 1 clone 5 (OMM 9904-111) and group F (consist of 4 clones/varieties: clone 1 (CMM 99008-3), clone 6 (MLG 10311), clone 7 (MLG 10311), and clone 8 (UJ5).

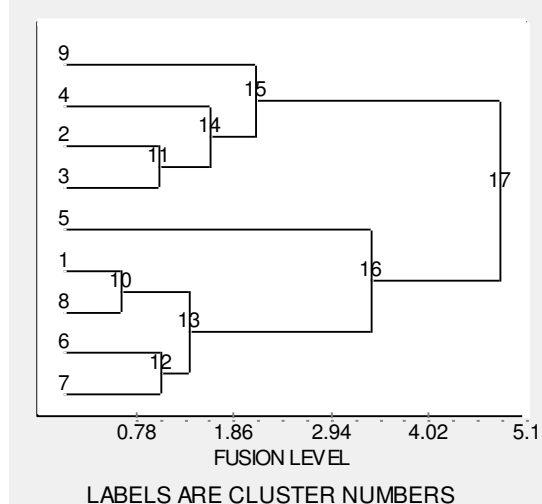
Table 1. Sidik ragam tergabung untuk hasil pati 9 bulan beberapa klon/varietas ubikayu di empat lokasi, MT 2004-2005.

Source	Degree of freedom	Mean Square
Environment(E)	7	37.047**
Error (a)	16	47.237
Clones (C)	8	233.371**
C x E	56	91.954**
Error (b)	128	23.807
Coefficient Variation (%)		13,17

Table 2. Mean tuber yield over years over locations, 2007-2009.

No.	Clone/variet	Mean Tuber yield over years and seasons t/ha
1	CMM 99008-3	32.15 e
2	OMM 9908-4	42.22 a
3	OMM 9904-70	39.27 bc
4	CMM 99023-12	36.79 cd
5	OMM 9904-111	34.27 de
6	CMM 99023-4	35.60 d
7	MLG 10311	40.38 ab
8	UJ5	36.26 cd
9	ADIRA 4	36.36 cd
	mean	37.04
	C.V. (%)	13.17

CLUSTER DENDROGRAM FOR GENOTYPES

**Fig. 1.** Cluster dendodram for genotypes

DISCUSSION

Interaction between clones and locations significantly affected fresh tuber yield in 9 months. It is natural law that genotype interacts with environment to produce phenotype. This phenomenon was also reported by Sholihin (2011a); (2011b) and (2009), and Kalkani and Sharma (2010).

Mean yield of OMM 9908-4 over years over locations was the highest, 42.22 t/ha, 15 % higher than UJ5 /Kasetsart 50), equal to Rp 4.460.800,-/ha or around US \$ 496, - if US \$ 1, = Rp 9000,-. There is possibility that the yield will be higher than this value if the environment and other input is better than the environment and other input used for produced 42,22 t/ha. Sholihin (2013) reported that clone OMM 9908-4 produced 89.48 t/ha.

Variety Adira 4 was reported as stable variety (Sholihin, 2009; 2011b). Clone 2 (OMM 9908-4), clone 3 (OMM 9904-70), clone 4 (CMM 99023-12) were in one group with variety ADIRA 4, so level of stability of these clones were similar to that of ADIRA 4 based on multi-variate analysis. On the opposite, clone 5 (OMM 9904-111), clone 1 (CMM 99008-3), clone 6 (MLG 10311), clone 7 (MLG 10311), and clone 8 (UJ5). There are two possibilities to explain stability of clone. High stability could be caused (i) clone is a hybrid and (ii) it has a genetic potential to perform well irrespective of the environment where they are grown.

Many industrial products are made from cassava, such as starch, sorbitol, fructose, glucose, crackers, and ethanol. Prospect of ethanol industry is good. The important thing in ethanol industry is supply of raw material. A good raw material is important in determining a good ethanol industry. It was reported that the yield potential of ethanol of OMM 9908-4 was 14472 liter/ha (Sholihin dan Sundari 2011).

CONCLUSION

1. Clone 9 (ADIRA 4), clone 2 (OMM 9908-4), clone 3 (OMM 9904-70), clone 4 (CMM 99023-12) were stable clones.
2. clone 5 (OMM 9904-111), clone 1 (CMM 99008-3), clone 6 (MLG 10311), clone 7 (MLG 10311), and clone 8 (UJ5) were not stable clones.

3. Mean yield of OMM 9908-4 over years over locations was the highest, 15 % higher than UJ5 /Kasetsart 50), equal to Rp 4.460.800,-/ha or around US \$ 496, - if US \$ 1, = Rp 9000,-.

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Effect of Medium and Explant source on Cassava Callus Induction

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ABSTRACT

Several researches in somatic embryogenesis of cassava have been reported but it has not been really applied for large scale propagation because the frequency of callus induction and regeneration is usually low. The successful of cassava somatic embryogenesis is vary depend on the genotype, explant source, hormones composition in media. This experiment tried to induce callus formation of cassava from several in vitro explants: leaf with adaxial-side up, adaxial-side down, petiole and stem. Explant sources cultured for callus induction in media MS basal medium supplemented with 30 g/l sucrose, MW vitamins, casein hydrolisat 100 ppm, picloram 25 ppm, supplemented with 25 or 50 ppm NAA as treatment. Different response of explants source to NAA treatment from those two accessions in callus induction as well as friable callus formation were found in this experiment. Callus induction of both accession achieved within 2 weeks from leaf with adaxial-side up and stem, while adaxial-side down and petiol produced callus formation within 4 weeks after treatments. Only accession 433 could produced friable callus on both NAA concentration, induced from leaf with adaxial-side up and stem.

Keywords : NAA, somatic embryos, *Manihot esculenta* Crantz.

INTRODUCTION

Cassava is a perennial woody shrub belongs to Euphorbiaceae and cultivated for its starchy tuberous roots. Several researches in somatic embryogenesis of cassava have been reported for over 3 decades. However, it has not been really applied for large scale propagation because the frequency of callus induction and regeneration is usually low (Wongtiem *et al.* 2011).

The use of somatic embryogenesis. Somatic embryogenesis is an efficient and reproducible regeneration system for propagation, transformation and long term conservation via cryopreservation (Puonti-Kaerlas, 1997; Raemakers, 1997; Zhou *et al.*, 2000; Taylor *et al.*, 2001). However, the conservation using somatic embryo is rare, due to low rates of primary embryo induction, highly genotype dependent nature of the whole embryonic system as well as the low rates of plant recovery (Danso and Ford Lloyd, 2002).

Type of explants. Multiplication and callus formation in cassava is vary depend on the genotype, explants sources and hormones composition in media (Sudarmonowati, *et al.*, 2002). Many explants features are known have different effect on culture initiation and transformation. Some explants sources namely shoot apical meristem (Zhang, *et al.*, 2000; Zhang, *et al.*, 2001; Hankuoa, *et al.*, 2005; Hankuoa, *et al.*, 2006), immature leaf lobes (Sofiari, *et al.*, 1997; Li, *et al.*, 1998; Zhang, *et al.*, 2000; Zhang, *et al.*, 2001; Groll, *et al.*, 2001; Hankuoa, *et al.*, 2005; Hankuoa, *et al.*, 2006; Saelim, et al., 2006; Priadi and Sudarmonowati, 2006), apical bud and lateral bud (Saelim, *et al.*, 2006) and shoot apices and shoots (Feitosa, et al., 2007) are common for somatic embryogenesis of cassava. But younger, more rapid growing tissue or tissue at early stage of development is the most effective (Rossin and Rey, 2011). In this experiment, we reported response of in vitro explants (leaf with adaxial-side up, adaxial-side down, petioles

and stems) to callus induction media supplemented with NAA 25 and 50 ppm.

MATERIALS AND METHODS

Plant materials. The in vitro collection of cassava accession 433 (genotype from open-pollinated cross of Adira) and 450 (genotype from open-pollinated cross of Muara) obtained from ICABIOGRAD field collections. Those accessions have been cultured in vitro and were maintained through 5 serial sub-culture on medium containing MS (Murashige and Skoog 1962) basal salts and vitamins supplemented with 30 g/l sucrose, incubated at a temperature of 24–25°C under a 16/8-h (light/dark) photoperiod with light provided by white fluorescent tubes at an intensity of 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Callus induction. In vitro explants used in this experiment: leaf with adaxial-side up (abaxial surface in contact with media), adaxial-side down (adaxial surface in contact with media), petiol and stem. Explants were excised and cultured on MS (Murashige and Skoog 1962) basal medium supplemented with 30 g/l sucrose, MW (Morel and Wetmore, 1951) vitamins contain thiamine 1 ppm, pyridoxine 1 ppm, nicotinic acid 1 ppm, biotin 0.1 ppm, myo-inositol 100 ppm and ca-panthotenate 1 ppm. Casein hydrolisat 100 ppm and picloram 25 ppm also supplemented into medium with 25 and 50 ppm NAA as treatment. The medium was adjusted to pH 5.8 prior to the addition of 2.5 g/l phytigel, autoclaving at 120°C, then cultured under dark condition at a temperature of 24–25°C.

RESULTS

Both cassava accessions successfully induced callus formation on all treatments in vary percentage (**Fig. 1**). In this experiment, callus inductions were successfully achieved within 2 weeks after treatment from stem and leaf with adaxial-side up, while adaxial-side down and

petiol induced callus formation within 4 weeks after treatments.

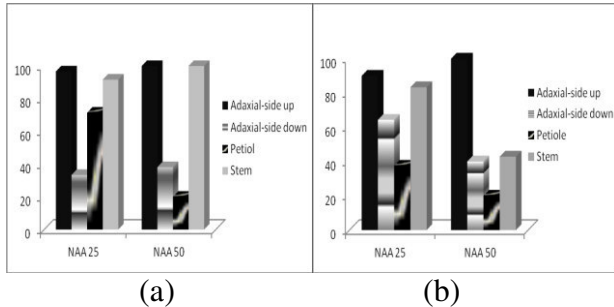


Figure 1. Effect of explant types and several NAA concentrations on percentage of cassava callus formation on: (a) accession 433 and (b) accession 450.

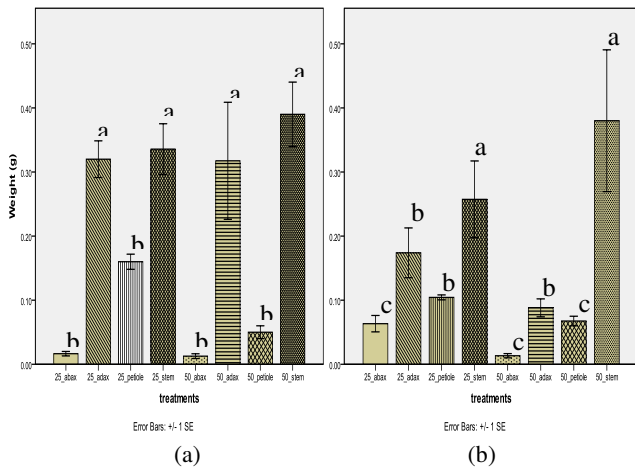


Figure 2. Weight of callus from different type of cassava explants on several NAA concentrations at 4 weeks after treatment: (a) acc. 433 and (b) acc.450.

No significant differences were observed between NAA concentrations to callus induction of cassava acc. 433. In contrast, explants type of acc. 433 exhibited higher percentage of callus induction as well as significant differences on weight of callus from stem and leaf with adaxial-side up (**Fig. 2**). The other accession (450) showed that combination of NAA 50 ppm and stem promoted low percentage of callus induction but produced the highest weight among others.

Result found in this experiment for both accessions showed that calli were produced in explants surface of leaf with adaxial-side up as well as stem, while calli from adaxial-side

down were produced around the cutting region only (**Fig. 3 and 4**).

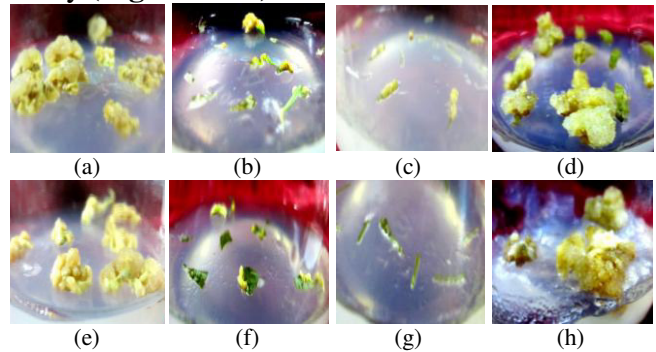


Figure 3. Cassava (acc 433) callus induction, upper row are treatments with NAA 25ppm and lower row are treatments with NAA 50 ppm, columns represent different type of explants: (a,e) adaxial-side up, (b,f) adaxial-side down, (c,g) petioles and (d,h) stems.

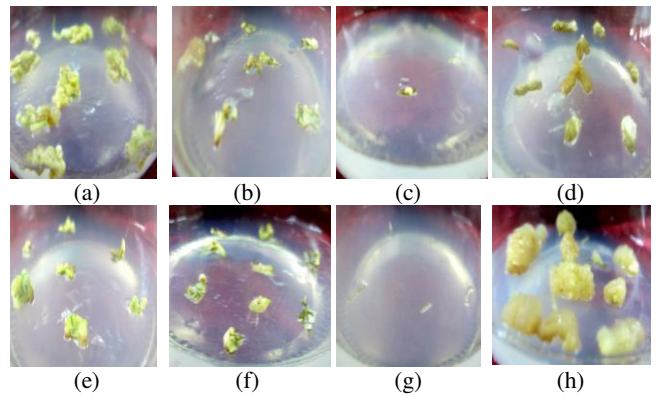


Figure 4. Cassava (acc 450) callus induction, upper row are treatments with NAA 25ppm and lower row are treatments with NAA 50 ppm, columns represent different type of explants: (a,e) adaxial-side up, (b,f) adaxial-side down, (c,g) petioles and (d,h) stems.

Non-embryonic callus which were characterized by their compact surface. It performed by all callus induced from acc 450, as well as callus induced from petiol and adaxial-side down in acc 433. In this study, only acc 433 could demonstrated a embryonic-friable callus, performed by explants from leaf with adaxial-side up and stem which were produced yellowish-green friable callus (**Fig. 5**).

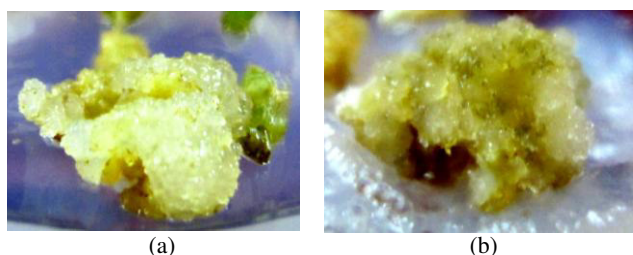


Figure 5. Friable and embryonic callus induced from medium supplemented with NAA 25 ppm (Acc 433): (a) stem and (b) leaf with adaxial-side up.

DISCUSSION

Different response of explants source to NAA treatment from those two accessions in callus induction as well as friable callus formation were found in this experiment. Callus successfully induced within 2 weeks from leaf with adaxial-side up and stem (**Fig. 1**). Those both explants of acc 433 also produced friable callus from medium supplemented with NAA 25 ppm (**Fig. 2**). In the contrary, explants from petiole and adaxial-side down were less-sensitive to auxin and gave slow response to callus induction (**Fig. 3 and 4**). Stamp (1987) and Raemakers *et al.* (1993) reported that embryos seem to emerge from the cell around the adaxial side of the explants. As well as Ibrahim, *et al.* (2008) proposed that the position of the explants should be considered as an important factor in the attempt to improve the frequency of somatic embryogenesis in cassava.

Accession 450 and 433 had different response to media formulation given in this experiment. Accession 450 might need different combination of induction agent to produce friable and embryonic callus. Previous study mentioned that the induction of FEC in cassava might be genotype dependent (Taylor *et al.* 2001 and Hankuoa *et al.* 2006). Recent study from Rossin and Rey (2011) also found differences between cultivars and type of explants in somatic embryos formation, as well as their response to the type of auxin.

According to Mapayi *et al.* (2013), some genotypes are grouped as recalcitrant, which no or low amenable response to plant growth regulator given. According to Sudarmonowati and Bachtiar (1995) and Raemakers *et al.* (1997), often it is possible to induce proembryonic structure, or even globular embryos on explants from recalcitrant cultivars, but these do not develop further to torpedo-shaped or maturing embryos.

CONCLUSION

Leaf with adaxial-side up and stem from in vitro explants gave the best response to callus induction, while petiole and adaxial-side down are not recommended due to their slow response on treatment media. Friable callus were only found in accession 433, produced from leaf with adaxial-side up and stem. In contrary, no friable callus was found in accession 450.

ACKNOWLEDGMENT

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Rapid Multiplication Procedure for Gadung's Tuber- Seeds Propagation

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ABSTRACT

Gadung (*Dioscorea hispida*) is belong to tuber food crops. In spite of that, gadung is also well known as a medical plant because of its diosgenin and dioscorine contents. Even though it has potential economic value, its cultivation and utilization in Indonesia is very low. Large quantities of planting material become a mayor constraint in its cultivation. Farmers usually need 30-50% of their harvest as planting material, impact to low consuming and marketing production. This experiment tried to answer the challenge of producing rapid multiplication tuber as planting material (tuber-seed) of gadung. Experiment was conducted at Cikeumeuh Experimental-Station, BB Biogen, Bogor on December 2011 - January 2013. Planting material was used gadung from Lampung province, collection of ICABIOGRAD (accession number: 354). The treatments were: mini tuber without cutting (20-100 of weigh), sett (cutting tuber) distinguished as small sett weighing 40-100 grams/each and mini sett which weighing 120-300 grams/each. Mini tuber promoted the highest sprouting rate than other treatments but no differences were found in harvest characters observed, namely tuber weight, total tuber per plant and tuber size. Small sett produced the most total tuber seeds on this experiment, which means that small sett could be applied to provide easy, fast and rapid seeds multiplication of gadung.

Keywords : *Dioscorea hispida*, small sett, rapid multiplication.

INTRODUCTION

Gadung (*Dioscorea hispida*) belongs to the genus *Dioscorea*, family Dioscoreaceae, the oldest groups of angiosperms. This genus are cultivated, or at least gathered, for food or pharmaceutical purposes (Norman et al, 1995).

Utilization. *Dioscorea* spp are widely utilized in African region as a main staple food crops, play an important role in economic, social and religious value. Indonesia, *Dioscorea* spp are not widely utilized and cultivated as much as cassava, potato or sweet potato. Several famous *Dioscorea* sp namely *D. alata* (ubi kelapa), *D. esculenta* (Gembili) and *D. hispida* (Gadung) mostly just cultivated in home garden or as intercrops in forest area.

Nutrient contents. Beside starch content, *Dioscorea* spp also contain important quantities of amino acids (aspartic acid, glutamic acid, alanine and phenylalanine), minerals (calcium, phosphorus and magnesium) and vitamins (ascorbic acid, beta carotene, thiamin and riboflavin) (Craufurd *et al.*, 2001). The tubers also contain alkaloids (sapogenins) and polyphenol which have genuine medicinal value and are used in many pharmaceutical preparations (Degras, 1993). Nevertheless, Farmers often encounter shortages of planting material and gadung cultivation is diminishing.

Mayor Constraint. Yam production, include gadung, are constrained by several factors including the limited availability and loss of planting material as well as the high cost of labour for operations such as land preparation, staking, weeding, droughts, disease epidemics, harvesting and storage (Okoro, 2008). The major constraint to increased yam production is scarcity and high cost of seed. Cost of seed may reach 40% or more in the total outlay for production (Okoli and Akoroda, 1995). The tuber which is the source of food is also the source of planting materials. Over 35% of total yams harvested was retained and used as planting sets for next year's production (Udoh *et al.* 2008).

1.1. Method for Rapid Multiplication. Kalu et al. (1989) assessed the seed yam production potential of three yam species (*Dioscorea rotundata*, *Dioscorea alata* and *Dioscorea cayenensis*) with setts cut from mother tubers and found that this technique reduced the number of tubers needed for planting system, and resulted in plants superior to those produced by the traditional method in all their production attributes, especially in *D. alata* and *D. rotundata*. But, the same technique never been reported in gadung.

MATERIALS AND METHODS

Planting material was used gadung from Lampung province, collection of ICABIOGRAD (acc.no:354). Experiment was conducted at Cikeumeuh Experimental-Station, BB Biogen, Bogor on December 2011 - January 2013. The treatments were: mini tuber without cutting (20-100 of weigh), sett (cutting tuber) distinguished as weighing mini sett 40-100 grams/each and small sett which weighing 120-300 grams/each. Tuber stored in a dark and moist-room about 4 months to break dormancy and stimulate germination.

Each planting material planted in 35x35 cm polybag. The composition of media was soil: compost=1:1. Bud initiation in polybag observed for a month and then transferred to the field, to induced plant growth and tuber development. Each planted in a raised bed which size of 1x5x0.5 m, contained 10 plants/row in completely randomized design. Fertilization done 2 times for 130kg urea/ha, 50kg SP36/ha, KCl 150kg/ha. 100g of dung/hole applied once before planting. Observations carried out on shoot length, number of branches, number of leaves and leaf segments. Harvest component on weight, number of tubers, tuber diameter and length were observed. Data analyzed with PAWS Statistic 18 and Duncan-test was performed to determine at what level of significance was estimated.

RESULTS

Shoot Initiation. After 1 month after planting, mini tuber induced shoots initiation higher than small sett and mini sett (**Fig. 1**).

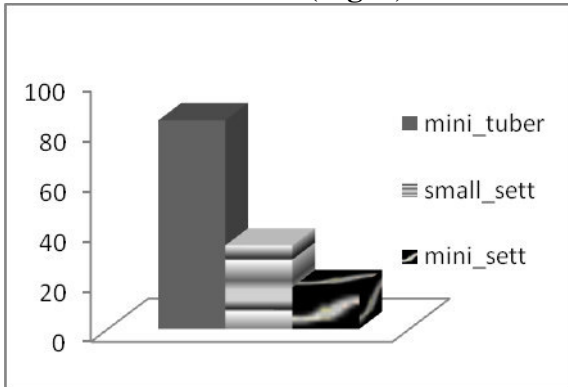


Figure 1. Shoot initiation (%) at 1 month after planting

Vegetative growth were promote in the field (**figure 2**). Data collected after 6 month (6 MAP) and were analyzed statistically. The results showed no statistical difference between treatments for plant height, number of shoot, number of leaves and number of nodes (**Fig. 3 and 4**).



Figure 2. Gadung plants in the field-experiment: (2a) Plant with control treatment (small-tuber without cutting) and (2b) field experiment at 3 MAP (month after planting).

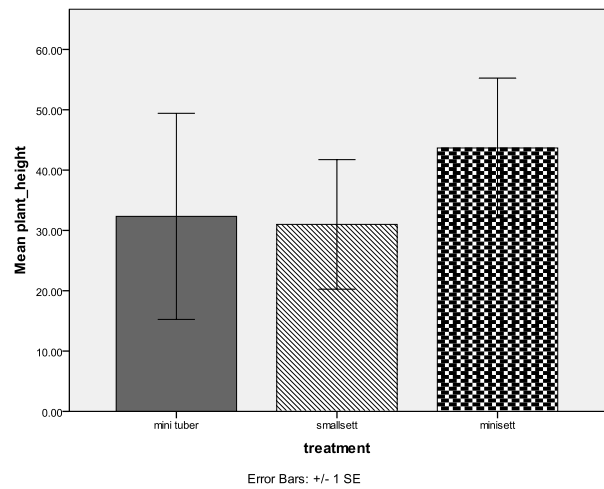


Figure 3. Effect of treatment on plant height at 6 month after planting

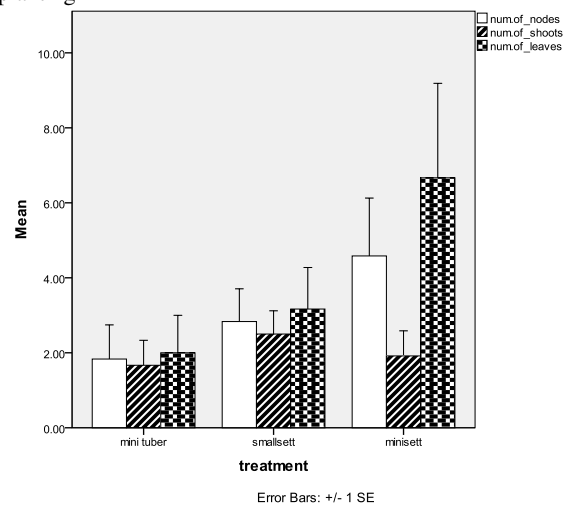


Figure 4. Effect of treatment on plant vegetative growth (number of nodes, number of leaves and number of shoots) at 6 month after planting.

Harvest components. Small-tuber and mini sett produced more tuber weight than small sett, in the contrary with number of tuber, small sett produced most tuber among others (**Fig. 5**). Observation on tuber produced per plant, tuber weight, diameter and tuber length showed no statistically difference between treatments (**Fig. 6 and 7**).

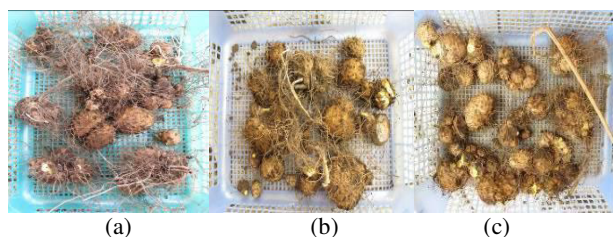


Figure 5. *Gadung's tuber from treatments: (a) mini tubers, (b) mini sett, (c) small sett*

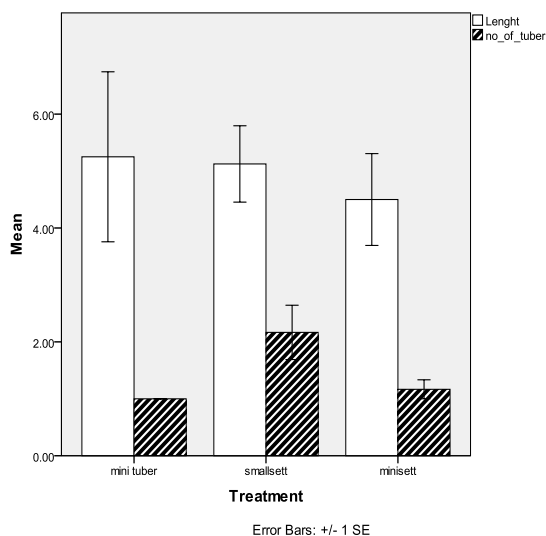


Figure 6. Harvest production: length (cm) and number of tuber produced from each treatment

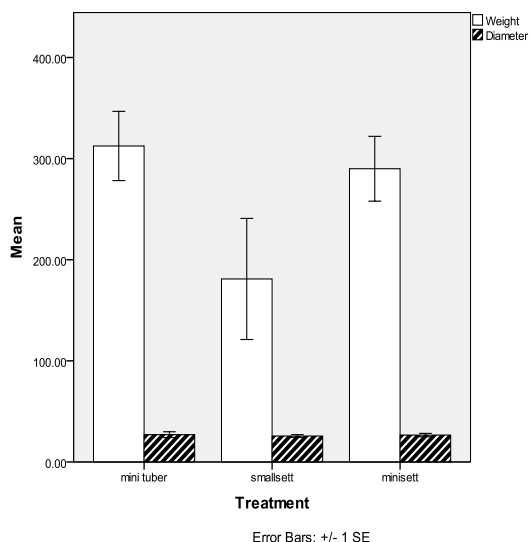


Figure 7. Harvest production: weight (grams) and diameter (cm) produced from each treatment

DISCUSSION

Due to **Fig. 1**, sprouting rate of mini sett and small sett were less than minituber, as well as uniformly time of sprout. This problem is attributed to the rotting and drying up of setts and the problem of apical dominance in tubers (Onwueme, 1982). Apical dominance is a phenomenon whereby tubers sprout first from the head region whether whole or cut-sett, followed by the middle portion and lastly from the tail region, due to greater concentration of the hormones which promote sprouting on the head region, results in non uniform sprouting of sett from various portions of the tuber (Okoro, 2008). Studies with mini sett cut from the head, middle and tail portions of seed yam tubers have shown that mini sett from the head region sprout earlier and achieve 50% germination before those from the middle portions (Okoli et al. 1982; Coursey, 1967). Ireland and Passam (1984) also found that growth inhibitors were concentrated in the outer layer of the tubers, in the meristematic and periderm layers; no inhibitors were found in the cortex.

During vegetative growth until 6 month after planting (**Fig. 3 and 4**), parameter observed (plant height, number of shoot, number of leaves and number of nodes) statistically showed no differences between treatments (small-tuber, mini-sett and small-sett). Un-uniformity in sprouting did not effect to plant growth for all treatments. But, interesting result found in generative growth (tuber production), that small sett produced highest number of tuber but lowest tuber weight, in the contrary with mini tuber and small sett (**Fig. 6 and 7**). This might be caused by harvest period related to sprouting time (Craufurd, et al., 2001). Because of technical reason, all plants in this experiment harvested in the same time, 9 month after planting. Some plant had been senescence and ready to harvest but others were not yet. Okoli, *et al.* (1982) stated that variations in the time of sprouting of the mini

setts were also affected the development of transplanted propagules and this, in turn affects the total tuber yield. Presently, tubers cut were planted without regard to the region of the parent tuber from which they were cut. This practice results in non-uniformity in sprouting and emergence, wide variations in tuber yields at harvest and in the overall crop performance (Kalu, 1989).

Akubuo (2002) also showed that some problems faced in the practice of planting a mixture of yam mini setts cut from the head, middle and tail portions of the tuber, will result in non-uniformity in sprouting and widely different germination rates and tuber yields.

CONCLUSION

Multiplication of gadung with small sett, mini sett and mini tuber resulted sufficient large tuber to serve as a seed tuber that used for another cycle multiplication. Production of food tubers will be produced from this seed tuber with tuber yields as good as traditional tuber seed (big tuber without cutting). Small sett (sett tuber weighing 40-100 grams) produced the most number of tuber among others, means provide most number of seed tuber to produce food tuber. With small sett technique, offer rapid seed multiplication which easy transported and distributed to farmers because of its small size of tuber seed.

ACKNOWLEDGMENT

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ISOLATION OF CELLULOLYTIC MOLD FROM SOIL OF TEAK FOREST IN KRESEK, MADIUN

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ABSTRACT

The research is conducted to isolation of cellulolytic mold from soil of teak forest in Kresek Madiun. Cellulolytic Mold is a type of mold that has a cellulase enzymes that can degrade cellulose containing materials like an organic wastes. Cellulose may be hydrolyzed using enzymes to produce glucose, which can be used for the production of ethanol, organic acids and other chemicals. Cellulose is expensive and contributes only 50% to the over all cost of hydrolysis due to the low specific activity. Celluloses provide a key opportunity for achieving tremendous benefits of biomass utilization. Therefore, there has been much research aimed at obtaining new microorganisms producing cellulase enzymes with higher specific activities and greater efficiency. Presently, work is aimed at screening and isolating cellulolytic mold from the soil of teak forest samples, identification of the isolates based on macroscopic and microscopic characteristic. The cellulolytic mold isolate were identified are *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizopus*. The cellulolytic mold that exhibited higher cellulase activity is *Aspergillus* sp. with $21,17 \pm 1,53$ mm of clear zone diametre and 0,68 of clear zone ratio. The cellulolytic mold that exhibited lowest cellulase activity is *Penicillium* sp. with $0,63 \pm 0,35$ mm of clear zone diametre and 0,06 of clear zone ratio.

Keyword : Cellulolytic mold, Degradation, Soil of Teak Forest

INTRODUCTION

Cellulose is considered as one of the most important sources of carbon on this planet and its annual biosynthesis by both land plants and marine algae occurs at a rate of 0.85×10^{11} tonnes per annum (Nowak *et al.*, 2005). Cellulose degradation and its subsequent utilisations are important for global carbon sources. The value of cellulose as a renewable source of energy has made cellulose hydrolysis the subject of intense research and industrial interest (Bhat, 2000). Over the years, a number of organisms, in particular fungi, possessing cellulose degrading enzymes have been isolated and studied extensively (Nowak *et al.*, 2005). Cellulose may be hydrolyzed using enzymes to produce glucose, which can be used for the production of ethanol (Olsson and Hahn Hagerdahl, 1996), organic acids (Luo *et*

al., 1997) and other chemicals (Cao *et al.*, 1997).

Enzymatic components act sequentially in a synergistic system to facilitate the break down of cellulose and the subsequent biological conversion to an utilizable energy source, glucose (Beguin and Aubert, 1994). Cellulases provide a key opportunity for achieving tremendous benefits of biomass utilization (Wen *et al.*, 2005). Therefore, there has been much research aimed at obtaining new microorganisms producing cellulase enzymes with higher specific activities and greater efficiency (Subramaniyan and Prema, 2000).

Cellulases have attracted much interest because of the diversity of their applications, and also for facilitating the understanding of mechanism of enzymic hydrolysis of plant carbohydrate polymers (Bhat *and* Bhat, 1997).

The major industrial applications of cellulases are in textile industry for 'biopolishing' of fabrics and producing stone washed look of denims, as well as in household laundry detergents for improving fabric softness and brightness. Besides, they are used in animal feeds for improving the nutritional quality and digestibility, in processing of fruit juices, in baking etc. Utilisation in deinking of paper is yet another emerging application. The cellulases that are used so far for the above mentioned industrial applications are those from fungal sources (Tolan and Foody, 1999).

Cellulolytic mold are produced in large amounts, which include all the components of a multi enzyme system with different specificities and mode of action, i.e. endoglucanases, cellobyohydrolases, (exoglucanases) and β -glucosidase, acting in synergism for complete hydrolysis of cellulose. The present work is aimed at screening and isolating cellulolytic fungi from the soil of teak forest and identification of the isolates based on macroscopic and microscopic characterization. Further, efforts were also made to optimize the cultural and environmental conditions for maximizing of yield of the enzyme. The present study was undertaken with the following objectives:

- To isolate mold from teak forest soil samples that produce cellulase enzyme using a selective medium after enrichment.
- To determine the ability of Cellulolytic mold to degrade cellulose

MATERIALS AND METHODS

Soil collected from teak forest in Kresek, Madiun with depth of 5-10 inches from the top and sieved through a 2 mm sieve constituted the soil sample. The samples were dispensed into bags and were brought to the laboratory and soil enrichment was done by adding 1g of cellulose. Enrichment broth with cellulose as carbon source and peptone as nitrogen source was used for isolation of cellulolytic mold. The selective medium i.e., Mandel's enriched medium with pH 5 was

employed to get desired fungi (Mandels, 1985).

The plate screening medium was used which contains Mandels mineral salts solution along with cellulose thus enabling the growth of many cellulase secreting fungi. The growth obtained on Mendel's enriched agar medium was isolated and inoculated into petri plates containing CMC (Carboxy methyl cellulose) agar medium. The strains isolated were then inoculated into the production medium to identify the ability of the strain for cellulase production under optimized conditions. The obtained pure cultures of the fungi were maintained at 29°C and then transferred to Potato dextrose agar (PDA) slants. The isolated fungi were identified based on the morphological, microscopic and macroscopic characterization.

RESULTS

Kresek is one of centra teak forest in Madiun. The location of this research is about 07° 41'53,07' S and 111° 37' 24,64'' E. Generally, the physicochemical characteristic of the soil showed with 40% of moisturity, 6,7 pH and 21 of C/N ratio. The condition of the soil is the general characteristic of forest. The mold that can be isolated from teak forest in kresek madiun are *Aspergillus* sp, *Fusarium* sp, *Penicillium* sp and *Rhizopus* sp. The genus that can be isolated is showed in **Fig. 1**. The soil sample contained considerable population of the cellulolytic mold. The mold grown on the selective media supported the growth of the mold by using cellulose as the carbon source (Khalid *et al.*, 2006). Efficient cellulolytic mold isolates were finally selected based on the zone of the clearing around the mold on Carboxy Methyl Cellulose agar (CMC agar) plates (Immanuel *et al.*, 2006). The appearance of the clear zone around the colony when the Congo red solution was added (Wood *and* Bhat, 1998), was a strong evidence that the cellulolytic mold in order to degrade cellulose (**Fig. 2**). The clear zone diametre of each mold are shown in **Table 1**.

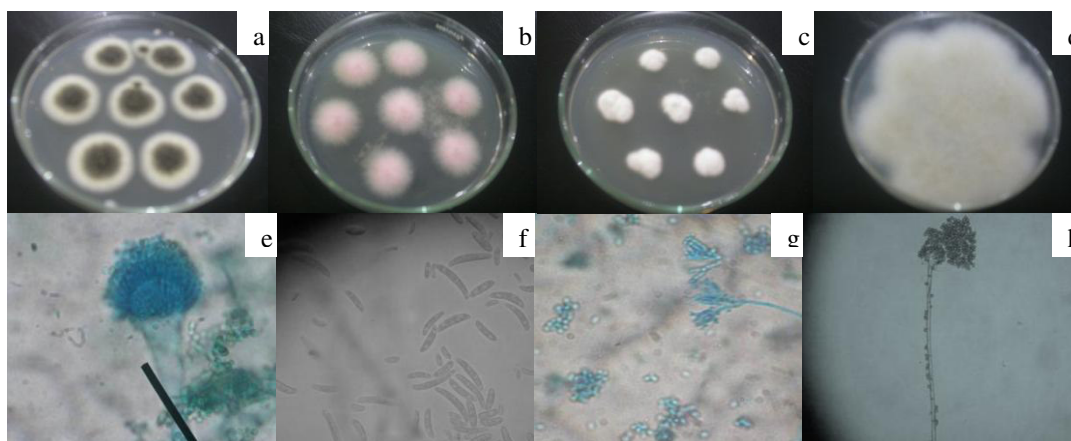


Figure 1. Morphological characteristic of cellulolytic mold in PDA medium a) *Aspergillus* sp., b) *Fusarium* sp., c) *Penicillium* sp., d) *Rhizopus* sp., e) *Aspergillus* sp. spore, f) *Fusarium* sp. spore, g) *Penicillium* sp. spore, d) *Rhizopus* sp. spore

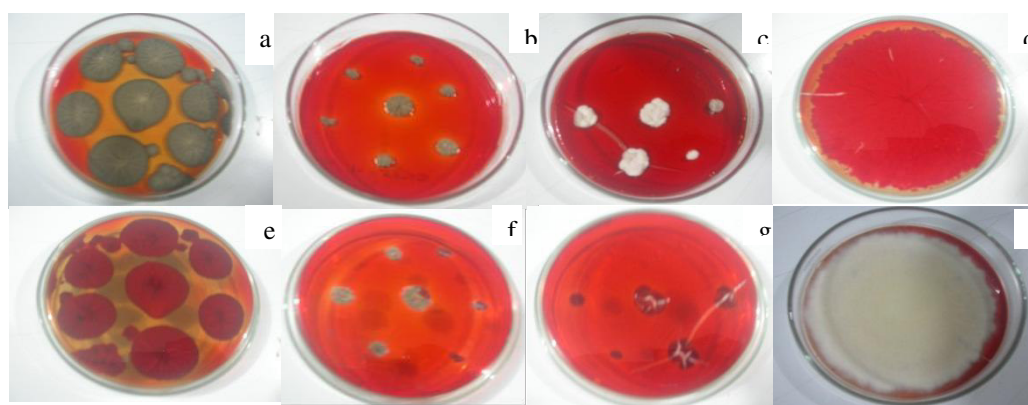


Figure 2. Cellulolytic Mold in CMC agar medium with Congo red solution a) *Aspergillus* sp looks foward, b) *Fusarium* sp. looks foward, c) *Penicillium* sp. looks foward, d) *Rhizopus* sp. looks foward looks foward, e) *Aspergillus* sp looks back, f) *Fusarium* sp. looks back, g) *Penicillium* sp. looks back, h) *Rhizopus* sp. looks back.

Table 1. The diametre of clear zone CMC agar with Congo Red solution

No	Genus	Clear zone (mm)	Colony Diametre (mm)	Ratio of clear zone
1	<i>Aspergillus</i> sp.	21,17± 1,53	31	0,68
2	<i>Fusarium</i> sp.	4,67 ± 2,08	9	0,52
3	<i>Penicillium</i> sp.	0,63 ± 0,35	11	0,06
4	<i>Rhizopus</i> sp.	8,00 ± 1,73	89	0,09

DISCUSSION

a. Isolation of the celulolytic mold

The teak forest soil sample contained population of the cellulolytic mold. The mold grown on the selective media supported the

growth of the mold by using cellulose as the carbon source (Khalid et al., 2006). Efficient cellulase producing mold isolates were finally selected based on the clearing zone around the mold on carboxy methyl cellulase agar (CMC agar) plates (Immanuel et al., 2006). The

appearance of the clear zone around the colony when the Congo red solution was added (Wood and Bhat, 1998), was a strong evidence that the fungi produced cellulase in order to degrade cellulose. Based on the research showed that the biggest diameter of clear zone is *Aspergillus* which then *Rhizopus*.

b. Morphological identification

The isolated mold was purified by repeated subculturing on the Potato Dextrose Agar medium at regular intervals and incubating at 29°C. The isolate was identified based on the colony morphology and microscopic observation like colony shape, color of colony, spore shape, hyphae, conidia, conidiophore etc.

The colonies size of *Aspergillus* sp. are medium, brown hyphae, hyphae's texture are smooth, rapid growth, yellowish brown hyphae in undersurface. Conidiophores $\pm 11 \mu\text{m}$ in length, conidiophores head diameter $\pm 63 \mu\text{m}$ conidiophores, brown vesicle and brown conidia, septate conidiophores. *Fusarium* sp. has a white hyphae initially. At the age of 2-3 days later the white spores appear yellow interspersed. Under the surface of the hyphae interspersed yellowish. The conidiophores in 45-50 μm in length, macroconidia $\pm 2, 6-5, 2 \mu\text{m}$ in length. The colonies of *Penicillium* sp. are white with dense hyphae at the first. At the age of 3-4 days the colonies changed to a dark green interspersed with white and form a vortex in the middle, sometimes showing a clear zone. Under the surface of the hyphae interspersed brown yellowish. The conidiophores $\pm 36 \mu\text{m}$ in length, the head of conidiophores $\pm 7 \mu\text{m}$ in length, has a black translucent vesicles and black conidia. The colonies of *Rhizopus* sp. like a thread with white color; the sections of sporangium and sporangiofora look certain form of black dots as pin, has a septate hyphae, multinucleated and have a stolon and dark color rhizoid if it is old.

CONCLUSION

Cellulose is the primary product of photosynthesis in environments and it is the

most abundant renewable bioresource produced in the biosphere (~100 billion dry tons/year). Cellulases produced by microorganisms are either cell associated or free form, metabolize the insoluble cellulose.

The mold was isolated from the soil and was enriched with the cellulase and the soil was diluted and inoculated on the Mendels medium (selective media for cellulase producing fungi) and the isolated was inoculated in to the selective Mendels mineral solution which was kept on the shaker. The mold capable to produce cellulase were identified are *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizopus*. The salient features of the present study are:

- The cellulolytic mold isolate were identified are *Aspergillus* sp, *Fusarium* sp, *Penicillium* sp and *Rhizopus* sp.
- The cellulolytic mold that exhibited higher cellulase activity is *Aspergillus* sp. with $21,17 \pm 1,53$ mm of clear zone diameter and 0,68 of clear zone ratio.
- The cellulolytic mold that exhibited lowest cellulase activity is *Penicillium* sp. with $0,63 \pm 0,35$ mm of clear zone diameter and 0,06 of clear zone ratio.

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***IN VITRO* SELECTION ON SUGARCANE CALUS CONDUCTED FROM GAMMA RAY IRRADIATION FOR ALUMINIUM TOLERANCE**

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ABSTRACT

Sugarcane (*Saccharum officinarum* L.) is the plant which has important role in Indonesia as main raw materials of granulated sugar. Sugar production in Indonesia was decrease caused of extreme season which cause of decreasing sucrose content of sugar cane crops. Increasing sugarcane production can be conducted by land extensification in outside of Java dominated by acid soils, especially podsollic red yellow. The problem of plant cultivation in that area was high aluminium concentration and low pH which cause plant growth decreased. High genetic variability was a main factor needed to conducted the new variety. Biotechnology *in vitro* culture was used to conducted new improved varieties beside of conventional breeding. The combination of mutation (gamma ray irradiation) with *in vitro* selection can increased a high genetic variability in somatic cells. Using *in vitro* selection the new charater was directed since culturing process. By the technology, many varieties were released with certain characters and the new characters were inherited to the next generation.. The sugarcane varities used as the explants was PS 864. Population of somatic cells irradiated with gamma rays with dosage of 0 – 50 Gray until conduct LD₅₀ dosage. These callus cultured on medium cuture added with AlCl₃.6H₂O to select callus tolerant to this condition. Regeneration of somatic cells population (somaclone) expectedly has new improved characters that tolerant to acid soil. The experiment showed that callus growth of PS 864 was very low because of phenol oxidation. LD₅₀ dosage for gamma irradiation treatment conducted at range of 20-30 Gy. Higher dosage of gamma ray irradiation caused higher cells damaged and callus regeneration. Callus regeneration after Al selection were 11.11 -100%.

Keywords: *Saccharum officinarum*, genetic improvement, gamma ray irradiation,
in vitro selection, aluminium tolerant.

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is an important crop of high economic value in many countries, especially in Indonesia, because of the high sugar content in the stalks. Until now the cane is used as the main raw material of sugar, nearly 70% of sweeteners derived from sugar cane, while the rest comes from sugar beets.

Increasing people population in Indonesia is very rapid impact on increasing sugar demand, on the other hand the domestic sugar demand can not be met, so the government must keep importing from other countries. Increased production of sugar cane can be done by utilizing the available dry land large enough in Indonesia, which is generally dominated by acid soils Podsollic Red Yellow, scattered mainly in wetter areas such as Sumatra, Kalimantan and Papua. However, of the approximately 148 million dry land in Indonesia, 102.8 million ha (69.4%) in the form of acid soils which are less suitable for growing crops (Zulfahmi, 2012). *Upland acid* generally has a low pH (<5.5) were associated with high Al content, high P fixation, exchangeable base content and low CEC, high content of iron and manganese, sensitive to erosion and poor biotic elements (Mulyani, 2006).

Plant growth in acid soil generally inhibited and productivity is very low, due to the high level of acidity (low pH), the availability of N, P, K, Ca, Mg, and Mo are low and Al and Mn concentrations reach toxic levels. According to Marschner (1995) this is caused by low pH and Al toxicity to roots thicken and short because the process of cell elongation inhibited so that the absorption of water and nutrients is reduced. Planting the sugar varieties tolerant to aluminium is the efficient and environmentally friendly approach.

High genetic diversity is one of the main factors in the improvement of the

characters of the plants. Increasing genetic diversity in plants that propagate vegetatively with conventional method is difficult. One technology that can be used to produce varieties is mutation induction and *in vitro* selection. The technique has been applied to get new varieties with superior characters. *In vitro* culture and mutagenesis has been widely used to improve the genetic diversity of plants, especially to get a plant that can be cultivated on marginal lands, such as acid land.. Both of these technologies are often combined to increase the chances of getting a new genotype (Ahloowalia et al., 2004; Mohan Jain, 2010).

New characters which are formed due to the induction of mutations is highly diverse due to random mutations formed. To direct characters changes can be selected using *in vitro* methods, thus allowing for the selection of candidates mutants generated by using specific selection agent. According Bidabadi et al., (2012), and Jayasankar et al., (2000), *in vitro* selection is very effective to speed up obtaining somaclones or mutants that are resistant to abiotic and biotic stress.

In vitro selection to obtain new varieties tolerant to acid soil can be done using $AlCl_3 \cdot 6H_2O$ as component selection and low acidity (about 4) (Short *et al.*, 1987, Purnamaningsih and Mariska, 2002, Purnamaningsih and Mariska, 2008). Regeneration in extreme environments are expected to generate new somaclones tolerant to aluminum and low pH (pH 4). This method has been proved to produce new varieties that are resistant to environmental stresses and the characters inherited by their progeny.

MATERIALS AND METHODS

Sugarcane varieties used are PS 862. The plant material used was a young sugarcane leaves taken stems of 4 month old cane plants. The explant sterilized using

alcohol, klorox, tween, betadin and sterile distilled water. The Outer leaves discarded until the deepest layers of leaves, and cut into pieces and grown on medium to induce callus formation.

Callus induction was performed using the strong activity of auxin, 2,4-D. Media formulations used for callus induction was MS + 2,4-D 3 mg/l + casein hydrolyzate 3 g/l. Callus formed sub-cultured every 2 weeks to stimulate callus proliferation and somaclonal variation. Variables measured were time callus formation, visual callus and callus structure.

Somatic cell populations subjected to gamma-ray irradiation at doses of 0, 10, 20, 30, 40, and 50 Gy. Radiation treated calli were immediately cultured on callus induction medium to eliminate the radiolysis hazards for 4 weeks. Sub-cultures done at least thrice at 15 days interval before using to further step. Observations were made of the percentage of callus alive, visual and colour of callus.

Callus treated gamma ray irradiation was transferred to callus induction media using $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ as component selection at level of concentration of 0, 100, 200, 300, 400, and 500 mg/l and low pH (4.0) with 20 replications. To bring out the toxicity of Al on the selection medium, macro salt from MS medium modified as follows: NH_4NO_3 content increased from 1650 mg/l to 2400 mg/l, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ reduced from 440 mg/l to 15 mg/l and KH_2PO_4 reduced from 170 mg/l to 13 mg/l. FeSO_4 28 mg/l used as a source of Fe. The medium used for selection was MS + 2,4-D 2 mg/l + Casein hidrolisat 3 g/l + component selection.

RESULTS

1. Callus induction

Initiation of callus begins at the age of 19 days after isolation, whereas callus obtained has crumb structure with a brownish color (**Table 1**). Callus obtained using 2,4-D 3 mg/l + Casein hidrolisat 3 g/l

was very low (41%) and have a crumb structure with yellowish or brownish white colour (**Table 1**).

Table 1. Callus initiation, diameter, structure and the colour of callus

Callus initiation (days)	Diameter (cm)	Callus structure	Colour of callus
19	0.5	friable	yellowish, brown

2. Induce mutation on callus using gamma ray irradiation and *in vitro* selection

Callus that had been treated with gamma irradiation give different responses depending on the dose of gamma-ray irradiation were given (**Table 2** and **Fig.1**). The results showed that the higher dose of gamma irradiation given percentage of the higher of callus, that is indicated by the colour of callus turn black and dry. Calli was still alive will then be transferred to the selection medium with the addition of aluminum to some extent with the concentration of media pH adjustment to 4.

Table 2. Percentage of callus alive after gamma ray irradiation treatment, 1 month

Dose of irradiation (Gy)	Percentage of callus alive	Color of callus
0	100	Yellowish
10	90	Yellowish
20	60	Yellowish
30	35	Yellowish, brown
40	30	Brown
50	15	Black

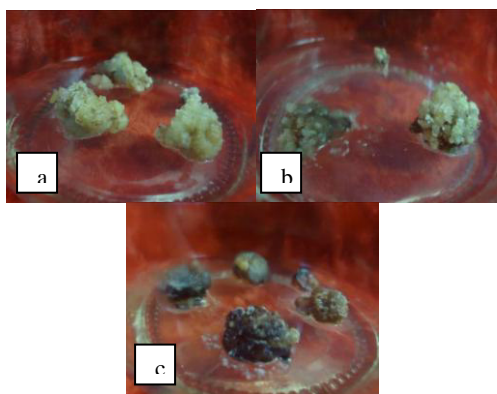


Fig 1. Callus growth after gamma ray irradiation treatment, 2 weeks, (a) 20 Gy (b) 30 Gy (c) 50 Gy

Calli were still alive after gamma-ray irradiation treatment has moved on selection media using $\text{AlCl}_3 \cdot 6 \text{H}_2\text{O}$ as component selection to select mutated cells and have the ability to tolerance to aluminum and low pH. Callus response on selection media varies depending on the ability of each cell to adapt to a given stress conditions. Cells are able to survive to aluminum stress and low pH regenerate forming shoots after 4 weeks on the selection medium, but if the cells can not survive, then the cells can not regenerate into prospective shoots. The candidate shoots grow and develop into shoots and have stems and leaves after the age of 8 weeks (**Fig. 3**). Percentage of callus regeneration of each treatment of aluminum concentrations ranged between 11.11 - 100% (Tables 3).

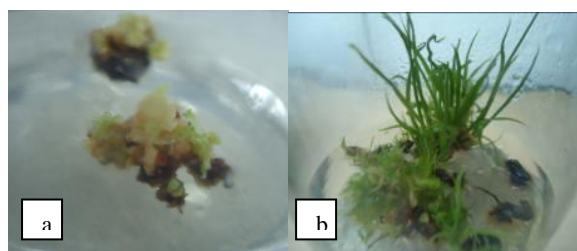


Fig3. Callus forming shoots regenerated on selection media a = 4 weeks, b = 8 weeks

Table 3. Regeneration of callus at various concentrations of aluminum

Dose of gamma ray irradiation (Gy)	Aluminium concentration (mg/l)	Regeneration of callus (%)
10	0	45
	100	32.5
	200	30.0
	300	16.67
	400	0
20	500	0
	0	52
	100	14.29
	200	0
	300	11.11
30	400	16.67
	500	0
	0	32.5
	100	0
	200	0
40	300	66.67
	400	16.67
	500	55.56
	0	30.15
	100	0
50	200	50
	300	100
	400	100
	500	75
	0	20.0
	100	15.0
	200	0
	300	0
	400	0
	500	0

DISCUSSION

Aluminium is considered as the main abiotic stress that causes 25–80% yield losses in various crop plants grown on soils containing excessive aluminium content (Singh *et al.*, 2010). The evaluation of aluminium tolerance in tissue culture may be more useful for breeding programmes, because selection is earlier and faster than in the field. Many studies in the recent years have tried to develop Al-

resistant plants through the use of tissue and cell culture. The first step of these methods was increasing genetic variation of plant using mutation and somaclonal variation, and the second step is selection of cell lines exhibiting enhanced tolerance to aluminium.

1. Callus induction

The key to success in this activity is determined by the amount of plant material available, namely callus. The more callus is available, then the chances of success of obtaining new mutants will be even greater. The first activity of this research was to conduct sterilization method of plant material. This stage is one of the crucial parts because if a combination of sterilization materials and time of sterilization was not right, the sterile callus obtained only slightly. The results of this research shows callus sterile conduct was only slightly. This was caused by phenols released by the injured tissue was very high. The more phenols released can inhibit the formation of callus and also usually the less callus sterile produced.

Callus formation was very low because callus produced from PS 862 released phenolic compounds that can inhibit the growth of explants forming callus. Presence of phenolic compounds in the culture medium made the medium color turn brown.. According to Shashi *et al* (2012) reducing phenol expression can be used antifenol compounds, such as ascorbic acid, citric acid or PVP (polyvinyl pyrrolidone). The use of PVP can reduce the accumulation of phenolic antioxidants in the medium and the inhibitory effect on the growth of explants can be reduced.

2. Induce mutation on callus using gamma ray irradiation and *in vitro* selection

Mutation induction done using gamma-ray irradiation at doses of 0-50 Gy. Gamma ray irradiation causes to the callus damaged. The higher dose of irradiation used, the level of damage to the cell will be higher (Table 2).

This was evident from the callus turns brown / black which usually indicates that the loss of visible callus forming shoots regeneration / plantlets. Gamma ray irradiation at a dose of 50 Gy produce surviving callus by 15%, while the other callus turns brown / black (**Table 2**). Table 2 shows that the LD₅₀ dose was 20-30 Gy. Patade and Suprasanna (2009) also states that the LD₅₀ dose on sugarcane callus occurred at a dose of 20 Gy.

Calli that was still alive after gamma-ray irradiation treatment had moved on selection medium using H₂O AlCl₃.6 H₂O as component selection to select cells that have the ability to mutate and tolerance to aluminum and low pH. Callus on selection medium response varies depending on the ability of each cell to adapt to stress conditions. In the early stages, the ability of the cell tolerance to aluminum stress can be seen from callus discoloration. The alive callus have yellow or brownish-yellow colour, whereas non-viable callus colour changed to brown or black (Table 3).

Regenerating callus of the age of 4 weeks were not fully develope into mature buds, depending on the ability of each cell to prevent from poisoning aluminium that accumulates on selection medium. If the cells do not have a tolerance mechanism to aluminium, the young shoots become brown and can not develop into mature shoots.

CONCLUSION

1. Percentage of callus formation of PS 862 was very low (41%) because phenol expression that inhibit the growth of callus.
2. The higher dose of gamma irradiation causes the greater cell damaged and cell deaths, as indicated by the colour change of callus.
3. LD₅₀ dose of callus was 20-30 Gy.
4. Callus regeneration in the treatment of *in vitro* selection using AlCl₃.6H₂O ranged between 11.11 -100%.

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SHOOT MULTIPLICATION OF TARO (*Colocasia esculenta* var. *Antiquorum*) THROUGH *IN VITRO* CULTURE

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ABSTRACT

Taro (*Colocasia esculenta* var. *Antiquorum*) is a tropical plant grown primarily for its edible tuber as source of calory and calsium with low carbohydrate. The tubers were served in any kind of food, medicinal, and diet food and drink for diabetes. There were some chance for Indonesia to export Taro to Japan about 45,000 ton per year. Result of *in vitro* experiment, shoot multiplication was still low. The aim of experiment is optimize of shoot multiplication of Taro. The experiment was done in Biology Cell and Tissue Division of ICABIOGRAD in 2011. Explant was shoot tip of tuber which sterilized and planted in MS medium. After shoot induction, shoots transfered to MS medium which combined with BA (0.5; 1 and 2 mg/l) and Thidiazuron (0,5 dan 1 mg/l). After multiplication, the shoots were transfer/sub culture to MS + IAA or NAA 0,5 mg/l for rooting formation and development. Acclimatization were done by transfer the plant to polibag content of soil and manure (1:1) in green house. Observation were done on: time of initiation, number of shoot per explant, number of leaf, number of root and percentage of survive plant after acclimatization. Result of experiment showed that initiation begin at 2-3 weeks after planting of explant. At 3 months after planting, the best media for shoot multiplication of Taro was BA 2 mg/l + Thidiazuron 1 mg/l with 1,88 cm shoot height, 3.5 number of shoot, 10.25 number of fresh leaves, 5.75 dry leaves, 2.5 number of root, and 0,38 cm of root length. In MS + IAA 0.5 mg/l the root was growth and developed better than that of in MS + NAA 0.5 mg/l. 80 % of seedling was survive in green house after acclimatization.

Keywords : *Colocasia esculenta* var. *Antiquorum*, shoot multiplication, *in vitro* culture

INTRODUCTION

Taro (*Colocasia esculenta* var. *Antiquorum*) is a bulbous plant with delicate flavors tubers are a source of high quality protein and can be presented in a variety of dishes such as soups, chips, ice cream and as a raw material other processed products, which turns it tastes good and tasty. Besides the fresh tuber as a source of Calcium and high calories, low carbohydrate content to be consumed as food diet is also good for diabetics. The starch can be used as an ingredient of food production / healthy beverages, such as thickeners (starch), pureed baby food, elderly, cakes and bakery, raw materials mixing wheat flour as a substitute for potatoes. In pharmacy / medicine it can be used as filler capsule and tablet. The fiber can function as a mixture of making jelly, ice cream, biscuit filling, soup preparations, fibrous drinks, puddings, foods and diet drinks for diabetics, etc.

Early Taro presence in Indonesia is in the occupation of Japan and called Japanese Taro or Satoimo (Anonymous, 2007). Taro is known by the public in the name of *Talas Bithek* in Toraja, and in Buleleng Bali *Keladi Salak* (LIPI, 2002 in Anonymous, 2007). Satoimo Indonesian-Japanese consortium in collaboration with Indonesian Chamber of Commerce, has initiated development Satoimo cultivation in Indonesia since 2003. Finally on February 16, 2006 until now, Satoimo of Indonesia has been exported to Japan (Anonymous, 2007).

Indonesian-Japanese consortium Satoimo prime export to Japan as a size 40 foot container weighing 25 tonnes. Each month will be exported 25 tons Satoimo that by the buyer, Global Seafood Limited and will be directly incorporated into the supermarkets in Japan (Anonymous, 2006). Open export opportunities for Satoimo production in Japan continued to decline as production

costs continue to rise. Moreover, because of seasonal factors that do not allow farmers to plant Japanese satoimo throughout the year.

Japanese taro cultivation promises huge profits and do not require complicated technology (Briliantono, 2006). Satoimo consumption in Japan reached 3 kg per capita per year. Assuming a population of approximately 120 million Japanese, hence the need Satoimo reach 360,000 tons per year. Meanwhile Satoimo production in Japan since 1996 continued to decline to below 2250,000 tons. As a result there is a shortage 110,000 tons, which is filled with imports from China that are capable of supplying 65,000 tons per year. So there is still a chance for Indonesia to fulfill the needs of Japan will Satoimo 45,000 tons (Anonymous, 2007; Briliantono, 2006). To fulfill the needs of the Indonesian Chamber of Commerce Satoimo target area of 70 acres of land scattered in various areas in West Java, East Java and Central Java by involving local farmers. Farms in 2007 is expected to increase significantly to be 1,000 hectares and in 2008 could increase to 3,000 hectares.

According to FAO (Anonymous, 2005) Taro or Satoimo can serve as food security (Food security) in some countries such as Africa, Asia and Oceania. Besides, Taro is also widely used in the social culture (Sosoio-cultural) as the food used in traditional events, traditional medicine and others, as well as a cash crop.

In vitro propagation has been carried out in China by using shoot buds explants (Du et al., 2006). At first, shoot buds were cultured on ½ MS semi-solid medium (Murashige and Skoog) combined with Thidiazuron (TDZ) and Benzylaminopurine (BAP) and naphthalene acetic acid (NAA) at various konsentrasi. selama 30 days. Best medium

was $\frac{1}{2}$ MS + 0.1 mg / l TDZ. Then in the sub-culture plantlets on MS liquid medium for 90 days. After 30 days the highest multiplication (2.5) was achieved on MS medium + 1.0 mg / l BAP + 0.5 mg / l NAA. After 90 days the highest multiplication (4.7) was achieved on MS medium + 3.0 mg / l BAP + 0.1 mg / l TDZ. Well-developed buds later in the sub culture on solid media for rooting MS + 1 mg / l BAP + 0.5 mg / l NAA + 500 mg / l active charcoal before transplanting.

Nyman *et al.* (1983) have examined the in vitro Taro. With in vitro selection techniques to salinity has obtained several resistant plantlets were alive at 10-70% artificial sea water. Nyman *et al.* (1986) also examined the taro zygotic embryos using light and scanning electron microscopy. Taro embryos cultured in media Linsmaier-Skoog (LS) without growth regulators. Embryo contained in the seed-containing hypocotyle-root axis and cotyledons undeveloped surrounded by two main types of cells that aleurone and starchy endosperm. In LS media embryos developed into plants only in the endosperm tissue. Embryo culture of plantlets taro can be developed if: 1. Inner and outer integument removed and 2. The existence of endosperm including the aleurone layer.

The research aims to optimize shoot multiplication method of Taro or Satoimo (*Colocasia esculenta* var. Antiquorum) through in vitro culture.

MATERIALS AND METHODS

The experiment was conducted at Biology Cell and Tissue Division of ICABIOGRAD in 2011. The experiment was begun by cutting young shoot or shoot tip of tuber as explant (**Fig.1**).



Figure 1. Taro/satoimo (*Colocasia esculenta* var. Antiquorum) as source of explant.

The explant then sterilized with alcohol 75 % for 5 minutes, chlorox 30 % for 20 minutes and 20 % for 15 minutes, washed with aquadest steril three times and soaked in betadine solution for 5 minutes. After that explant was planted in MS and VMW medium in laminar air flow. After shoot induction, the shoots were transferred/sub culture to the best medium which combined with BA (0.5; 1 and 2 mg/l) and Thidiazuron (0,5 dan 1 mg/l). After multiplication, the shoots were transferred/sub culture to MS + IAA (0,5; 1 mg/l) or NAA (0,5; 1 mg/l) for rooting formation and development. Acclimatization were done by transfer the plantlet to polybag content of soil and manure (1:1) in green house. Observation were done on: time of initiation, number of shoot per explant, shoot height, number of leaves (fresh and dry), number of root, root length, and percentage of survive plant after acclimatization.

RESULTS

Result of experiment showed that initiation begin at 2-3 weeks after planting of explant in MS medium. In VMW medium, the explants were not initiated to produce shoot. Shoot formation of explant marked with swelling bud formation with green small lump (**Fig. 2**).



Figure 2. Shoot initiation of Taro/Satoimo in MS media.

At three months after planting the highest shoot was 2,20 cm in media BA 1 mg/l + Thidiazuron 0.5 mg/l, but number of shoot was only 2,5. The highest number of shoot was 3,5 cm in media BA 2 mg/l + Thidiazuron 1 mg/l even the shoot height only 1.88 (Fig. 3 and 4).

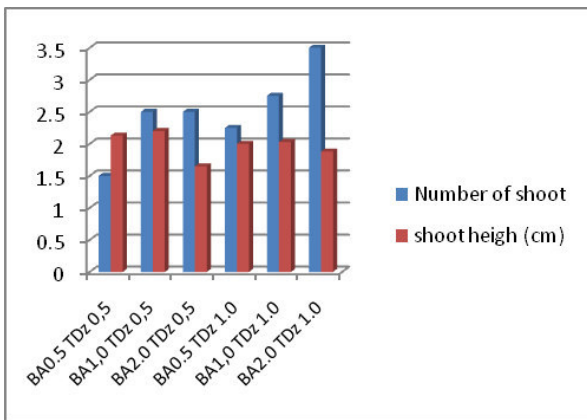


Figure 3. Effect of treatments on number of shoot and shoot height



Figure 4. Multiplication of Taro/Satoimo

The leaves of Taro in tissue culture were easy to be dry and falling down. Fig. 5 showed that the highest of fresh leaves and dry leaves was 10.25 and 5.75 respectively achieved by media BA 2 mg/l + Thidiazuron 1 mg/l. At three months after planting the

plantlet of Taro begin to produced root even in a short length. The biggest number of root in BA 2 mg/l + Thidiazuron 1 mg/l media (2.5), but the root length only 0.38 cm. The longest root of Taro in 3 month cultured was 2,58 in BA 0,5 mg/l + Thidiazuron 0.5 mg/l (Fig. 6).

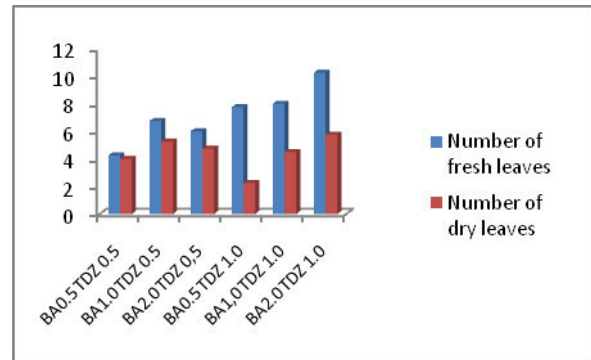


Figure 5. Effect of treatments on number of fresh and dry leaves.

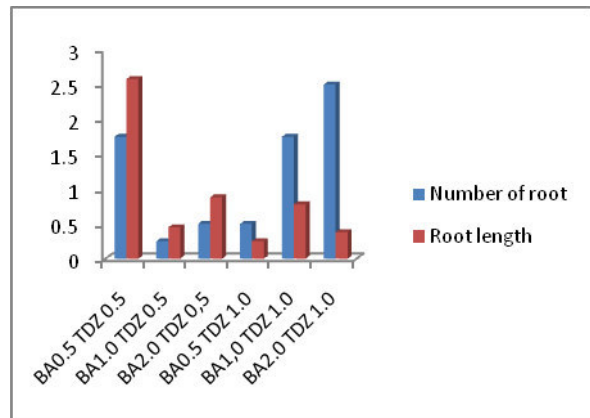


Figure 6. Effect of treatments on number of root and root length.

Since the root at 3 months after plating was quite short, plantlet from the best media for shoot formation (BA 2 mg/l + Thidiazuron 1 mg/l) were transferred to media for root induction MS media combined with IAA (0.5 ; 1 mg/l) and NAA (0.5 ; 1 mg/l). The result of root induction showed that for 3 months after sub culture, root of Taro in MS + IAA 0.5 mg/l was growth and developed better than that of in MS + NAA 0.5 mg/l

(Fig. 7). After complete with stem, leaves and root, plantlets of Taro were acclimatized in green house by planted in polybag which contained with soil and manure (1:1). One month after acclimatization, there were about 80 % of seedling was survive in green house.

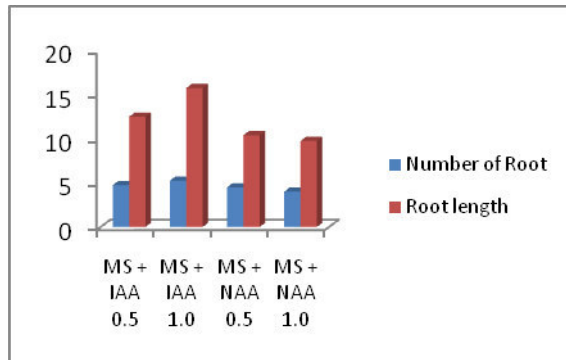


Figure 7. Effect of IAA and NAA on number of root and root length.

DISCUSSION

Taro shoot initiation observation indicated that the initiation starts at 2-3 weeks after planting on MS medium. In the method of micro propagation through tissue culture, growth and development of the explants is influenced by the media formulation (basic medium consisting of macro and micro nutrients, vitamins, and plant growth regulators, amino acids and other organic and inorganic components). MS (Murashige and Skoog) basic medium has been widely reported to be effective used in tissue culture (Jalaja et al., 2008). In addition to plant micropropagation using basic MS media often added growth regulators Benzyladenine (BA) and Thidiazuron. Benziladenine is a plant growth regulator belonging to the activity of cytokinine and have a strong power. When applied to crops are not at risk to humans and the environment (Anonymous, 2010). The 6-Benzylaminopurine or benzyladenine (BAP = BA) is the first generation of synthetic

cytokines that influence the response of plant growth and development and can regulate flowering, fruit formation through stimulation stimulates cell division, and inhibit the kinase enzymes in plant respiration (Kianamiri and Hassani, 2010). At 3 months after panting, the highest number of shoot was 3.5 in media BA 2 mg/l + Thidiazuron 1 mg/l with 10.25 number of fresh leaves, 5.75 dry leaves, 2.5 number of root, and 0,38 cm of root length. This is consistent with the results of Balachandran et al, (1990), which states that the cytokinins (BA or kinetin) is a critical factor in the shoot multiplication occurs also in the propagation of various medicinal plants and ornamental plants (Mariska and Gati, 1995; Mariska *et al* ., 1996 and Nandang, 1993). Thidiazuron (1-phenyl-3- (1,2,3-Thiadiazol-5-yl) urea; TDZ) is one of the several substituted ureas that have been investigated recently for their cytokinin-like activity. TDZ is known to be more active than zeatin for stimulating the growth when added to a tissue culture medium at a low concentration. Response appears to depend on the type of crop plants, parts of plant, phase of development, the concentration of growth regulators, the interaction between hormones, and environmental factors (Salisbury and Ross, 1992). Growth regulators BA / BAP belonging to the cytokinin which useful for shoot growth in plants grown in vitro.

From the observations show that during the 3 months of each explant can produce 3.5 buds/shoots that can be propagated into the plant. If the in vitro propagation is stable, there is no contamination, no other factors interfere, then the amount of culture generated in 1 year can be calculated geometrically as follows: 1 year = 4 x 3 months. So every 1 cultured in 1 year can be multiplied into $(3.5)^4 = 150.06$ new shoots. Acclimatization success = 80%. It means that seedlings can be produced from a single explant = $80\% \times 150.06 = 120.05$ seedlings. Conceivably, if we start to plant 100 explants can produce

12005 seedlings. Besides profit rapid seed multiplication by tissue culture in addition to saving space and time as well, the resulting seed will be :

- True to type
- Uniform
- Free of disease

In MS + IAA 0.5 mg/l the root was growth and developed better than that of in MS + NAA 0.5 mg/l. The auxins commonly used in plant tissue culture media are 1H-indole-3-acetic acid (IAA), 1H-indole-3-butyric acid (IBA), (2,4-dichlorophenoxy) acetic acid (2,4-D), and 1-naphthaleneacetic acid (NAA). The only naturally occurring auxin found in plant tissues is IAA. IAA is a natural auxin more likely to be recognized by receptors in plants. NAA is a synthetic auxin which have high activity, so that even if given the same concentration of IAA, may inhibit the effect (feedback inhibition), so the number of root and root length not higher than IAA 1 mg / l. Mixture of soil and manure (1:1) was the optimum media for acclimatization of some plant, so 80 % of seedling was survive in green house after acclimatization.

CONCLUSION

- Initiation of Taro through *in vitro* culture begin at 2-3 weeks after planting of explant.
- The best media for shoot multiplication of Taro was BA 2 mg/l + Thidiazuron 1 mg/l with 1,88 cm shoot height, 3.5 number of shoot, 10.25 number of fresh leaves, 5.75 dry leaves, 2.5 number of root, and 0,38 cm of root length in 3 months after planting.
- In MS + IAA 0.5 mg/l the root was growth and develop better than that of in MS + NAA 0.5 mg/l.
- After acclimatization 80 % of seedling was survive in green house.

- Shoot multiplication of Taro by *in vitro* culture was more profitable in terms of time, area, and the quality of the resulting seedlings.

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PRELIMINARY FIELD TRIAL OF BIO-6 PATCHOULI CLONE IN THREE LOCATIONS

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ABSTRACT

Some of promising clones of patchouli plant (*Pogostemon cablin* Benth.) which tolerant to drought and high oil content was produced by combination of mutation induction and somaclonal variation in 2007. The clones were tested in green house in 2008. Bio-6 clone is one of the clones which tolerant to drought and high oil content. The experiments were done in 2011 in three locations (Citayam, Pemalang and Cicurug). The aim of experiment was to get production data, oil and patchouli alcohol content of patchouli clone Bio 6. The parent variety (Tapak Tuan) was planted as control, and local Pemalang variety also planted in Pemalang. Randomize Block Design were used with 4 replications. The size of plot was 7.5 m x 5 m and plant spacing was 75 cm x 50 cm. Observation every month were: Percentage of survive plant, plant height, number of leaf, number of branch, and diameter of canopy. Dry plant weight, oil and patchouly alcohol content were observed at harvesting time. Result of harvesting plant at 4.5 months in three locations showed that most of yield components of Bio-6 were significantly higher than that of Tapak Tuan variety and local Pemalang. In Citayam oil content of Bio-6 (3.72 %) and patchouli alcohol (31.35%) were higher than that of Tapak Tuan. In Pemalang oil content of Bio-6 (2.56) and patchouli alcohol (34.24%) were higher than that of local Pemalang. In Cicurug oil content of Bio-6 was also higher than that of Tapak Tuan. Dry plants weight of Bio-6 clone in Citayam (4133.33 kg), Pemalang (9460.00 kg), and Cicurug (4031.33 kg) were higher than that of Tapak Tuan and Local Pemalang. From the result showed that Bio-6 clone was more tolerant to drought compare with Tapak Tuan and Local Pemalang varieties.

Keywords : Patchouly plant (*Pogostemon cablin* Benth.), Field trial, Bio-6 clone

INTRODUCTION

Patchouli (*Pogostemon cablin* Benth.) Is a plant essential oil or Patchouli oil (from the Tamil language *Patchai* (green) and *ellai* (leaves), because oil is distilled from the leaves). The smell of Patchouli oil known as 'heavy' and 'strong' and has been used for centuries as a perfume and material incense or incense in eastern tradition, and is also used as a binder other essential oils. Patchouli oil is widely used in cosmetics, perfumes, soaps, antiseptic and insecticide (Kadir, 2007). Patchouli oil price is the highest when compared with other essential oils. World market today require at 1200-1400 tones of patchouli oil a year with the average increasing trend. Supplied demand of patchouli oil 80-90% was from Indonesia Dirjenbun, 2006). Biggest importer of patchouli oil is currently the United States with no less than 210 tons of patchouli oil needed on average per year. Other importing countries include the UK, France, Switzerland, Germany and the Netherlands (Administrator, 2012). Patchouli plant can grow in low or high elevation with optimal height 10-1200 meters above sea level, rainfall between 2500-3500 mm / year and evenly distributed throughout the year, the temperature 24 - 28⁰C, more than 75% humidity, sunshine intensity enough, fertile soil and loam rich in humus (Nuryani *et al.*, 2001). In certain areas such as Tasikmalaya, patchouli cultivated on dry land, thus the development of patchouli is very relevant to the potential dry land which large enough in Tasikmalaya compared with wetland. Development of patchouli plant has a dual purpose, in addition to increasing the income of farmers and improving productivity of dry land which widely spread in Tasikmalaya region, while utilization is not maximized.

There are three species Patchouli in Indonesian, *Pogostemon cablin* Benth., *P.*

hortensis Backer, and *P. heyneanus* Benth. Among the three species *P. cablin* was widely cultivated known as *Nilam Aceh* with a high oil content (2.5%), while the other two species were not cultivated commercially because the yield and quality of oil is low (<2.0%) which out of quality standards of trade (Nuryani *et al.*, 2001). Mariska and Purnamaningsih (2007) produced some excellent clones which drought tolerant and have a higher oil rate compared to control (3.2%) through mutations induction and somaclonal variations. In 2008 the clones were tested in the greenhouse and produced some drought tolerant clones, one of them is Bio-6 (Pitono *et al.*, 2008). Bio-6 Patchouli clone which drought tolerant and oil content of 250 kg / ha / year is used further in this study. Seed requirement of patchouli plant for each unit area is 20,000 seedlings / ha (Nuryani, *et al.*, 2005). These obstacles can be overcome through tissue culture as well as the seed can be reproduced at any time, the resulting seedlings are also not limited in number due to the high rate of multiplication (Hobir *et al.*, 1992). The purpose of this study was to get production data and the oil content of patchouli alcohol in 3 test sites patchouli cultivation.

MATERIALS AND METHODS

Bio-6 patchouli clone has been tested for its tolerant to drought in 3 locations: 1. Cicurug (West Java) with 550 m height above sea level and rainfall 2000-3000 mm as controls; 2. Citayam Experimental Station (West Java) with 120 m height above sea level and is a dry area; 3. Pemalang (Central Java), as production centre of patchouli with frequent droughts. In addition to Bio-6 clone, improved varieties of patchouli which already spreads to farmers field (Tapak Tuan) also been planted for comparison. In Central Java Local variety also used beside Tapak Tuan. Complete randomized design with four

replications was used with plot size was 7.5 m x 5 m, and row spacing was 75 cm x 50 cm. 120 kg N + 80 kg P₂O₅ + 100 kg K₂O per hectare were used as fertilizer. Maintenance and control of pests and diseases conducted as the recommendation. Each plot was taken 10 samples of plants and observed each month: plant height, number of leaves, number of branches, diameter of the canopy. At harvesting time was observed: fresh and dry matter weight (Stem, branch and leaves), oil and patchouli alcohol content, and oil yield (kg / ha / year).

RESULTS

1. Citayam

Two weeks after planting in Citayam, there was a difference growth between Bio-6 clone was still green and Tapak Tuan var. was turning yellow. (**Fig. 1**). Dried and dead plants were replaced with new seedlings.



Figure 1. Patchouli growth of Bio-6 clone (left) and Tapak Tuan (right) 2 weeks after planting and drought in Citayam.

Percentage of survive plants per plot and some agronomic characters at 1 to 4 months in Citayam listed in **Tables 1 and 2**. **Table 2** showed that the percentage of survive plants, number of leaves, number of branches and canopy diameter of Bio-6 clone was higher than that of Tapak Tuan variety. The growth was difference between Bio-6 clone and Tapak Tuan variety (**Fig. 2**).

Table 1. Some agronomic characters of Tapak Tuan and Bio-6 clone at 1-3 months after planting in Citayam.

Variety/Clone	Month after planting		
	1	2	3
Tapak Tuan			
% survive plant	72,5	70,2	63,2
Plant height (cm)	24,0	32,9	31,5
Number of leave	69,5	151,1	46,9
Number of branch	10,8	17,5	21,6
Canopyd diameter cm)	16,6	28,7	32,9
Bio-6 clone			
% survive plant	99,75	95,5	90,7
Plant height (cm)	30,35	46,3	56,3
Number of leave	95,0	222,2	514,6
Number of branch	13,8	26,1	57,2
Canopyd diameter cm)	23,0	38,4	54,0

Table 2. Some agronomic characters of Tapak Tuan and Bio-6 clone in at 4 months after planting in Citayam.

Variety/Clone	% survive plant	Plant height (cm)	Number of leaves/ plant	Nu. of branch / plant	Diameter of canopi (cm)
Tapak Tuan	35,7 b	33,67 b	437,1 b	51,27 b	35,95 b
Bio 6	66,7 a	61,95 a	947,8 a	118,7 a	61,78 a

Expl: Numbers which followed by same characters in the same column were not significantly different at 5% DMRT.



Figure 2. Patchouli growth of Bio-6 clone (left) and Tapak Tuan variety (right) in Citayam.

At 3 months after planting, the plants attacked by rot disease that caused crop withered and eventually dead. Number plants per plot was decreased so harvesting time was done at 4.5

months and it was still be able to analyze the oil content and patchouli alcohol. Number of crops harvested, wet and dry yield (dry matter production) weight listed in **Table 3**.

Table 3. Number of crops harvested, wet and dry yield weight at harvesting time in Citayam.

Variety/Clone	Numb. of harvesting plant	Fresh yield.weight (kg)	Dry yield weight (kg)
Tapak Tuan:			
sampel	40	7,4	0,7
Plot	107	16,2	1,5
Total	147	23,6	2,2
Per plant		0,18	0,02
Bio 6:			
sampel	40	30,7	2,8
Plot	184	118,4	12,7
Total	224	149,1	15,5
Per plant		0,77	0,07

The number of plants that should be 400 plants per plot Tapak Tuan variety only 147 and Bio-6 clone was 244. Oil and patchouli alcohol content of Tapak Tuan and Bio-8 clone in Citayam at harvesting 4.5 months are listed in **Table 4**. From Table 4 showed that oil content of Bio-6 clone (3.72%) and patchouli alcohol content (31.35%) were higher than Tapak Tuan (2.48% oil content and 25.25% patchouli alcohol).

Table 4. Oil and patchouli alcohol content of Tapak Tuan and Bio-8 clone in Citayam at harvesting time (4.5 months)

Variety / Clone	Oil content (%)	Patchouli alcohol (%)	Oil Production (kg/Ha/ 1 x harvest)	Assumptions 3 times harvest (kg/Ha/tahun)
Tapak Tuan	2,48	25,25	3,64	10,92
Bio 6	3,72	31,35	38,44	115,32

Expl. Analysis by Destilation GC Metode.

2. Cicurug

Rainfall data in Cicurug listed in **Table 5**.

Table 5. Rain fall data in Cicurug

Month	Number of rain (days)	Volume (mm)
Juni	6	129
Juli	6	206
Agustus	3	9,8
September	5	30
Oktober	18	450,7

Observation of Patchouli plant growth at 1 to 3 months after planting were listed in **Table 6**.

Table 6. Some agronomic characters of Tapak Tuan and Bio-6 in at 1-3 months after planting in Cicurug.

Variety/Clone	Month after planting		
Tapak Tuan	1	2	3
% survive plant	94,5	100,0	92,0
Plant height (cm)	23,1	30,4	38,2
Number of leave	19,8	45,8	95,1
Number of branch	3,4	6,9	8,1
Canopyd diameter (cm)	20,4	27,2	44,97
Bio-6 clone			
% tan hidup	95,75	94,2	89,7
Tinggi tan. (cm)	27,5	44,2	60,5
Jumlah daun	19,9	53,1	141,8
Jumlah cabang	3,3	7,5	11,2
Diameter kanopi (cm)	21,7	38,7	67,3

Table 6 showed that at the beginning of growth until 3 months, % of survive plant, plant height, number of leaves, number of branches and canopy diameter did not differ greatly between Tapak Tuan variety and Bio-6 clone, but after 4 months the growth started to diverge (**Fig. 3**). Bio-6 clone had a plant height (78.45 cm), number of leaves (393.5), and canopy diameter (86.75 cm) was higher than Tapak Tuan (plant height was 51.11 cm, number of leaves was 222 , 9, and canopy diameter = 63.55 cm) (**Table 7**).



Figure 3. Patchouli growth of Bio-6 clone (left) and Tapak Tuan variety (right) in Cicurug.

Table 7. Some agronomic characters of Tapak Tuan and Bio-6 at 4 months after planting in Cicurug.

Variety/ Clone	% survive plant	Plant height (cm)	Number of leaves/ plant	Nu. of branch / plant	Diamete r of canopi (cm)
Tapak Tuan	92,00 a	51,11 b	222,9 b	11,90 a	63,55 a
Bio 6	89,75 a	78,45 a	393,5 a	16,97 a	86,75 b

Expl: Numbers which followed by same characters in the same column were not significantly different at 5% DMRT.

3. Pemalang

There were 3 (varieties and clone) used in Pemalang: Tapak Tuan, Bio-6 clone and Local Pemalang variety. Rainfall data showed in **Table 8**). The farmers used to plant patchouli in Pemalang covered with rice straw mulch to reduce evaporation (**Fig. 4**), but Patchouli plant growth looks real difference among Bio-6 clone, Tapak Tuan and local Pemalang varieties. The highest % of survive plant, plant height, number of leaves, number of branches and the canopy diameter was Bio-6 clone, followed by Tapak Tuan and local Pemalang variety. (**Table 9**).

Table 8. Rain fall data in Pemalang

Month	Number of rain (days)	Volume (mm)
June	4	169
July	6	163
August	3	56
September	0	0



Fig.4. Patchouli plant which covered with rice straw mulch to reduce evaporation in Pemalang

At 4 months, % of survive plant and canopy diameter of Bio clone 6 is higher than Tapak Tuan and local Pemalang variety (**Table 10**).

Table 9. Some agronomic characters of Tapak Tuan, Bio-6 and Local var. at 1-3 months after planting in Pemalang.

Variety/Clone	Month after planting		
	1	2	3
Tapak Tuan			
% survive plant	100,0	85,5	80,5
Plant height (cm)	21,5	21,6	22,0
Number of leaf	16,2	85,8	89,0
Number of branch	3,2	6,7	7,6
Canopyd diameter cm)	11,1	18,6	20,4
Bio-6 clone			
% survive plant	100,0	98,0	95,0
Plant height (cm)	20,0	27,2	32,7
Number of leaf	26,1	96,8	113,8
Number of branch	2,6	6,0	7,3
Canopyd diameter cm)	15,0	24,3	20,2
Local Pemalang var.			
% survive plant	dead	90,0	70,0
Plant height (cm)	-	15,0	24,1
Number of leaf	-	45,6	66,3
Number of branch	-	4,9	5,0
Canopy diameter cm)	-	13,4	15,0

Table 10. Some agronomic characters of Tapak Tuan, Bio-6 and Local variety at 4 months after planting in Pemalang.

Variety/ Clone	% survive plant	Plant height (cm)	Number of leaves/ plant	Num. of branch / plant	Diameter of canopi (cm)
Tapak Tuan	77,5 ab	29,050 a	122,400 a	8,75 a	25,58 ab
Bio 6	90,1 b	37,775 a	138,050 a	9,40 a	34,75 b
Lokal Pemalang	57,5 a	28,850 a	84,425 a	6,15 a	17,90 a

Expl: Numbers which followed by same characters in the same column were not significantly different at 5% DMRT.

To compensate Citayam harvested at 4.5 months, in Pemalang also partially harvested at the 4.5 months. Plants were harvested for analysis only partially oil and patchouli alcohol content. The results of total yield/ dry matter weight (leaves and branches), the estimated dry matter weight per ha, oil content and Patchouli alcohol content listed in **Table 11**. Patchouli plant which harvested at 4.5 months had low of oil and patchouli alcohol content. The next harvesting for Cicurug and Pemalang were done at 5.5 months. Production data, the oil and patchouli alcohol testing from 3 locations listed at Table 12.

Table 11. Total yield/dry matter weight (stem, leaves and branches), the estimated dry matter weight per ha, oil content and Patchouli alcohol content of Tapak Tuan, Bio-6 and Local Pemalang var.

Variety/C lone	% survive plant	Plant height (cm)	Number of leaves/ plant	Nu. of branch / plant	Diameter of canopi (cm)
Tapak Tuan	77,5 ab	29,050 a	122,400 a	8,75 a	25,58 ab
Bio 6	90,1 b	37,775 a	138,050 a	9,40 a	34,75 b
Local Pemalang var.	57,5 a	28,850 a	84,425 a	6,15 a	17,90 b

Expl: Numbers which followed by same characters in the same column were not significantly different at 5% DMRT.

Table 12. Production data, the oil and patchouli alcohol content in 3 locations.

Varietas/Klon	Citayam	Pemalang	Cicurug
Tapak Tuan			
Number of harvested plant	147	252	240
Fresh yield weight (kg)	23,6	77,97	119
Dry yield weight (kg)	2,2	12,77	19,97
Oil content (harvesting at 4,5 months)	2,48	3,42	1,47
Patchouli alkohol content (harvesting at 4,5 months)	25,25	35,25	34,98
Assumption of yield /dry matter production (kg/ha)	586,67	4010,00	1331,33
Oil content (harvesting at 5,5 months)		1,80	0,72
Patchouli alkohol content (harvesting at 5.5 months)		31,34	41,96
Klon Bio 6			
Number of harvested plant	224	291	329
Fresh yield weight (kg)	149,1	153,73	376,3
Dry yield weight (kg)	15,5	29,15	60,47
Oil content (harvesting at 4,5 months)	3,72	2,56	2,24
Patchouli alkohol content (harvesting at 4,5 months)	31,35	34,24	32,12
Assumption of yield /dry matter production (kg/ha)	4133,33	9460,00	4031,33
Oil content (harvesting at 5,5 months)		2,09	1,64
Patchouli alkohol content (harvesting at 5.5 months)		31,17	34,23
Lokal Pemalang			
Number of harvested plant		170	
Fresh yield weight (kg)		41,02	
Dry yield weight (kg)		7,22	
Oil content (harvesting at 4,5 months)		1,99	
Patchouli alkohol content (harvesting at 4,5 months)		31,03	
Assumption of yield /dry matter production (kg/ha)		2220,00	
Oil content (harvesting at 5,5 months)		2,83	
Patchouli alkohol content (harvesting at 5.5 months)		29,10	

DISCUSSION

1. Citayam

Two weeks after planting in Citayam, there were drought because there is no rain. There was a difference between the growth of Patchouli Bio-6 are still green and Tapak Tuan var. was turning yellow. From these circumstances indicate that Bio-6 clone more tolerant to drought than Tapak Tuan. Four months after planting the percentage of survive plants, number of leaves, number of

branches and canopy diameter of Bio-6 clone was higher than that of Tapak Tuan variety. This was due to the lack of rain long enough (3 months) although occasional watering at first, but was running out of water so that the plants begun to dry up, withered and dead. The growth was difference between Bio-6 clone and Tapak Tuan variety. Harvesting at 4.5 months was actually still too early. Patchouli first harvest is usually done at 5-6 months after planting in order high oil content and alcohol. According to Hobir (personal communication) patchouli with oil content from 2.5 to 3.0% in can produce 80 kg / ha for 1time harvest. With 3 times harvest/year the oil content was 160-240 kg / ha / year. With drought conditions and stem rot disease in Citayam for Tapak Tapak Tuan decreased 93.175% (from 160 to 10. 92 kg / ha / year). While Bio-6 clone was decreased 27.925% (from 160 to 115.32 kg / ha / year). From these results proved that the Bio-6 Clone still can survive under drought (3 + months of no rain).

2. Cicurug

In Cicurug at the beginning of growth until 3 months % of survive plant, plant height, number of leaves, number of branches and canopy diameter did not differ greatly between Tapak Tuan variety and Bio-6 clone. That was due to still enough water in first month with high rain fall (June and July). Four months after planting the growth started to diverge. Bio-6 clone had higher plant height, number of leaves, and canopy diameter than that of Tapak Tuan. That was due to a long drought, there was no rain in 2 months on July and August.

3. Pemalang

Patchouli plant growth in Pemalang looks real difference among Bio-6 clone, Tapak Tuan and Local Pemalang varieties. That caused of there were only little rain until 4 months, even in

September there was no rain at all and even the plant was covered with rice straw mulch to reduce evaporation. At 4 months, % of survive plant and canopy diameter of Bio-6 clone is higher than Tapak Tuan and local Pemalang variety. Its proved that Bio-6 clone was more adapted in dry area with low rain fall and without irrigation water.

CONCLUSION

- Harvesting at 4.5 months in three locations showed that most of yield components of Bio-6 were significantly higher than that of Tapak Tuan variety and local Pemalang.
- In Citayam oil content of Bio-6 (3.72 %) and patchouli alcohol (31.35%) were higher than that of Tapak Tuan.
- In Pemalang oil content of Bio-6 (2.56 %) and patchouli alcohol (34.24%) were higher than that of local Pemalang.
- In Cicurug oil content of Bio-6 was also higher than that of Tapak Tuan.
- Dry plants weight of Bio-6 clone in Citayam (4133.33 kg), Pemalang (9460.00 kg), and Cicurug (4031.33 kg) were higher than that of Tapak Tuan and Local Pemalang.
- From the result showed that Bio-6 clone was more tolerant to drought compare with Tapak Tuan and Local Pemalang varieties.

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Grafting Technology for Sustainable Improvement of Tomato Production: A Field Study in Kediri, East Java

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Abstract

Tomato is one of important vegetable commodities because it has economic value and high nutrition. However, tomato production is very sensitive to climate change. Soil-borne diseases are the main constraint of tomato production in low land; and the diseases have been reduced productivity of tomato. Grafting technology is expected to deal with such constraint. Tomato, which is grafted onto selected eggplant rootstocks, is able to reduce the incidence of diseases attack it. This study is to analyze grafting technology on tomato with different types of rootstocks. The study was conducted during the rainy season in January – May 2013 in Kediri. Three treatments, including control, were applied. A variety of tomato is “Permata”, which was grafted on two different types of eggplant rootstocks EG203 and EG195. The control is tomato without grafting. The results show that grafted tomato onto eggplant EG203 provides better performance than that onto EG195, in terms of plant growth and yield. Tomato without grating shows the worst performance, because of death in early stage. To sum up, grafting technology can improve the production of tomato in Kediri, and other areas where soil-borne diseases are the main problem.

Keywords: grafting technology, tomato, eggplant rootstocks

Introduction

Tomatoes are difficult to grow during the hot – wet season. Flooding, waterlogged soils, diseases, and high temperatures can significantly reduce yields. Lack of cultivars tolerant or resistant to increasingly important soil biotic and abiotic stresses, together with the prohibition of the use of methyl bromide for soil disinfestations, has led to worldwide renewed interest in vegetable crops grafting (Blets OS *et al*, 2005). Eggplant (*Solanum melongena* L) is widely cultivated in tropical and temperate regions around the world and is amenable to grafting (Bletsos *et al*, 2003). Kediri is one city in East Java has potential to develop vegetables, because has structure

sandy and clay soil and normal of acidity. Yield of other crops seem normal, except tomato because of severe pest and disease problem, yield of tomato was only 20 t/ha. From the survey results revealed *Fusarium* was the major diseases on tomatoes since the lost to such diseases reached 60% in Kediri especially in Pagu sub district. Based on a survey conducted in Pagu Sub-district, farmers could not cultivate tomato because tomato grown in the location frequently died before producing fruit. According it's reason and then need to be carried out research on Grafting technology for sustainable improvement of tomato product. Grafting of vegetable crops is used to provide resistance to soil pests and pathogens, to increase the tolerance to abiotic stresses, to improve

water or nutrient uptake, or to enhance the vigour of the scion (Davis *et al*, 2011). Grafting has been highly effective at overcoming abiotic stresses as well, which can indirectly lead to increased yield in a number of ways. Over 1/3 of all the irrigated land in the world is affected by salinity, and this technique could be instrumental in decreasing yield losses (Rivero, 2003). Grafting has also been utilized in order to reduce the effects of flooding in areas where a wet season may occur (Black, 2003). This technique has been shown to offer effective tolerance to soil temperature extremes. Because soil tends to heat and cool much more slowly than the aerial temperature, roots are exposed to extreme temperatures for a longer period of time than the above ground structures (Rivero, 2003). In both of the cases, the growing season may be extended in either direction, resulting in better yield and economic stability through the year. This study is to analyze grafting technology on tomato with different types of rootstocks.

Material and Method

The assessment was conducted in the village of Kediri Regency Ceiling wins district with an altitude of 200 m above sea level in the month of January to May 2013, with the use of hybrid varieties of tomato seedlings Jewel, the eggplant seedlings derived from AVRDC (Asean Vegetable Research Development Centre) in Taiwan there are 2 kinds of strains are strains and strain EG 195 EG 203 as rootstock. Assessment consisted of the following 3 treatment where each treatment consisted of 6 replicates (beds) and consists of 20 beds each plant are:

1. Tomato varieties with a gem in the grafting rootstock eggplant strain EG 203
2. Tomato varieties with a gem in the grafting rootstock eggplant strain EG 195
3. Tomato varieties at Jewel without grafting.

Methodology used in accordance (Black et al, 2011) tomato scion and eggplant rootstock stems must be the same diameter 1,6-1,8 mm. To achieve this, sow the eggplant approximately three days before the tomato. Cut the eggplant above the cotyledons at a 30⁰ angel. Start the cut as high on the stem as possible. Cut the tomato stem at a 30⁰ angle, slightly above the cotyledons or first true leaf. It is critical that the tomato scion diameter matches the eggplant stem diameter. Select a place on the stem to the tomato scion ton achieve the proper diameter. Slide a 10 mm long latex tube (2.0 mm inner diameter and cut at a 30⁰ angel) over the scion stem. Make sure that the cut angles of the tube and scion are parallel. Push the scion about halfway into the tube. Slice the scion (now fitted with the latex tube) over the eggplant seedling stem. Again, make sure that the cut angles of the tube and rootstock stem are parallel. Gently push the scion and rootstock together. If have kept all of the cuts parallel, then it can be certain that the scion and rootstock are in complete contact with one another. The tube will stay on the seedling until it naturally hardens, splits, and falls off in the field. Move the grafted seedlings immediately into the shaded chamber. Recommended temperatures are 25-32^{0c}. Keep a shallow layer of water in the polyethylene floor liner and keep the doors closed to maintain high humidity (>85%RH). Place seedling trays on bricks to support the plants above the water line. The grafted seedlings may wilt initially but will become upright within three days. Four to five days after grafting, begin the hardening process by peeling away the top (silver) layer of shade net material. Drain the water out of the floor pan. Open the chamber's plastic-covered door, but keep the screen door closed to prevent insect infestation. Maintain these conditions for two to three days. Move the grafted plants out of the chamber and place them into a Screenhouse. Nine days after grafting,

apply a foliar application of 0,3-0,4 % urea solution , or 1 g per liter of foliar Nitrophonska (20 N-19 P₂O₅.19K₂O), or the equivalent of a similar soluble fertilizer. Hold the plants in the screen house for seven to eight days for further development and hardening. The entire process takes 30-33 days from sowing. Once the process is complete valve does not need to be taken will be hardened, split and fall to the ground by itself.

Observation variables include: plant height (cm), number of leaves, the weight of its fruit each plot (g) and intensity of the disease. The data obtained from the study were analyzed using analysis of variance level of 5% and if there are real differences continued Duncan multiple range test (DMRT) level of 5%.

Result and Discussion

The study showed high growth of tomato plants with varieties of gem grafted rootstock strains eggplant rootstock EG 203 and EG 195 eggplant strain were not significantly different. Gem varieties of tomato plant growth without grafting showed the most growth in the short appearance Both strains of eggplant EG 203 and EG 195 is recommended eggplant strain of AVRDC is resistant to damage caused by flooding, bacterial wilt, root knot nematode (caused by *Meloidogyne incognita*), and tomato *Fusarium* wilt (caused by *Fusarium oxysporum* f.sp . *lycopersici*) grafted so with two strains of tomato plants with the eggplant, showing higher growth compared with no grafting tomato plants. Grafting tomato plant growth is higher than the one without grafting cause is this behavior of roots endowed by grafting was also highlighted by Rochdi *et al.*, 2005. Rivero *et al.*, 2003 have explained this effect by improvement of the meristematic activity.

This observation confirms the findings of Lee (1994) and Ioannou *et*

al.,2002 who have emphasized a tendency of grafted plants to attain a larger stem diameter. This was confirmed also from the results of research Radhouani and Ferchichi (2010) That grafting of muskmelon has positive effects on the performance by improving vegetative growth due to vigourous roots that favoured considerable uptake of water and nutrients and rate of growth deduced, especially, from high values of RGR and NAR. Consequently, production was earlier and higher. These effects are dependent on choice of suitable rootstocks and condition of crop growth. To the number of leaves produced spliced with eggplant tomato strain EG 203 produces the highest leaf, then a level below the rootstock tomato eggplant and tomato strains of EG 195 without grafting produces leaves the least.

Percent of virus attack on tomato plants grafted good eggplant stems EG 203 and EG 195 was not significantly different between 40% -50% lower than without grafting tomato plants ranged from 60% virus attacks. This is explained well in (Kawaguchi *et al.*, 2008). Despite the high soil infestation (4290 nematodes kg⁻¹ soil), 100% of survival was observed in plants grafted on to eggplant rootstocks survival ranged from 25 to 100%. High infestation with nematodes, together with the incompatibility manifested with tomato rootstocks may be the cause of the death of plants after transplant grafted onto our tomato rootstocks. On the other hand, as reported by other authors (Boiteux and Charchar, 1996), we have found important differences among *S.melongena* accessions for resistance to nematodes. In our case, although an exhaustive evaluation of nematode resistance in these eggplant accessions is needed, the intermediate G1 values in hybrids derived from the three *S. Melongena* varieties, indicate that these materials are promising materials as sources of variation breeding new eggplant cultivars or rootstocks with improved nematodes resistance (Daunay, 2008). The

yield of tomatoes with resistant rootstocks was 4 times that of the susceptible times. This particular line was also identified for use against bacterial wilt in Germany and similar results were found

(Grimault,1994). Several Hawaiian lines (Hawai 7996-7998) have been identified as suitable candidates for resistance to bacterial wilt (Oda,1999).

Table 1. Average Plant Height, number of leaves,% virus attacks tomato plants and fruit weight

Treatments	Plant Height	% Virus Attack	Leaves Number	Fruit weight
Tomato rootstock eggplant grafted strain EG 203	80.615 a	0.408 a	36.692 a	7853.8 a
Tomato rootstock eggplant grafted with strain EG 195	76.923 a	0.567 a	35.846 b	5771.4 b
Tomato permata varieties without grafting	62.538 b	0.608 b	24.615 c	2970.7 c

Note: These numbers are followed by the same letter in the same column are not significantly different by DMRT at 0.05 levels.

For fruit weight obtained grafted gem varieties of tomatoes with eggplant rootstock EG 203 strains produce higher weight than the gem varieties of tomato rootstock eggplant grafted with EG strain. Gem varieties of tomato plants without grafting produces the lowest fruit weight on his every plot. Pagonyi *et al.*, (2005) reported that when Lemance F1 was grafted onto Beaufort rootstock, increased yield was caused mainly by higher average yield fruit weight. In similar studies (Khah *et al*, 2006), fruit weight of grafted plants was found to be higher than in non grafted plants. The results of the study showed that tomato grafting on suitable rootstocks had positive effects on the yield. In grafted combinations, the total fruit yield per plant increased significantly in comparison with that of the non grafted plants. (Ibrahim *et al* 2001) observed similar results in grafted and non grafted tomato plants. These investigators suggested that the higher yield of fruit from grafted tomato plants was most likely an effect of the vigorous root system of the rootstock. Grafting has proved to be an efficient tool for increasing the yield, disease resistance and quality of a number of vegetable crops (Rivero *et al*,2003) According to Lee (1994), the increased yield of grafted plants is also believed to

be due to enhanced water and mineral uptake. In the other study all selected rootstock “Efialto”, Herman and Maxifort for this are characterized by a strong generative tendency and high disease resistance. Rootstock efficacies are influenced by compatibility to the selected scion, existing disease pressure, and climate conditions. Our grafting technique and subsequent plant handling procedure were successful in all three variants, “Tamaris”, “Efialto”, “Tamaris”,Herman”,and “Tamaris Maxifort”. The productivity of the grafted tomatoes was increased, which agreed with previously published results (Pagonyi *et al*, 2005)

Conclusion

Grafting is a valuable management tactic for tomato growers. This practice originated as a way to ensure fruit quality weight,tomato plant growth, and while keeping disease resistance high. As rootstock tomato grafted most suitable for eggplant tomato strain EG is 203. EG strain 195 also showed growth, fruit weight and% virus attacks better than a tomato that does not grafted.

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The Effect of Metal Ion Cu²⁺ for Development and Secondary Metabolites (Stigmasterol and Sitosterol) Callus of Purwoceng (*Pimpinella alpine* Molk.)

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ABSTRACT

Purwoceng (*Pimpinella alpine* Molk.) is a plant native to Indonesia which has a variety of benefits. Purwoceng reported to have many phytochemical content of which is stigmasterol and sitosterol. Alternative methods to produce secondary metabolites *in vitro* is using tissue culture and elicitation techniques using metal ions Cu²⁺. The research aims to determine the effect of callus growth and increased levels of stigmasterol and sitosterol with the delivery of metal ions Cu²⁺ with different levels of concentration. observations include changes in morphology and weight of callus in media with concentrations of Cu²⁺ 0 µL (control), 20 µL, 30 µL, and 40 µL. stigmasterol and sitosterol levels tested using column chromatography method and the results were analyzed descriptively. The results showed that administration of metals with various concentrations of Cu²⁺ influence on the development of callus the higher the concentration of Cu²⁺, the more intense the color that produced callus and signifies the high production of secondary metabolites produced. the higher weight of callus at 40µM Cu²⁺ concentration of about 0.29 g. The highest stigmasterol and sitosterol result from treatment media which concentrations of Cu²⁺ 40µM 1695,620 and 3128,739 ppm.

Keywords

Metal ion Cu²⁺, Purwoceng (*Pimpinella alpine* Molk.), Sitosterol dan Stigmasterol

INTRODUCTION

Purwoceng (*Pimpinella alpine* Molk.) Is an herbaceous plant of commercial. This is native to Indonesia which live in endemic in mountainous areas such as the highlands of Java. Part of the plant known Purwoceng has various benefits is the root.

Allah has explained in a letter Asy Syuara 'paragraph 7 of the many useful herbs on earth. (Q.S. Asy-Syu'araa' 26 :07)

أَوَلَمْ يَرَوْا إِلَى الْأَرْضِ كَرَاهَتْ فِيهَا مِنْ كُلِّ زَوْجٍ كَرِيمٍ ﴿٢٦﴾

Meaning: "And if they do not pay attention to the earth, what is the amount of earth that we grow in a wide variety of plants are good?"

Drug efficacy resulting from Purwoceng plant roots are as an aphrodisiac and blood circulation. Phytochemical studies on the roots purwoceng test known to have that on there purwoceng root phytochemical compounds such as alkaloids, triterpenoids, flavanoid, steroids (stigmasterol and sitosterol) and glycosides. Tissue culture is an alternative source to produce bioactive plant substance. By culturing plant biotechnology (in vitro) provide several advantages than conventional cultivation (in vivo). Excess in vitro is not affected by the climate and do not require extensive space (Ogita *et al.*, 2009).

Purwoceng callus growth was conducted to obtain the active compounds contained in the culture. Content of secondary metabolites in callus cultures and cell culture is relatively low (Mantell & Smith, 1983). Therefore necessary the tissue culture method for increase the content of bioactive secondary metabolites.

One technique that has been developed is the elicitation technique.

Accumulation of secondary metabolites in vitro can be enhanced by using a variety of methods, such as by providing radiation treatment, ray, given the fungal pathogen eg, disturbed growth through reduction of nutrients, toxic compounds in the form of provision of metal ions Cu^{2+} , Mg^{2+} , Zn , and others. Elicitation is a technique to simultaneously induce phytoalexin formation, secondary metabolites konstitusif or other secondary metabolites that are not normally accumulate. Elicitor is an external factor that is used to improve secondary metabolites (Siregar, 2006). Elicitor is a biological and non-biological compounds that lead to increased production of phytoalexin (Buitelaar *et.al.*, 1991).

Added of metal ions Cu^{2+} in media culture is one of elicitor to increase secondary metabolites. Muryanti (2005) Cu^{2+} ions play a role in defense responses in plants by inducing genes and increases the formation of secondary metabolite pathways. This as abiotic elicitor signal transduction in plant defense systems against stress due to growing environmental stresses. Elicitor Cu^{2+} acts as an co factor enzyme to be attached to the side of the non-enzyme protein in a bid to boost metabolism terpnoid types of secondary metabolites, steroids, from isoprene pathway.

The purpose of this study was to determine the effect of metal ions Cu^{2+} to the development of callus and content of secondary metabolites such as stigmasterol and sitosterol levels in callus purwoceng (*Pimpinella alpine*)

MATERIALS AND METHODS

This study was experimental study using qualitative and quantitative descriptive methods. The treatment used the difference concentration metal ion Cu^{2+} on callus subculture medium purwoceng (*Pimpinella alpine* Molk.) concentrations of Cu^{2+} 0 μL (control), 20 μL , 30 μL , and 40 μL

The tools used in this study is the beakers, petri dishes, stir bar, bottle culture, section instruments (scalpel, tweezers, scissors), LAF (Laminar air flow), analytical balance, pipettes, sterilizer (autoclave, light spirits , and spraying alcohol), pH mater, refrigerator, shelves culture, hot plate, tissue paper, aluminum foil, column chromatography apparatus.

The research material includes explants used in this study is the callus from plants Purwoceng (*Pimpinella alpine* Molk.) that in the induction medium 2,4-D. Materials for sterilization is liquid detergent, alcohol 70%, 5.25% Clorox, and sterile distilled water. MS medium (Murashige and Skoog), a plant growth regulator 2,4-D (2,4 - Diclorophenoxy acetic acid) with a concentration of 6 mg / l and Cu²⁺ metal ions with concentrations of 0, 20, 30, and 40 µL.

RESULTS

Effect of Cu²⁺ Ion Metals in Development of Callus Morphology and Secondary Metabolites

1. Callus colour

Indicator in tissue culture is one that callus morphology can be seen from the color of the callus. Color change after subcultured callus on media with the addition of the metal ions Cu²⁺ at the age of 4 weeks in Table 1.

Table 1. Colour of callus subcultures on media with addition metal ion Cu²⁺

Explant	Concentration Cu ²⁺	Callus color	
		Awal	Akhir
Leaf	0 µM (E0)	green	green to yellow
	20 µM (E20)	green transparant	Brown+
	30 µM (E30)	green transparant	Brown transparant
	40 µM (E40)	green	Brown++

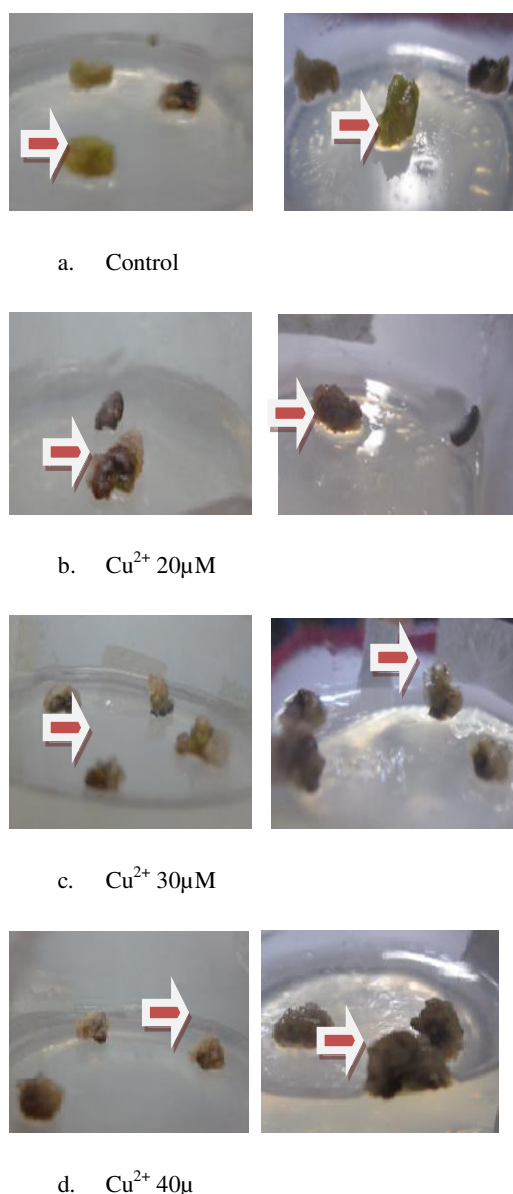


Figure 1. Callus in several treatments subcultures on media with addition metal ion Cu²⁺

2. Callus texture

Texture callus on the addition of metal ions Cu²⁺ unchanged. Texture callus formed is compact callus. Compact callus which can be caused by several things including due to the original cells divide decreased proliferation activity. This activity affected the natural

auxin found on explant origin (Santosa and Nursandi, 2002).

According to Street (1993) is an arrangement compact callus cells are tightly segregated and difficult to it.

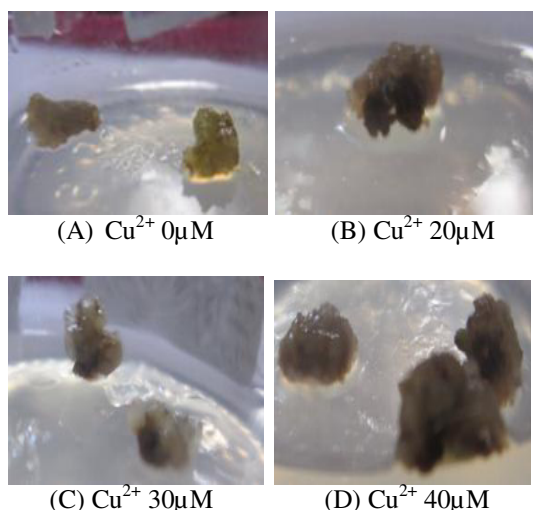


Figure 2. Texture callus in several treatments subcultures on media with addition metal ion Cu²⁺

3. Callus weight

Callus weight differences that occur due to differences condition in each callus growth. The following data callus weight change with the addition of the metal ions Cu²⁺ at the age of 4 weeks after subculture:.

Table 2. Calus weight in several treatments subcultures on media with addition metal ion Cu²⁺

Concentration	Callus weight (g)	
	Early	Later
Cu ²⁺ 0 μM	0.1	0,24
Cu ²⁺ 20 μM	0.1	0,19
Cu ²⁺ 30 μM	0.1	0,22
Cu ²⁺ 40 μM	0.1	0,29

From the data obtained callus was highest weight on treatment with the addition of 40μM Cu²⁺ with callus weight of 0.29 grams. Whereas the control treatment without the addition of Cu²⁺ treatment had a greater weight of heavy callus callus on treatment of Cu²⁺ and Cu²⁺ 20 μM is equal to 0.19 grams and 0.24 grams.

4. Secondary Metabolites Stigmasterol and Sitosterol Callus Purwoceng (*Pimpinella alpine*)

Testing of secondary metabolites using column chromatography method. Data presented results of secondary metabolites in the table below:

Table 3. Stigmasterol and Sitosterol in several treatments subcultures on media with addition metal ion Cu²⁺

Concentration	Content of secondary metabolites	
	Stigmasterol (ppm)	Sitosterol (ppm)
Roots	1124.17	2002.67
Cu ²⁺ 0 μM	1373.628	2443.800
Cu ²⁺ 20 μM	1539.607	2545.830
Cu ²⁺ 30 μM	1609.122	2856.512
Cu ²⁺ 40 μM	1695.620	3128.739

From the diagram above it can be seen that the content of secondary metabolites such as stigmasterol and sitosterol produced the highest concentrations of Cu²⁺ at 4 μL. With levels of stigmasterol and sitosterol 3128.739 1695.620 ppm. Compared the content of secondary metabolites in plants herbaceous rootsit can be seen that the content of secondary metabolites in plants herbaceous purwoceng lower than the results of secondary metabolites in callus.

Provision of metal ions Cu²⁺ on callus produce secondary metabolites are higher than the control and herbaceous plants.

DISCUSSION

Brown color that occurs in callus showed the synthesis of phenolic compounds. Zhao *et al.*, (2001) the synthesis of phenolic compounds triggered by stress or disturbance in plant cells. Stress or disturbance on plant cells due to reduced nutrients in the media

Brown color intensity positively correlated with oxidative enzyme hyperactivity. The increase in enzyme activity associated with the defense reaction of tissue oxidative stress. So it is assumed that occur on callus browning is due to the stress experienced by a callus that due to the stress of metal ions Cu^{2+} . Change colour of the callus also depends on media development.

Ariningsih (2003) stated that this condition is caused significant accumulation of phenols in callus as a result of Cu^{2+} ion absorbs a more than adequate. Stress are given in the media on callus callus will change color to indicate older than callus fresh. The older the color changes of callus on a medium shows the activity of secondary metabolite biosynthesis more higher

Callus will produce secondary metabolites during callus cells decreased activity and decreased cell division. Aisyah (2007). Texture is a compact callus callus texture that have cleavage to the stationary phase that tends to compact callus growth slow when compared with the callus crumb. Callus crumbs that have cells that are easily separated and tend to have the power to perform proliferation or cell division faster. So the compact callus can be produced secondary metabolite production is higher than in callus crumbs.

Slowing the growth of callus on the media due to callus elicitation can adapt to the new media (Sutini, 2008). Additionally callus condition still in lag phase towards linear growth phase and the linear phase of secondary metabolite formation is taking place.

Metal ions have antagonistic properties of the cells in the presence of inhibition of

absorption of ions when one of the other ions in excess conditions. If excess Cu^{2+} ions are absorbed by the cells resulted in cells lacking Ca^{2+} contained in the cell.

Kusuma (2011) that the increased production of secondary metabolites contained in the callus as their interactions with the host pathogen and stress that can induce phytoalexin production and other metabolites.

Sutini (2008) elicitation should be the optimization, such as, concentration, time and dosage of elicitation. The addition of Cu^{2+} in tissue culture to a certain dose capable of affecting the accumulation of secondary metabolites. this is cause the metal ions Cu^{2+} can serve as a spur to the enzyme activity, cell membrane and thus affect the Ca^{2+} + metabolism, the metabolism and cell growth.

Ali *et al* (2006) elicitor role of metal ions Cu^{2+} can be through the first two lines which can lead to oxidative stress in callus and the second is as an enzymatic cofactor in the formation of compounds stigmasterol and sitosterol. Under conditions of stress metal ions Cu^{2+} role in the defense response in plants by inducing genes and increase the formation pathways of secondary metabolites.

The abiotic elicitor function as signal transduction in plant defense systems against stress due to environmental stresses to produce secondary metabolites (Muryanti, 2005).

Role of Cu^{2+} on the metabolism of steroids can stimulate enzymatic process that goes through the track mevalonic acid. Initially the metal ions can penetrate the cell membrane, then this entry elicitor in plant metabolic reactions and form the primary and secondary metabolites. In the process of formation secondary metabolites Cu^{2+} will stimulate mRNA through an increase in the transcription of genes involved in the formation of phytoalexin and other metabolites

Hudoyono (2004) elicitor Cu^{2+} also acts as a cofactor that will be attached to the side of the non-protein enzyme metabolism

boosters secondary metabolites types of terpenoids and steroids from isoprene pathway

Enzymes that can stimulate the formation of steroid compounds and terpenoids include IPP isomerase enzyme, synthetase GPP, FPP synthetase, squalene synthetase, and squalene epoksidase which can apply to the mevalonic acid pathway. Callus color and texture can also influence the occurrence of elevated levels of stigmasterol and sitosterol on callus purwoceng. The addition of the metal ions Cu^{2+} then change color indicating an increase in callus secondary metabolites. Callus color change indicates that the more concentrated the production activity of the higher secondary metabolites.

Compact callus texture which can result in higher production of secondary metabolites, this is due to the compact callus showed decreased activity of cell division and cell growth. Aisyah (2007) which states that the callus will produce secondary metabolites during callus cells decreased activity and decreased cell division. The content of secondary metabolites are also affected by callus weight. The higher weight of callus on providing ion $40\mu\text{M}$ Cu^{2+} metal is 0.29 g can produce levels of sitosterol and high stigmasterol..

CONCLUSION

The addition of Cu^{2+} at a concentration of $40\mu\text{M}$ produced the best callus weight is 0.29 grams and produces stigmasterol and sitosterol best with small doses of stigmasterol and sitosterol 3128,739 1695,620 ppm ppm.

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Use of Compost and Coconut Water as Media for Plant Tissue Culture Propagation

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ABSTRACT

In vitro culture techniques provide many advantages, but not easy to implement, if the cost is limited. Compost is the result of the fermentation of organic waste is easy to obtain and the chemical content of the compost is also a nutrient in the form of mineral salts. Coconut water is result from coconut cultivation is quite a lot available in the neighborhood, its unique chemical composition of sugars, vitamins, mineral, amino acid and growth regulators. The object of the experiment was to find out the composition of compost and coconut water as medium to regenerate axillary buds of patchouli in tissue culture technique. Media treatment used compost, with several concentration level (2.5g, 5g and 10g) combined with 15% coconut water. The results showed the treatment (media with compost combined with coconut water) and media only compost (2.5g and 10g) growth the axillary buds of patchouli to shoot. However, this research has not shown any shoots multiplication.

Keywords : compost, coconut water, media, tissue culture.

INTRODUCTION

The organic waste which most people are not valuable items, abundant due to the increasing population, can be composted. Compost contains nutrients such as minerals, vitamins and there is also the possibility of growth regulators. Coconut water is traditionally used as a growth supplement in plant tissue culture. The wide applications of coconut water can be justified by its unique chemical composition of sugars, vitamin, minerals, amino acids and growth regulators.

The development of tissue culture in Indonesia is quite rapid. Almost all commodities that have the potential to be developed was propagated through in vitro culture (Lestari, 2008). Some of the commodities that have been propagated and developed with this technique include: coffee, cocoa, tea, teak, sandalwood, aloe, abaca banana, plantain, orchids, palm oil, anthurium, nepenthes and potatoes.

The success of this technique is influenced by the interaction of several factors i.e explants, carbon source, exogenous growth regulators, environment and composition of medium.

Murashige and Skoog (MS) medium is an example basic medium of the most commonly used medium for the propagation of plants. Privileged MS medium was nitrate content, high potassium and ammonium. In addition to the basic medium MS is also known to B5 medium (Gamborg); DKW medium (Driver and Kuniyuki) for woody plants, Vacint and Went for orchid plants and N6 basic medium for rice, etc.

Mineral salts are mixed by the experts to make tissue culture medium can of course also use a number of natural ingredients is also much that is obtained in the fermented organic waste into compost. Compost is a natural fertilizer (organic) made from green materials and other organic materials are

intentionally added to speed up the process of decay, such as manure.

Compost is useful for all types of plants, include ornamental plants, vegetable plants, fruit trees, crops and plantation crops. Compost contains organic-C, total N, as well as P and K nutrients in the form of compounds and K_2O , P_2O_5 (Sulistiyawati *et al*, 2008). Based on the content, compost possibility can be used as an alternative components in tissue culture medium.

Coconut water as food reserves (endosperm) contain vitamins, amino acids, nucleic acid phosphorus, and plant growth regulators auxin and gibberellin besides some nutrients. Therefore, coconut water has the ability to drive cell growth, proliferation and differentiation processes. The optimum concentration of coconut water are given in vitro culture was 15% (Tulecke, *et al*. 1961).

Van Staden and Drewes (1975) reported that the coconut water is known contain zeatin belongs to a group of plant growth regulators cytokinin. Cytokines have the ability to encourage cell division and differentiation of certain tissues in the formation of buds shoot and root growth.

Coconut water affect the growth of orchids in increased growth of protocorm like bodies (Widiastoety and Santi 1994). Coconut water can increase the amount of ginger shoots on shoot multiplication in tissue culture (Seswita, 2010).

The aim of this study is to get the composition of compost and coconut water as component medium in tissue culture propagation.

MATERIALS AND METHODS

The experiment was carried out at the laboratory of Tissue Culture of Nasional University Jakarta from September to November 2012.

Tools used include: laminar air flow, autoclaves, ovens, bottle cultures, pH meters,

scales, Bunsen lamps, scalpel, hand sprayer, measuring cups, stove, pipettes, etc.. Media materials used are MS medium, in the form of solid, refined compost, coconut water, sugar, agar and distilled water. The plant material as a source of explants was axillary buds of patchouli (**Fig. 1**).



Figure 1. Patchouli plants as a source of explants.

Sterilization equipment

Culture bottles, glass tools, medium, aquadestilata sterilized using an autoclave at 121°C pressure at 171 psi (pounds per square inch) for 30 minutes. Petri dish, scissors and scalpel wrapped with paper and sterilized in oven at temperature of about 80-100°C for 2 hours.

Preparation of medium

Media used was MS medium plus 15% coconut water as the first control (K1) and the MS medium without coconut water as the second control (K2).

Solution pipetted from stock in accordance with the appendix table. Then added 30 g sucrose and 15% coconut water for K1, whereas for K2 without coconut water. Medium solution was added distilled water become 1 L. pH was adjusted to 5.6-5.8 range

with the addition of 0.1 N HCl or 0.1 N NaOH solution. The solution was added for 8 g agar. Furthermore, heated on the stove to boil. After that it was poured into a culture bottle. The next process, media sterilization in an autoclave for 20 minutes at a temperature of 121°C pressure of 15-17 psi.

Media treatment was used compost, and coconut water with the following concentration:

2.5 g compost + 15% coconut water (IM-1)

5 g compost + 15% coconut water (IM-2).

10 g compost + 15% coconut water (IM-3).

2.5 g screened compost + 15% coconut water (IM-4).

5 g screened compost + 15% coconut water (IM-5).

10 g screened compost + 15% coconut water (IM-6).

2.5 g screened compost (IM-7).

5 g screened compost (IM-8).

10 g screened compost (IM-9).

2.5 g compost (IM-10).

5 g compost (IM-11).

10 g compost (IM-12).

15% coconut water (IM-13).

All treatments added 30 g sucrose g respectively, pH solution adjusted to 5.6-5.8, then all solution was added for 8 g agar. Furthermore, heated on the stove to boil. After that, it was poured into a culture bottle. The next process all treatments media was sterilization in an autoclave for 20 minutes at a temperature of 121°C pressure of 15-17 psi.

Planting

Explants were prepared and then transferred into a sterile Petri dish and then cut right to take one axillary buds along approximately 1 cm (**Fig. 2**). Then implanted in the bottle. Bottles that have been planted with explants prepared in culture shelves.



Figure 2. Explants form axillary bud in culture bottles.

Data analysis

The experiment was arranged in completely randomized design with 5 replication. Parameters observed were numbers of the shoots, high shoot, numbers of leaves and length of leaf.

RESULTS

The state of culture in the first week is still good and not contaminated. The explants in flasks for all treatments was seen alive, marked the color of explants same with the first grown. In the third week explants grow in to shoot (**Fig. 3**). About 97.3% bottle looks uncontaminated and the explants grow into shoots.



Figure 3. Explants grow into shoots at the age of 21 days.

Fig. 4 shows the explants grow to form a shoot, except the treatment with the addition of 5g compost does not proliferate to form new shoots.

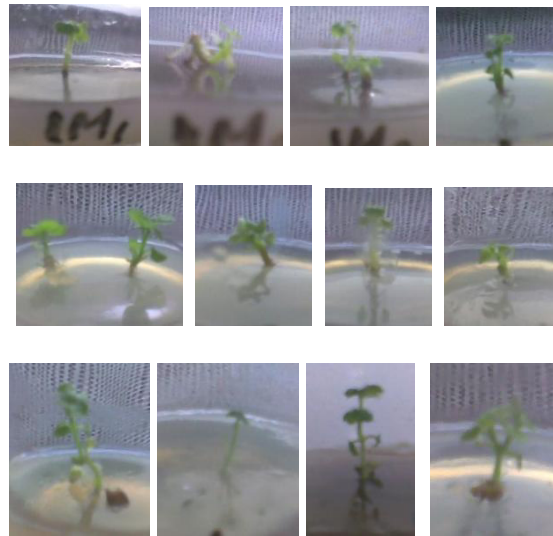


Figure 4. Axillary buds in all treatments grew forming shoots age of 63 days, unless treated IM-11.

Table 1 below shows the average height of shoots, number and length of leaves of plantlets obtained from proliferating explants.

Table 1. The average height of shoots, number and length of leaves.

Treatment	High shoots (mm)	Number of leaves	Leaf length (mm)
IM-1	3.6	1.6	0.8
IM-2	1.6	0.4	0.2
IM-3	8	2.4	0.8
IM-4	7.6	3.2	2
IM-5	11.6	3.8	3.2
IM-6	2	0.8	0.4
IM-7	3.4	1.6	0.8
IM-8	1	0.8	0.4
IM-9	10.6	3.2	2.2
IM-10	1.6	0.4	0.2
IM-11	0	0	0
IM-12	3	1.2	0.2
IM-13	4.4	1.6	0.8
K1	4.4	2.8	0.8
K2	4.8	2	0.8

Growth of explants forming buds in addition, there is also form roots and callus.

Treatments that form the root is treated IM-4; IM-5; IM-6; IM-7; IM-8; IM-9; IM-12; IM-13 and K2. Explants showing callus formation is the control (K1).

DISCUSSION

Capability proliferates explants were characterized by greater explants, showed in the medium containing compost and coconut water have plant growth regulators. Plant grows needs mineral nutrients in addition to plant growth regulators which will drive the pattern growth of explants. Auxin and cytokinin are growth regulators that commonly used in tissue culture. Both of these growth regulators play a role in directing the growth of a piece of tissue into shoots or roots. Auxin and cytokinin may come from pieces of tissue (endogenous growth regulators), and from the medium of coconut water and compost (exogenous growth regulators).

The presence of plant growth regulators on the compost is possible because at the moment there is the role of microbial fermentation process that produces a particular stage of plant growth regulators. As with the addition of coconut water, intentionally done because coconut water also contains plant growth regulators (auxin, cytokinins and gibberellins) (Tulecke, *et al.* 1960; Van Staden and Drewes, 1975).

In the orchid plant propagation using coconut water showed a growth of protocorm like bodies (Widiastoety and Santi, 1994), as well as research Seswita, 2010 showed that coconut water can increase the amount of ginger shoots on shoot multiplication in vitro. Giving 10% coconut water can speed up and increase the number of shoots in vitro culture of orchids *Paraphalaeonopsis serpentilingua* from Borneo. Giving 7.5% coconut water produces the most number of shoots (11) in the same study (Mukarlina, *et al.*, 2010).

The results are listed in the table above, statistically, all treatments showed no

significant differences. Growth model equations obtained as follows $y = 0.083 + 0.017 + \text{compost} + 0.300 - 0.233 \text{ coconut water filtration compost}$. This means that for every 1.0g of compost added, the culture that grew up 1.7%, the culture with coconut water that grows up 23.3% and in the media who screened compost to grow up 30%. The addition of compost 10g showed shoot more long than compost 2.5g. Giving coconut water also increases the percentage of explants growth.

High model equations derived shoots equation $y = 7.430 + 0.377 \text{ compost} - 0.796 - 0.200 + \text{coconut water filtration compost}$. It is for each added 1.0g of compost increased shoot height 0.377mm. In cultures with high coconut water shoots down 0.200mm, the height of filtered media shoots increased 0.796mm. As for the length of the leaves, screened compost significantly increased the leaf length of 1.25 mm.

The formation of buds, roots, and callus showed that in media have growth regulators maybe auxin and cytokinin. It is generally known explants forming buds will proliferate if the ratio cytokinin is higher than auxin. Conversely, if more auxin than cytokinin it will form roots. When the amount of auxin and cytokinin are relatively equal, will form a callus.

CONCLUSION

Compost and coconut water can be used for growth medium in vitro culture techniques. The best composition of compost and coconut water could not be determined. Explant from whole composition compost and coconut water have not been able to proliferate to form many shoots.

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Appendix Table. The composition of MS medium manufacturing stocks.

Stock	Compound	Concentration in MS medium (mg / L)	Weighting ingredients	Volume stock (mL)	Volume decision to 1 L Medium (mL)
A	NH ₄ NO ₃	1650	16500	200	20
B	KNO ₃	1900	19000	200	20
C	CaCl ₂ .2 H ₂ O	440	4400	200	20
D	H ₃ BO ₃ KH ₂ PO ₄ KI Na ₂ MoO ₄ .2H ₂ O CoCl ₂ .6H ₂ O	6.2 170 0.83 0.25 0.025	620 17000 83 25 2.5	100	1
E	MgSO ₄ .7H ₂ O MnSO ₄ .H ₂ O ZnSO ₄ .7H ₂ O CuSO ₄ .5.H ₂ O	370 16.9 8.6 0.025	37000 1690 860 2.5	100	1
F	Na ₂ EDTA FeSO ₄ .7H ₂ O	37.3 27.8	373 278	100	10
G	Thiamine-HCl Nicotinic acid Pyridoxine-HCl Glysin	0.1 0.5 0.5 2	10 50 50 200	100	1
H	Myoinositol	100	1000	200	20

Polymorphisms of *Insuline-like growth factor-I* (IGF-1) and *Pituitary Positive Transcription Factor-1* (Pit-I) Genes and their Effect on Growth Traits in Indonesian Native Chickens

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ABSTRACT

Indonesian native chickens, as known as non-race chicken, have large variations in morphology, i.e., feather color, skin color, beak, body shape, production performance, growth, and reproduction. Those variations are the results of rearing system and uncontrolled mating over generations, as well as factors of environmental adaptation. Chicken growth is determined by genetic and environmental factors. The growth is controlled by polygene, such as Pituitary Positive Transcription Factor 1 (Pit-1) and Insuline-Like Growth Factor 1 (IGF-1). This research was aimed at exploring polymorphism in Pit-1 and IGF-I genes and the effects on growth in Indonesian native chickens. Four races of Indonesian native chickens, i.e., Kampong, Sentul, Pelung, and Kedu, were used as samples. Broiler chickens were applied as comparison. Based on PCR-RFLP using PSt-I enzyme, there were two genotypes detected in Indonesian native chicken IGF-1 gene (the genotypes were denoted as AA and AB); while in Broiler IGF-1 gene were found three genotypes (AA, AB, BB). AB and BB genotypes related to large body weight. Heterozigosity in Indonesian native chickens were between 0.27-0.32, while Broiler chicken was 0.46. Value of Hardy-Weinberg equilibrium was 2.73 – 3.34. Pit-1 gene was conserved in all test animals, except Sentul chicken. Nucleotide sequences were 99% similar among those, there was only one nucleotide substitution in Sentul chicken that were defined as dissimilarity. *Anser anser* was used as outgroup in Pit-1 gene sequence, the genetic distance between chickens and *Anser anser* was 53-59%. Therefore, we concluded that variation in growth gene was low.

Key words : Indonesian native chicken, Broiler, IGF-1, Pit-1, Polymorphism

INTRODUCTION

Indonesian native chicken as known as non-race chicken is an important commodity maintained by most local people, especially in rural areas. As Indonesian germ plasm livestock, local chickens need to be maintained and purified as well as utilized optimally to provide animal protein (Sulandari *et al.* 2007).

Indonesian native chickens, as known as non-race chicken, have large variations in morphology, i.e., feather color, skin color, beak, body shape, production performance, growth, and reproduction (Jafendi, 2007). Those variations are the results of rearing system and uncontrolled mating over generations, as well as factors of environmental adaptation. Indonesian native chickens consist of Kampong, Pelung Kedu, and Sentul.

Chicken growth is determined by genetic and environmental factors. Environmental factors consist of food, water, climate, and other maintenance facilities. Those environmental factors are not inherited. On the other hand, genetic factor influencing growth is controlled by multigenes and pass down to the next generations. Genetic information is necessary to identify genetic quality of livestock for further consideration in selection and crossbreeding. Basic research such as molecular analysis and phenotypic observation is important in order to explore genetic information.

Pituitary Positive Transcription Factor 1 (Pit-1) gene is one of the genes determining growth. This gene is related to animal growth and productivity, as well as controlling gene expression encoding growth and prolactin hormone (Miyai 2005). Pit-1 gene is a positive regulatory factor in specific transcription to express gene encoding growth hormone (GH),

prolactin (PRL) and *Thyroid Stimulating Hormone- β* (TSH- β) (Bodner *et al.* 1988; Miyai 2005).

Jiang *et al.* (2004) reported that mutation in Pit-1 gene of 8 week aged-Chinese local chicken significantly influenced body weight. Nie *et al.* (2005) studied 23 SNP in Pit-1 gene and found 57 nucleotide deletion/insertion, those were related to body weight.

Other growth controlling- gene is *Insulin-like growth factor I (IGF-I)* gene that is the major factor in enhancing animal growth hormone polypeptide (Kita *et al.*, 2005; Li *et al.* 2008). IGF-1 mediates stimulation in mitosis, metabolism in protein deposition, protein metabolism, regulating function of several organs (Zhou *et al.* 2005). Some results of research on the IGF-1 gene polymorphism is associated with growth have been reported in chickens (Sco *et al.* 2001; Kita *et al.* 2005; Li *et al.* 2009), in sheep (Zhang *et al.* 2008), and in cattle (Curi *et al.*, 2005; Siadkowska *et al.*, 2011; Maskur *et al.*, 2012). According to Abbasi dan Kazemi (2011), IGF-1 gene is growth candidate gene in animal; it influences development and growth, such as somatic growth. Research results of Li *et al.* (2008) found that the IGF-1 gene has a very significant effect on body weight, egg weight in Xinghua chickens. Lei *et al.* (2005) analyzed chicken IGF-1 gene facilitated by PCR-RFLP using *Pst*-1 restriction enzyme, they found *single nucleotide polymorphism* (SNP) in *5' region*. There were three genotypes (AA, AB, BB) in chicken IGF-1 gene. According to Mu'in (2010), native chickens in Papua showed significant effect of genotype BB on body weight. Research on role of IGF-1 gene on some Indonesian native chicken growth and broiler chickens comparisons has not been done. Therefore, the study of the genetic diversity using Pit-1 and IGF-1 gene as molecular marker and the

effect on growth will be the basis for the identification and characterization of native chickens genetic resources in supporting sustainable farm in Indonesia. This study aimed at assessing genetic diversity of Indonesian native chickens based on the Pit-1 and IGF-1 genes that will be used as genetic markers, and reviewing the relationship between phenotypic morphology of native chickens and one of the genes growth determinant.

MATERIALS AND METHODS

Research procedure

Morphologic measurements were carried out from the age of DOC (Day Old Chicks) to five months for native chickens, while broilers were measured only until the age of two months.

Following that, blood samples (each of 25 individus per race) were taken out at the age of two months for molecular analysis and preserved in alchohol absolut. The analysis of molecular was conducted in Laboratory of Animal Molecular Biology in Center for Research in Natural Resources and Biotechnology (PPSHB), Bogor Agricultural University.

Molecular Analysis

Isolation of total DNA from blood samples of native chickens and broilers was performed following the method of Duryadi (1993). Primers of the exon 6 (AJ236855) used in this study are based on Nie *et al.* (2008) i.e., E6 Pit-1 F: 5'- GGCAC TTTGGAGAACA AAGC-3' and E6 Pit-1 R : 5' CTCGTGGTGCTC CTTGATAA-3' and primers of the IGF-1 gene are based on Li *et al.* (2008), i.e., IGF-I F: 5'-GACTATACAGAAAGAACCCAC-3' and IGF-I R: 5- TAT CA CTCAAGTGGCTCAGT-3. PCR conditions as follows: initial denaturation for 5 minutes at a temperature

94⁰C followed with 35 cycles of denaturation 94⁰C for 45 seconds, annealing at a temperature of 55⁰C for 90 sec (for Exon6-Pit-1) or 56⁰C for 90 sec (for IGF-1), elongation at a temperature of 72⁰C for 60 sec , and then terminated by the extension for 5 min at 72⁰C.

PCR products of IGF-1 were cutting with RE-enzyme Pst I (5' . CTGCA ▼ G.3') . The mixture was incubated at a temperature of 37⁰C for 16 hours. Cutting products were then migrated using 2% agarose gel at the voltage of 100 volts for 30 minutes. DNA marker of 100 bp sized used as a standard to measure the size of PCR band integrity. Results of electrophoresis was analyzed with UV-Transilluminator. Beside that PCR products of IGF-1 were sequenced to know nucleotide mutation position of RE-enzyme Pst-I.

Based on Li *et al.* (2008), A allele was represented as a band with size 621 bp and not cut by the restriction enzyme Pst-1, while B allele was represented as a band with size 364 bp and 257 bp that was a cutting product from 621 bp fragments. Therefore, BB genotype was indicated as double bands with fragments 364 bp and 257 bp; AA genotype was represented as single band that fragment of PCR product was not truncated); while AB genotype consisted of three bands with size 621 bp, 364 bp, and 257 bp.

PCR products of Exon6-Pit-1 were sequenced in two direction forward and reverse.

Genetic Diversity Analysis

Results of RFLP analysis with restriction enzyme Pst-1 of IGF-1 are grouped based on cutting point of the enzyme Pst-1. The cutting point determined genotype of each chicken sample. Allele and genotype frequencies of the IGF-I gene were analyzed using Nei's method (1987), as follows:

Frequency of A allele = frequency of AA genotype + ½ frequency AB genotype
 Frequency of B allele = frequency of BB genotype + ½ frequency AB genotype
 Frequency of AA genotype = $\frac{\sum \text{AA genotype}}{(\sum \text{individuals in the observed population})}$
 Frequency of BB genotype = $\frac{\sum \text{BB genotype}}{(\sum \text{individuals in the observed population})}$
 Frequency of AB genotype = $\frac{\sum \text{AB genotype}}{(\sum \text{individuals in the observed population})}$

Pit-1 nucleotide sequence in exon-6 were aligned with Clustel W of MEGA-5 (Tamura et al., 2011) to analyze Single Nucleotide Polymorphism (SNP) at each individus of all samples.

Morphologic Diversity Analysis

Phenotypic performances were observed by measuring body weight. Measurement body weight in broiler chickens only implemented until the age of 8 weeks, whereas the native chicken applied up to the age of sexual maturity, i.e., at the age of 28 weeks. The live weight of the studied native chicken age is 1, 2, 3, 4 and 5 months, corrected towards the average of live weight native male studied chicken. The objective was to eliminate the factor of sex differences. Life weight correction was as performed following by Mu'in *et al.* (2010) method, as follows:

(a) Analysis on correction value of live weight of chickens at each age (A_{ki}) was determined by dividing the average of live weight of male chickens at certain age (R_{Ji}) with the average of weight life of female chicken at certain age (R_{Bi}). Therefore,

$$AK_i = RJ_i / RB_i, \text{ where } i = 1, 2, \dots, 4.$$

(b) Corrected live weight of female chicken at certain age (BBT_i) is live weight of female chickens at the certain observational age (BBB_i) multiplied by AK_i ; or corrected live weight of male chicken at certain observational age (BBJ_i) multiplied by one. Therefore, female chicken $BBT_i = BBB_i \times AK_i$; while the male chicken $BBT_i = BBJ_i \times 1$ where $i = 1, 2, \dots, 4$.

RESULTS

Fig. 1 is showing the results of electrophoresis genotyping of IGF-I were found in some samples of chickens.

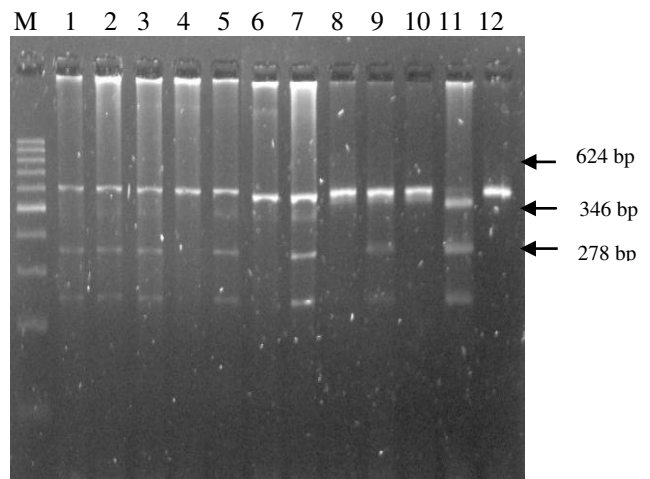


Figure 1. Electrophoresis Results PCR-RFLP Pst-I locus IGF-I of studied native chickens

Description: Lanes 1: DNA marker 100 to 1000bp); lanes 1-3 5.7 9, and 11 AB genotyping PCR products (621 bp 364 bp and 257 bp); lanes 4,6, 8, 10, and 12: AA genotype (621 bp)

Table 1 presents the frequency of allele, genotype frequency of IGF-I, heterozygosity and the χ^2 test results.

Table 1. Allele and genotype frequency and χ^2 test for IGF-I phenotype native chicken population studied

Clump	Frequency Genotip (n)			Frequency Allele		X ² hit	X ² tab
	AA	AB	BB	A	B		
Kampung Chicken	0.69 (11)	0.31 (5)	-	0.84	0.16	3.32	3.84
Pelung Chicken	0.63 (10)	0.37 (6)	-	0.81	0.19	3.34	3.84
Sentul Chicken	0.69 (11)	0.31 (5)	-	0.84	0.16	3.32	3.84
Kedu Chicken	0.69 (11)	0.31 (5)	-	0.84	0.16	3.32	3.84
Broiler	0.25 (4)	0.69 (11)	0.06 (1)	0.59	0.41	2.73	3.84

Weight calculation results of native chicken aged one, two, three, four, and five months to clumps chicken, IGF-I genotype (AA and AB) and their interactions are presented in **Table 2**

which shows that the AA genotype at all ages and all clumps have the weight lower body than chicken with genotype AB

Table 2. The average live weight of studied native chickens age 1, 2, 3, 4, and 5 months.

Umurbulan)	Genotip	Sentul (gr)	Kedu (gr)	Kampung (gr)	Pelung (gr)	Broiler (gr)
1	AA	278.50±14.15 ^a	246.22±14.15 ^a	244.83±14.15 ^a	298.29±14.84 ^b	802.20 ^a
	AB	265.00±20.98 ^{ab}	237.81±20.98 ^b	280.28±20.98 ^a	288.58±19.15 ^a	842.30 ^b
	BB	-	-	-	-	887.40 ^c
	Interaksi	Ns	ns	Ns	ns	
2	AA	388.02±19.14 ^c	407.32±19.14 ^b	400.65±19.14 ^b	481.74±20.07 ^a	1820.56 ^a
	AB	405.41±28.39 ^a	413.39±28.39 ^a	444.97±28.39 ^b	462.62±25.92 ^a	1870.42 ^b
	BB	-	-	-	-	1905.25 ^b
	Interaksi	Ns	ns	Ns	ns	
3	AA	513.57±19.75 ^b	510.73±19.75 ^b	530.41±19.75 ^b	670.82±20.71 ^a	
	AB	529.68±29.29 ^c	565.19±29.29 ^b	584.06±29.29 ^b	638.41±26.74 ^a	
	BB	-	-	-	-	
	Interaksi	Ns	ns	Ns	ns	
4	AA	648.72±20.37 ^c	734.71±20.37 ^b	672.16±20.37 ^c	825.46±21.37 ^a	
	AB	655.44±30.22 ^c	739.63±30.22 ^b	730.46±30.22 ^b	811.62±27.58 ^a	
	BB	-	-	-	-	
	Interaksi	Ns	ns	Ns	ns	
5	AA	765.42±21.56 ^c	926.62±21.56 ^b	860.21±21.56 ^b	1015.09±22.61 ^a	
	AB	783.39±31.98 ^c	912.69±31.98 ^b	919.39±31.98 ^b	1008.99±29.19 ^a	
	BB	-	-	-	-	
	Interaksi	Ns	ns	Ns	ns	

Note: Different superscript letters in the same row states significantly different (P < 0.05), ns = non-significant

PCR results of Pit-1 gene in the local chicken in Fig. 2

kimura-2 parameters from the nucleotide bases, are presented in Table 3.



Figure. 2. PCR results of Pit-1 gene in the local chicken
 Note: 1 = Marker 100 bp; 2-3 = SN606 - SN623 (Sentul chicken) 4-5 = KD53 – KD87 (kedu chicken) 6-7 = BRO59- BRO712 (broiler chicken) 8-9 = P7 - P22 (pelung chicken) 10-11 KM5-Km6 (kampong chicken)

Cluster genetic distance of Sentul chicken, kedu, kampong, pelung, and broiler can be compared on the basis of genetic distance of

Table 3. Kimura 2 parameter genetic distance Pit1 gene exon 6 in five clusters of chicken research along the 179 nt and geese cluster (ANS)

ANS	SN606	SN632	KD53	KD87	BRO59	BRO712	P7	P22	KM5	KM6
ANS										
SN606	0.053									
SN632	0.053	0.000								
KD53	0.059	0.006	0.006							
KD87	0.059	0.006	0.006	0.000						
BRO59	0.059	0.006	0.006	0.000	0.000					
BRO712	0.059	0.006	0.006	0.000	0.000	0.000				
P7	0.059	0.006	0.006	0.000	0.000	0.000	0.000			
P22	0.059	0.006	0.006	0.000	0.000	0.000	0.000	0.000		
KM5	0.059	0.006	0.006	0.000	0.000	0.000	0.000	0.000	0.000	
KM6	0.059	0.006	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Phylogeny tree construction in Figure 3

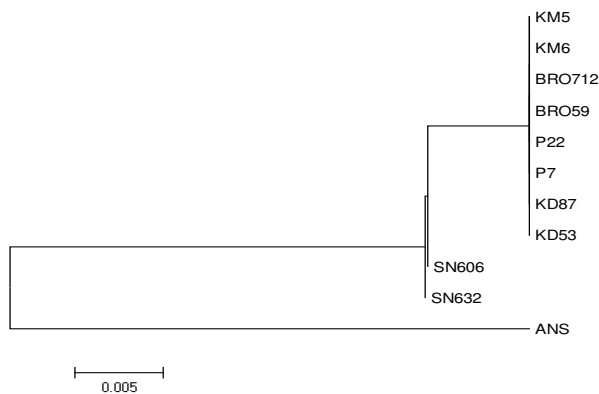


Fig. 3. Phylogeny tree construction

DISCUSSION

Amplification of the specific DNA fragments genetic marker on native chicken and comparison chicken has been successfully performed. The resulting PCR product after sequencing later in alignment using Mega 4 with a yield of 624 bp and these results differ slightly to those designed by Li *et al.* (2008) ie 621 bp.

Digestion results with restriction enzyme Pst-I of PCR products of all samples produced two alleles that can be clearly distinguished ie the allele A is a ribbon size 624 bp and allele B are two ribbons size 346 bp and 278 bp. The result is slightly different from that reported with the previous investigators (Wang *et al.*, 2004; Li *et al.*, 2008). Allele A was shown with the failure of the restriction enzyme Pst-I found the sequences of DNA that are recognized in all the PCR products, so that the enzyme does not cut the PCR product. As a result, size of PCR products before and after the cut with Pst-I restriction enzyme remain the same ie 624 bp. In contrast, allele B is indicated by the success of Pst-I found the sequences of DNA that are recognized (5'-CTGCA ▼ G-3 ') in all PCR products and managed to cut the PCR product into two fragments size 346 bp and 278 bp. The specific DNA

fragment was containing SNP (single nucleotide polymorphism). Point mutations in specific DNA fragments of IGF-I gene is due to substitution (transversion) a nucleotide guanine (G) with Thymin (T) was detected using the Pst-I. This is not in accordance with the results of Li *et al.* (2008) ie results of substitution are cytosine (C) with Thymin (T). Figure 1 is showing the results of electrophoresis genotyping of IGF-I were found in some samples of chickens.

Results of this study indicate ie there are only 2 genotypes AA and AB in the sample, while the native chickens in broiler chickens found 3 genotypes AA, AB, and BB. However, there are interesting things ie AB genotype frequency in broiler chickens is greater than the genotype AB of native chickens.

Conversely, native chickens AA genotype frequency greater than the frequency of genotype AA broilers (Table 1).

Table 1 presents the frequency of allele, genotype frequency of IGF-I, heterozygosity and the χ^2 test results. Based on allele frequencies of IGF-I seen that on native chicken, allele A has a value large enough that the frequency between 0.81-0.84 (average of 0.83), whereas the value of broiler chickens fekuensi its allele A 0.60 is lower than The Indonesian native chicken (average of 0.83). Allele B frequency in broiler chickens is 0.410 higher than the frequency of allele B at the native chicken of Indonesia, which is an average of 0.165. The same phenomenon, also found in populations of exotic chickens: Lohmann (broiler), has allele B for 0791 is higher than the allele A frequency is 0209 (Wang *et al.* 2004).

Chi-square analysis results in Table 1 show that in Kampong chicken, Kedu, Sentul, Pelung, and broilers are in Hardy-

Weinberg equilibrium because X^2 count is smaller than table (not significant). A population expressed in Hardy-Weinberg equilibrium when showed no selection, migration, mutation or genetic drift on to the native chicken three clumps as stated Abbasi and Kazemi (2008). This indicates that in the native population of and broiler chickens in the study area, there were no factors that disrupt genetic equilibrium in the population. Heterozygosity values of all five types of chickens showed the value less than 0.5, so it is said to have low gene diversity. Javanmard *et al.* (2005) stated that if the value of heterozygosity in a population less than 0.5, then it is concluded to have low gene diversity.

Polymorphic Genes of IGF-I effects on the growth of native chickens

Calculation results of body weight average of studied chickens at the age of one, two, three, four, and five months grouped by gender before corrected towards males indicate that male native chickens live weight was higher than female native chickens at all ages observation ($P < 0.05$). This is in accordance with the results of Daikwoet *et al.* (2011) studied at Nigerian native chicken, and Mu'inet *et al.* (2010) at Papuan native chicken, which males body weight were higher than the females. Sex hormones factors (sex steroids) is the cause of male and female differences in growth (Hammond *et al.* 1984), so resulting in a clear sexual dimorphism.

Weight calculation results of native chicken aged one, two, three, four, and five months to clumps chicken, IGF-I genotype (AA and AB) and their interactions are presented in Table 2 which shows that the AA genotype at all ages and all clumps have the weight lower body than chicken with genotype AB. This is in line with the statement of Mu'inet *et al.* (2010) that the body weight of chicken

with genotype AA is lower than the weight chicken with the genotype AB at native chicken Papuan. Pelung chicken body weight at all ages with the AA genotype was higher than the Sentul chicken, Kampong chicken, and Keduchicken ($P < 0.05$), at the age of one and two months. Furthermore genotype AB in Pelung chickens age of three, four, and five months had higher body weight than the three other native chickens ($P < 0.05$) However, between Sentul, Kedu and Kampong chicken did not show differences in body weight were in either genotype AA or AB at the age of one to five months. This is in accordance with the statement of Nagaraja *et al.* (2000) that IGF-1 genotype did not give different weight in chickens aged 140, 265, and 365 days. The highest body weight of broiler chickens were in genotype B, then AB, and AA as research results of Wang *et al.* (2004) in Lohman chickens. The results of four analysis variables distinguishing were in local chicken clumps obtained only two variables, namely length of the back and chest circumference showed significant results (Nurcahya 2012, unpublished data). Dorsal length measurement results based on genotyping of IGF-1 showed that in genotype AA had lower back length compared to the length back in genotype AB in each age and clump of chicken. Length analysis results of back length and chest circumference in Pelung chickens were higher than Sentul, Kedu, and Kampong chicken in both AA and AB genotype ($P < 0.05$). Furthermore, the Kedu, Sentul, and Kampong chicken showed no difference in back length and chest circumference both genotypes AA and AB at the age of one to five months.

Pit-1 gene amplification of exon 6.

PCR amplification results in each local chicken, which is kedu chicken, kampong chicken, sentul chicken, and broiler

chicken using primers MR1 gene Pit-1 is presented in Figure 3. The figure shows that the Pit-1 gene fragment exon 6 produces consistent bands of 179 bp.

Alignment of nucleotide sequences and characterization of Pit-1 gene exon 6.

From 10 results of sequences consist of 2 sequences in each group of chicken obtaining results of parallel along 179 nucleotides.

Analysis of phylogeny.

Cluster genetic distance of Sentul chicken, kedu, kampong, pelung, and broiler can be compared on the basis of genetic distance of *kimura-2 parameters* from the nucleotide bases, are presented in Table 3.

Table 3 treated with MEGA 4 program resulting in diagram of phylogeny tree as can be seen in Figure 3. The figure shows that there are two major groups (clusters) of the studied clusters and one group for comparison, the nucleotide sequences of goose. Sentul chicken clumps separated in its own group (B), while the rest are clustered in another group. .

Based on the alignment results showed that as many as 178 nt conserved value and variabed value by 1 nt (Appendix 3), whereas the goose cluster parallel of 179 nt showed conserved value, 173 nucleotides and 6 nt variabed value.

Results of the genetic distance matrix can be seen in Table 2 showed that the genetic distance between Sentul chicken (SN606 and SN632) and kedu chicken, pelung, broiler and kampong was 0.006. This is not in accordance with the study results of Sartika (2004) with morphometric methods between Sentul chicken, kampong, and pelung chicken had a close relationship. Further genetic distance in

kedu, pelung, and kampong chickens (KM5) and the broiler were 0.000, indicating a close relationship. However, from the results of genetic distance 0.006 is a relatively small figure that is still a relatively close genetic distance. This is not in line with the results of Zhang *et al.* (2010) showed that among the Chinese broiler local chickens had a much genetic distance meaning Pit-1 gene different from the china chicken Pit-1 gene broiler chickens, as well as research results of Azmi *et al.* (2000) between Malaysia and local chicken broilers.

The resulting phylogeny tree construction showed that chicken clusters formed two main branches of research and the main branch of the family which was the benchmark down. Chicken group research, namely Sentul chicken (SN606 and SN623) is one group, while the other group is the group of kedu chicken, kampong, pelung and broiler. These results are not consistent with the study results of Nurcahya *et al* (unpublished data) with morphometric methods showed three groups in which each group is pelung chicken, broilers, and local chickens (kampong chicken, Sentul and kedu chicken) .

Phylogeny tree construction associated with the analysis of genotype on IGF-1 gene showed a grouping. It can be seen in the grouping SN606 and SN632 chickens (Sentul chicken) was genotype AA, while the chicken KM (kampong), P (pelung), KD (kedu), BRO (broiler) genotype AB.

Results should be presented in a logical sequence in the text, tables and figures. Repetitive presentation of the same data in tables and figures should be avoided. The results should not contain material appropriate to the Discussion. All tables, graphs, statistical analyses and sample

calculations should be presented in this section.

Conclusion

There was genetic variation in IGF-1 gene, so that each individual can be sorted out by different genotypes. The differences were due to point mutations in Pst-1 restriction sites of the bases guanine (G) became base Thymin (T). There are only two genotypes of IGF-1 in the native chicken ie genotypes AA and AB with successive frequency of 68.75 and 31.25% respectively. The frequency of allele A in native chicken (0.83) is higher than broiler chicken (0.59). AB Genotype in Pelung chicken at the age of four and five months had the appearance of greater body weight than genotype AA fellow either in same clumps or different ones (Sentul, Kampong, and Kedu chicken).

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ANTICANCER ACTIVITY OF BANGLE HANTU (*Zingiber ottensii* Val.) RHIZOMES ON BREAST CANCER CELL LINES MCF-7

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ABSTRACT

Bangle Hantu (*Zingiber ottensii* Val.) is one of underutilized Zingiberaceae plants which are abundantly grow in Indonesia. It is not commonly used in Indonesian traditional medicine nor as spice in Indonesian culinary system. To increase its utilization, in this study we investigated anticancer activity of the methanolic extract of the rhizomes on human breast cancer cell line MCF-7. The antiproliferative effect was measured by comparing the rate of proliferation (doubling time) of cells treated with extract to untreated or control cells using *in vitro* tetrazolium salt (MTT) assay, and the ability to induce apoptosis was observed using acridine orange-ethidium bromide staining. The results showed that the methanolic extract significantly inhibit the proliferation of MCF-7, showed by no proliferation occurred in cells treated with 20 µg/mL extract (the lowest concentration used in the experiment) while only 25% cells left alive after 24 hours incubation with 80 µg/mL extract (the highest concentration used in the experiment). The extract also showed ability to induced apoptosis of MCF-7 cells. From the results we concluded that the methanolic extract of the rhizome of *Zingiber ottensii* Val. have anticancer activity and could be developed further as source of novel natural anticancer agent.

Keywords

Anticancer, *Zingiber ottensii*, MCF-7, antiproliferative, apoptosis.

INTRODUCTION

Plants have been a source of medicine for thousands of years and phytochemicals continue to play an essential role in medicine. Zingiberaceae is a large family comprises more than 1000 species, and many of its member were famous as spices and medicinal plants as well. These plants thrives in tropical region such as Indonesia. One of the plants which abundantly grows wild in Indonesia is Bangle Hantu (*Zingiber ottensii* Val.). Despite of its abundance and wide distribution, Bangle Hantu is still underutilized, it is not commonly used in Indonesian traditional medicine nor as spice in Indonesian culinary system, maybe due to its unpleasant taste and odour. Very rare information was found about the use of this plant as traditional medicine, some of which mention about the use of Bangle Hantu as pain reliever, and sometimes use to cure fever and cough especially for children (Sinaga *et al*, 2000).

Rhizomes of Bangle Hantu contain essential oils, flavonoids, steroids, tannins, and other bioactive compounds. Flavonoids are well known for its strong antioxidant activity and some of them have been shown to have significant anticancer activity (Chahar *et al*, 2011; Naphong *et al*, 2013; Vijayalakshmi *et al*, 2013). Essential oil in Bangle Hantu rhizomes contains mainly zerumbon (37 to 40.1%), terpinen-4-ol (11.2 to 16.8%), α -humulen (5.6 to 10.9 %) and sabinen (6.5-7.2%) (Malek *et al*, 2005; Thubthimthed *et al*, 2005). Zerumbon isolated from *Zingiber zerumbet* L. had been shown to inhibit the growth of Human HeLa Cervical Cancer Cells, pancreatic cancer cells Panc-1, and HepG2, and also induced their apoptosis (Sakinah *et al*, 2007; Abdul *et al*, 2009; Zhang *et al*, 2012). In addition, the rhizomes of Bangle Hantu also contain a cysteine protease, called zingipain, which has

antiproliferative activities against fungi and human malignant cell lines (Karnchanatat *et al*, 2011). Sinaga *et al* (2011) reported the strong cytotoxicity of the methanolic extract of Bangle Hantu rhizomes against human breast cancer cell lines MCF-7 with IC₅₀ value of 60 mg/ml, as strong as the methanolic extract of the rhizomes of *Zingiber zerumbet* L. (lempuyang gajah) and much stronger than methanolic extract of the rhizomes of *Nicolaia speciosa* L. (kecombrang). Several research groups have shown that *Zingiber zerumbet* rhizomes extract has strong anticancer activity (Rashied and Pihie, 2005; Ruslay *et al*, 2007; Yob *et al*, 2011). Therefore it is very intriguing to know whether Bangle Hantu, the close relative of *Zingiber zerumbet*, also has strong anticancer activity, especially in inhibiting proliferation and induced the apoptosis of cancer cells, so it can be used as source or raw material for a novel natural anticancer drug.

Materials and Methods

Plant material, cell line and chemicals

Fresh rhizomes of *Zingiber ottensii* Val. were obtained from BALITTRO (Balai Penelitian Rempah dan Tumbuhan Obat) Cimanggu Bogor, West Java. MCF-7 cell line were obtained from CCRC (Cancer Chemoprevention Research Center) Gadjah Mada University, Yogyakarta. BSA (Bovine Serum Albumin), PBS (Phosphate Buffer Saline), MTT Solution, Ethidium Bromide and Acridine Orange were purchased from Sigma-Aldrich Corp, St. Louis, MO, USA. DMEM (Dulbecco's Modified Eagle Media), FBS (Foetal Bovine Serum), 1% (v/v) Penicilline-Streptomycine Solution, and Tripsin-EDTA 0,25% were purchased from Gibco, Invitrogen Corporation, Grand Island, NY, 14072, USA.

Preparation of plant extract

Slices of fresh rhizomes of Bangle Hantu (ca 10 kg) were sun dried for 2 days and autoclaved in an electric oven at 40°C for 5 days. The dried rhizomes slices were grinded and sieved with a 18 mesh sieve. The dried powdered (1000 g) of the rhizomes were extracted in room temperature with methanol (1.5 L) for 24 hours and then filtered. The process of extraction was repeated three times, and then the filtrat collected was concentrated by rotary vacuum evaporator.

Antiproliferative activity assay

Antiproliferative activity was measured by comparing the rate of proliferation (doubling time) of extract-treated cells with untreated or control cells by means of growth inhibition assay using *in vitro* tetrazolium salt (MTT) as described recently (Hamedeyazdan et al, 2012). Stock extract solution were prepared by dissolving 5 mg of extract with 100 mL DMSO. Stock solution was then diluted with DMEM medium to obtain a series of test extract solution.

MCF-7 cells were maintained in a humidified incubator with 5% CO₂ for 24 hours at 37°C. When the cells were 80-90% confluent, they were harvested by treatment with a solution containing 0.25% trypsin, thoroughly washed and resuspended in supplemented growth medium and seeded at a density of $\sim 5 \times 10^3$ per well. After 24 hours, to each well was added 100 μ L test solution with varying concentrations range from 20-80 μ g/mL, and then incubated for 6, 12, 24, 48 and 72 hours. 1.25% DMSO solution was used as control. At the end of incubation, the culture medium was removed by carefully aspirated, and cells were washed with 100 μ L PBS (Phosphate Buffer Saline). To each well then added 100 μ L of culture medium and 10 μ L of 5 mg/mL MTT solution, and the cells were reincubated for

next 6 hours. Viable cells will react with MTT to form purple-blue formazan. The reaction was stopped by MTT stopper reagent (Sodium dodecyl sulfate), shaken in shaker plate for 10 minutes, then incubated overnight in the dark at room temperature. Finally the absorbance of formazan in each well was measured with an ELISA reader at 595 nm. The resulting growth data represents the net outcome of cell proliferation. The cell viability (%) was obtained by comparing the absorbance between the samples and a negative control.

Apoptosis induction assay

The induction of apoptosis was observed by acridine orange/ethidium bromide (AO/EB) staining to visualize nuclear changes and apoptotic body formation that are characteristic of apoptosis as described by others (Ćurčić *et al*, 2012; Lakshmi *et al*, 2011) with a slight modification. MCF-7 cells grown on coverslips inserted in 24-well microplate to obtain a density of 5×10^4 cells/well and incubated until 50-60% confluent. After the cells were incubated with the extract for 48 hours with 1.25% DMSO as control solution, culture medium was removed by carefully aspirated, and cells were washed with PBS (Phosphate Buffer Saline). Cells were then stained with acridine orange-ethidium bromide solution and allowed to stand for 5 minutes, and then immediately observe under fluorescence microscope (Zeiss MC 80). Viable cells showed normal bright green nuclei, early apoptotic cells showed condensed green nuclei, dead cells (late apoptotic cells) showed condensed red-orange fluorescens nuclei, while necrotic cells showed normal red-orange fluorescens (McGahon *et al*, 1995). Number of apoptotic cells were observed and counted to quantify apoptosis.

Results

Antiproliferative activity of *Zingiber ottensii* Val. rhizomes

Methanolic extract of *Zingiber ottensii* Val. rhizomes possessed strong antiproliferative activity as shown in Figure 1. Observations were carried out for 72 hours at 0, 6, 12, 24, 48 and 72 hour, and the concentration of the extract used was around its cytotoxic-IC₅₀ value (60 µg/mL) revealed from previous experiment (Sinaga *et al*, 2011). All concentration of extract used in this experiment (20-80 µg/mL) significantly inhibited the proliferation of MCF-7 cells.

The lowest concentration of Bangle Hantu rhizome's extract used in the experiment (20 µg/mL) had significantly inhibited the proliferation of MCF-7 cells from the beginning of incubation until the end of experiment, while the highest concentration (80 µg/mL) of extract showed strong cytotoxicity with only 25% cells left alive after 24 hours incubation, and almost all the cells dead after 72 hours incubation (Figure 1). At the same time, solvent-treated cells (negative control) showed an increase in cell's number (data not shown), means the proliferation occurred normally.

Apoptosis induction activity of *Zingiber ottensii* Val. rhizomes

Double staining with acridine orange and ethidium bromide revealed that methanolic extract of *Zingiber ottensii* Val. rhizomes had the ability to induce apoptosis on MCF-7 cell lines, showed by orange-red fluorescent cells (indicating by red arrow), that are characteristic of apoptotic cells (Fig. 2B).

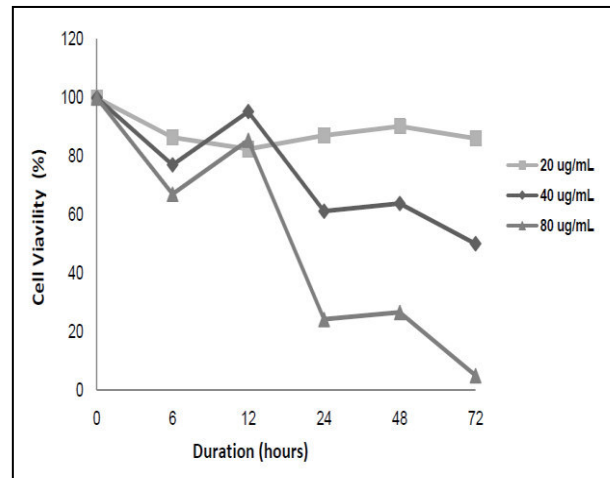


Figure 1. Inhibition of MCF-7 cells proliferation by methanolic extract of Bangle Hantu rhizomes. All concentration used in this experiment were effective in inhibiting the proliferation of the cells.

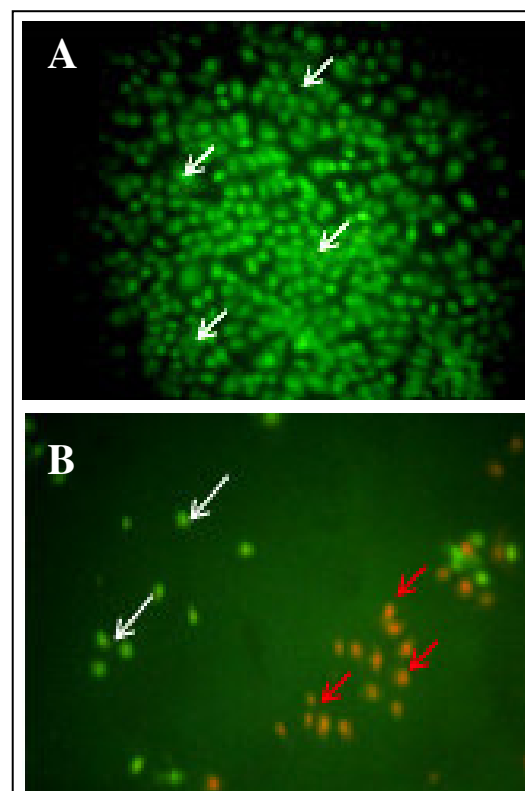


Figure 2. Treatment with methanolic extract of Bangle Hantu rhizomes induced apoptosis of MCF-7 cells. The untreated cells appeared in uniformly bright green colour indicating the normal living cells (A), while some of the treated cells had undergone apoptosis showed by orange-red fluorescent indicated by the red arrows (B).

Some cells underwent nuclei condensation shown by the yellow color in the nucleus, indicating early events of apoptosis, while the untreated cells showed uniformly bright green colour indicating viable cells (Figure 2A). The data obtained showed that MCF-7 cells were undergoing apoptosis after incubation for 24 hours, and increasing the concentration would increase the apoptosis.

Discussion

Rapid and uncontrolled proliferation is main characteristic and the primary key in the progression of tumor or cancer. Therefore, the ability of a substance to inhibit or suppress the proliferation of cancer cells is an important feature or property of a potential cancer drug. In the search for new cancer drugs derived from nature, in the present study we investigated the antiproliferative activity of methanolic extract of Bangle Hantu rhizomes on human breast cancer cell line, MCF-7, and also its ability to induce apoptosis of the cells.

Figure 1 clearly showed that the cells treated with methanolic extract of Bangle Hantu rhizomes showed a decrease in viable-cell's number from the beginning of the experiment until 72 hours incubation. At the lowest concentration used in the experiment, i.e. 20 $\mu\text{g/mL}$, the cells failed to double its number. It means that during the experiment some of the cells died, while proliferation did not occur or significantly reduced.

The higher the concentration, the stronger the cytotoxicity of the extract. 24 hours incubation in 40 $\mu\text{g/mL}$ extract caused cells dead with only 60% of the original number of viable cells left, while 24 hours incubation in 80 $\mu\text{g/mL}$ extract only left 25% of viable cells. At the end of the experiment, i.e. at 72 hours incubation with 80 $\mu\text{g/mL}$ extract, almost no viable cells could be observed (**Fig. 1**). These data corroborate the results of cytotoxicity

assay that had previously been conducted by Sinaga et al (2011), and revealed the antiproliferative activity of methanolic extract of Bangle Hantu rhizomes. However, in this experiment, the doubling time of MCF-7 cells treated with Bangle Hantu extract could not be calculated due to the very strong toxicity of the extract. Therefore the determination of doubling time should be done with other methods, such as by AgNOR staining or by using a lower concentration of extract, i.e. below 20 $\mu\text{g/mL}$.

Apoptosis is a genetically directed process of cell self-destruction, also called *programmed cell death*. Malignant or cancer cells generally lack of apoptosis. Induction of apoptosis is a useful marker for screening compounds for subsequent development as possible anticancer agents. Therefore it is very interesting to know whether methanolic extract of Bangle Hantu rhizome also posses ability to induce apoptosis.

Further experiment by double staining the MCF-7 cells with acridine orange and ethidium bromide demonstrated that the dramatically growth inhibition of methanolic extract of Bangle Hantu rhizomes on MCF-7 cells was caused, at least partly, by the apoptosis induced by the extract. Figure 2B showed some of the treated cells had undergone apoptosis shown by orange-red fluorescent. Acridine orange is a vital dye that stain both live and dead cells, whereas ethidium bromide stain only those cells that have lost their membrane integrity or apoptotic cells. Viable cells will appear uniformly green, while apoptotic cells underwent membran-blebbing and would incorporate ethidium bromide and therefore stain orange (Kasibhatla *et al*, 2006).

Results of the experiments revealed the potentiality of substances contained in the methanolic extract of Bangle Hantu rhizomes to strongly inhibit the proliferation of human breast cancer cell lines, MCF-7, and also

significantly induced the apoptosis of those cells. This means that methanolic extract of Bangle Hantu rhizomes have potential anticancer activity.

However, the extract used in this experiment was crude methanolic extract. It is necessary to isolate the active substances from the extract to determine which substance or substances, among abundant bioactive substances contained in the extract, are the most potent to be developed further as novel anticancer agents.

Conclusion

The study concludes that methanolic extract of the rhizomes of *Zingiber ottensii* Val. have anticancer activity due to its strong antiproliferative activity and ability to induced apoptosis in MCF-7 cell lines. Therefore it should be studied and developed further as source of natural anticancer agent.

Due to the lack of data on anticancer activity of *Zingiber ottensii* Val. rhizomes, it is necessary to conduct further research to examine the anticancer effect of the extract and its fractions on various cancer cell lines and to conduct *in vivo* study as well. It is also important to reveal the mechanism of action of anticancer compounds contained in the plant's part.

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ANTIOXIDANT ACTIVITY AND TOTAL FLAVONOIDS CONTENT OF CURCUMA RHIZOME EXTRACT

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ABSTRACT

The family of Zingiberaceae, including the genus *Curcuma*, has been used since hundreds of years ago as ingredient of traditional medicines. Various scientific studies to support its use as traditional medicine was already done. One of the most prominent biological activity possessed by the family of Zingiberaceae was the antioxidant activity. The aim of this research was to examined the antioxidant activity and flavonoid content of rhizome's extract of *Curcuma heyneana*, *Curcuma mangga*, *Curcuma aeruginosa*, *Curcuma phaeocaulis* and *Curcuma purpurascens*. The antioxidant activity was determined by DPPH method and total flavonoid content was determined by colorimetric method. The antioxidant activity of *Curcuma* rhizome's extract in this study ranged from very strong to weak. *C. purpurascens* had a very strong antioxidant activity (EC₅₀ value of 36.30 ppm) and also the highest flavonoids content measured as quercetin (14.27%). Based on correlation analysis ($R^2 = 0,6573$), there is a positive correlation between total flavonoid content with antioxidant activity of the extract.

Keywords

Antioxidant, flavonoid, *Curcuma*, *rhizome*.

INTRODUCTION

Plants of Zingiberaceae family has been used since hundreds of years ago as source of traditional medicines, including plants belong to the genus *Curcuma*. Various scientific studies has been done to support its use as traditional remedies. One of the most prominent biological activity of plants belong to Zingiberaceae family is the antioxidant activity (Vankar et al, 2006; Chompo *et al*, 2012; Kantayos and Paisooksantivatana, 2012; Sattar *et al*, 2013).

Antioxidants are compounds capable to either delay or inhibit the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species. Antioxidants are involved in the defense mechanism of the organism against pathologies associated to the attack of free radicals. (Pisoschi and Negulescu, 2011). Free radicals can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital, make them very reactive and capable of reacting with important biomolecules, such as proteins, lipids, and DNA. Free radicals damage contributes to the etiology of many chronic health problems such as cardiovascular and inflammatory disease, cataract, and cancer (Lobo *et al*, 2010).

Recently, antioxidants have attracted considerable attention in relation to free radicals and oxidative stress, cancer prophylaxis and therapy, cardiovascular diseases and other degenerative diseases. Antioxidants prevent free radical induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition (Lobo *et al*, 2010). In addition to endogenous antioxidant defense systems which naturally present in human body, endogenous antioxidant is necessary to

improve the body's resistance against degenerative diseases.

Endogenous antioxidant can be either synthetic or natural. Synthetic antioxidants are recently reported to be dangerous to human health. Thus the search for effective, nontoxic natural compounds with antioxidative activity has been intensified in recent years. Dietary and medicinal plants are major source of natural antioxidants.

Many researches has revealed the antioxidant activity of dietary and medicinal plants, including the plants belong to Zingiberaceae family. Antioxidant activities of rhizomes of *Alpinia allughas*, *A. galanga*, *A. smithiae*, *A. vittata*, *Hedychium coronarium*, *Vanoverberghia saskiana*, *Zingiber cassumunar*, *Z. chrysanthum*, *Z. officinale*, and *Z. zerumbet* had been reported (Vankar et al, 2006; Chen et al, 2008; Pal et al, 2011; Rout et al, 2011; Julie and Ernest, 2012; Sattar et al, 2013). Anget et al (2013) reported the antioxidant activity of heat stable protein isolated from aqueous extracts of rhizomes of *Curcuma aeruginosa*, *C.amada*, *C. aromatica*, *C. brog*, *C. caesia*, *C. malabarica*, *C. rakhakanta* and *C. sylvatica*. Protein extracted from *C.brog*, *C.amada*, and *C.caesia* had low IC₅₀ values of 0.70, 0.73, 0.80 respectively, showing high DPPH scavenging activity which were comparable with that of *C. zedoaria* (IC₅₀ 0.84). Antioxidant activity of ethanolic extract of *Curcuma longa*, *C. zedoaria*, *C. angustifolia*, *C. aromatica*, and *C. amada* had also been reported. Antioxidant activity of those species except *C.angustifolia* had been found to have strong correlation with curcumin and phenol content. However *C.angustifolia* may be active due to high aromatic oil content like eugenol, palmitic acid and camphor (Nahak and Sahu, 2011). *Curcuma longa* or turmeric is a famous medicinal plants, and it has strong

antioxidant activity especially in its essential oil (Liju *et al*, 2011).

In attempt to search more source for natural antioxidant, in this work we evaluated the antioxidant activity of rhizomes of five species of *Curcuma*, i.e. *Curcuma heyneana*, *C. mangga*, *C. aeruginosa*, *C. phaeocaulis* and *C. purpurascens*. Since antioxidant activity of plant's extracts often related to its flavonoids content (Grassi *et al*, 2010; Brunetti *et al*, 2013), in this work we also determined the total flavonoids content of the extracts, and evaluated the correlation between antioxidant activity and flavonoids content of the extracts.

MATERIALS AND METHODS

Preparation of crude rhizome extract

Rhizomes of *Curcuma heyneana*, *C. mangga*, *C. aeruginosa*, *C. phaeocaulis* and *C. purpurascens* were obtained from BALITTRO (Balai Penelitian Tanaman Rempah dan Obat), Bogor, West Java.

The dried rhizomes were powdered using a grinder and extraction was done at room temperature. About 100 g of dried powder of the rhizomes were soaked in methanol (1 L, 98%) for 2-3 days, and then filtered through Whatman filter paper No.1. The filtrates obtained were concentrated under vacuum on a rotary evaporator at 50°C and stored at 4°C for further use. The stock solution of crude extract (5 mg/mL) was prepared by dissolving a known amount of dry extract in 98% methanol. The working solution (75, 100, 250, 500 and 750 ppm) of extracts were prepared from stock solution by suitable dilution.

DPPH Radical Scavenging Activity Assay

The antioxidant activity of the rhizome extract was assessed on the basis of the radical

scavenging effect of the stable 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical activity as described recently (Pal *et al*, 2011; Chompo *et al*, 2012). Working solutions of the extract were prepared in methanol. Ascorbic acid was used as standard in 1-100 ppm solution. 0.002 % of DPPH was prepared in methanol and 1 mL of this solution was mix with 1 mL of sample solution and standard solution separately. These solution mixtures were kept in dark for 30 minutes and optical density (OD) or absorbance (A) was measured at 517 nm using UV-Vis Spectrophotometer. Methanol (1 mL) with DPPH solution (0.002%, 1 mL) was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below:

$$(\%) \text{ inhibition} = \frac{\text{Ab} - \text{As}}{\text{Ab}} \times 100$$

where: Ab = Absorbance of blank
As = Absorbance of sample

Linear regression analysis (Origin 6.0 version) was used to calculate the IC₅₀ values.

Determination of total flavonoid

The total flavonoid content was measured by aluminium chloride colorimetric assay as described recently (Hossain *et al*, 2011). An aliquot (1 mL) of extracts or standard solution of quercetin (20, 40, 60, 80 and 100 ppm) was added to 10 mL volumetric flask, containing 4 mL distilled deionized water (dd H₂O). To the flask was added 0.3 mL 5% NaNO₃. After 5 minutes, 0.3 mL 10% AlCl₃ was added, and after 6 minutes more, 2 mL 1 M NaOH was added and the total volume was made up to 10 mL with dd H₂O. The solution was mixed well and the absorbance was measured against a prepared reagent blank at 510 nm with an UV-Vis Spectrophotometer. The measurement was carried out in triplicate and the results were averaged. The data of the total flavonoid

contents of the dry rhizome extracts were expressed as % of quercetin, calculated using the formula given below:

$$\% \text{ Flavonoids (as quercetin) } = \text{As} \times \frac{\text{Aq}}{\text{W}}$$

where: As = Absorbance of sample
 Aq = Absorbance of quercetin
 W = sample weight

RESULTS

Yield of Extraction

Yield of extraction was expressed as weight (g) of crude extract per 100 gram of powdered-dried plant material. The yield of crude extract from Curcuma rhizomes by using methanol as solvent varied between 7.08% - 11.52%. As shown in **Table 1**, the highest yield generated from *C. purpurascens* and the lowest is from *Curcuma mangga*.

Total Flavonoid Content

Total flavonoids content of five methanolic extract of Curcuma rhizomes measured as quercetin are presented in **Table 2**. The value ranged from 1.35 to 14.27%. The highest was *Curcuma purpurascens* rhizome’s extract, while the lowest was *C. aeruginosa*.

Table 1. Yield of Extracts

Species	Local Name	Yield (%)
<i>C. heyneana</i>	Temu giring	11,10
<i>C. mangga</i>	Temu mangga	7,08
<i>C. aeruginosa</i>	Temu ireng	7,26
<i>C. phaeocaulis</i>	Temu jingga	10,78
<i>C. purpurascens</i>	Temu pinggang	11,52

Table 2. Total Flavonoid Content of Curcuma species

Species	Total Flavonoids as Quercetin (%)		
	1	2	Mean
<i>C. heyneana</i>	1.94	1.98	1.96

<i>C. mangga</i>	2.14	2.15	2.15
<i>C. aeruginosa</i>	1.37	1.32	1.35
<i>C. phaeocaulis</i>	5.23	5.18	5.21
<i>C.purpurascens</i>	14.14	14.39	14.27

Antioxidant Activity

Antioxidant activity of rhizomes of five Curcuma species determined using DPPH method are varied, the EC₅₀ values ranged from 36.30 to 199.71 ppm (**Table 3**). EC₅₀ value is concentration of sample required to scavenge 50% of DPPH radicals. The lowest EC₅₀ value, means the strongest antioxidant activity, was belong to rhizome’s extract of *Curcuma purpurascens* (36.30 ppm), while the highest value belong to rhizome’s extract of *Curcuma heyneana* (155.68 ppm).

Table 3. Antioxidant activity of Curcuma species

Species	Antioxidant Activity (EC ₅₀) (ppm)		
	1	2	Mean
<i>C. heyneana</i>	153.16	158.20	155.68
<i>C. mangga</i>	89.47	91.36	90.42
<i>C. aeruginosa</i>	199.57	199.85	199.71
<i>C. phaeocaulis</i>	112.96	108.88	110.92
<i>C.purpurascens</i>	36.34	36.26	36,30

DISCUSSION

In this work we used methanol as solvent for extraction of bioactive substances from the rhizomes. Methanol is a popular solvent for extraction of bioactive substances from plant material, due to its quite small molecular structure, so it can penetrate all plant tissues to pull out the active ingredient. Methanol also has the ability to dissolve almost all organic compounds, both polar and non-polar compounds. Another advantage of methanol is

that it is volatile so it easily separated from the extract. The disadvantage of methanol as solvent for extraction is due to its ability to dissolve wide range polarity of substances, so it is not selective. Yield of extraction with methanol usually higher compare to other solvent, such as ethanol, acetone, chloroform, and ethyl acetate. Extraction of Ginger (*Zingiber officinale*) with methanol give highest yield compare to acetone and chloroform (Ghasemzadeh *et al*, 2011). Similarly, the methanolic extract of *A. wilkesiana* and *S. scrabrum* gave the highest yield (14.67% and 17.23%, respectively), while the ethylacetate extract gave the least yield (2.73% and 4.13% respectively) (Anokwuru *et al*, 2011).

Flavonoids are plant's secondary metabolites with variable phenolic structures. More than 4000 varieties of flavonoids have been identified, most of them posses important bioactivity, such as antioxidant and anticancer activity. One of the best-described flavonoids is quercetin. Quercetin is found in abundance in onions, apples, broccoli, and berries. In *Curcuma* species, the most well known flavonoid is curcumin, the principal curcuminoid of turmeric (*Curcuma longa*). The main flavonoid content in *Curcuma* plants studied in this work is still unknown.

Methanolic extract of *Curcuma purpurascens* rhizomes had the highest total flavonoids content (measured as quercetin) among the species investigated in this work, very much higher (14.27%) than the others (1.35-5.21%). This is in line with the antioxidant activity of the extracts, as shown ini Table 3. Methanolic extract of *Curcuma purpurascens* rhizomes showed the lowest EC₅₀ values (antioxidant activity), i.e. 36.30 ppm, means the highest antioxidant activity, while the other four species ranged from 90.42 to 199.71 ppm (**Table 3**). According to Zuhra *et al.* (2008), a substance is said to have very strong

antioxidant activity if the EC₅₀ is less than 50 ppm, strong if EC₅₀ ranged from 50 to 100 ppm, moderate if EC₅₀ ranged from 100 to 150 ppm, and weak if EC₅₀ ranged from 151 to 200 ppm. According to the criteria, *C. purpurascens* could be stated as having very strong antioxidant activity, followed by *C. mangga* (strong), *C. phaeocaulis* and *C. heyneana* (moderate) and *C. aeruginosa* (weak).

DPPH radical scavenging activity assay based on scavenging of DPPH through the addition of an antioxidant that decolourizes the DPPH solution. The degree of colour change is proportional to the concentration and potency of the antioxidants. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test (Krishnaiah *et al*, 2011). However, to obtain a better and closer approach to its expected use, as a natural antioxidant for human use, it is necessary to conduct *in vivo* antioxidant assay especially for the potential one.

From five *Curcuma* species studied in this work, the best performance, in terms of antioxidant activity and flavonoids content was *Curcuma purpurascens*. Therefore, this species could be developed further as a potential source of natural antioxidant for human use.

Based on correlation analysis ($R^2 = 0,6573$), we revealed a positive correlation between total flavonoid content with antioxidant activity of the extract, suggested that the antioxidant activity of the extract might be due, at least partly, to the presence of flavonoids. This results in line with other works previously reported (Nahak and Sahu, 2011; Khan, 2012; Gopal, *et al*, 2013).

CONCLUSION

Antioxidant activity of methanolic extract of five *Curcuma* species; i.e. *C. heyneana*, *C. mangga*, *C. aeruginosa*, *C. phaeocaulis* and *C. purpurascens* varied from very strong to weak with EC₅₀ values varied from 36.30 to 199.71 ppm. *C. purpurascens* had the strongest antioxidant activity in line with its highest flavonoid content (14.27%). The antioxidant activity of the extract significantly correlated with the total flavonoid content.

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ANTIFUNGAL AND PRELIMINARY PHYTOCHEMICAL SCREENING OF LEAF EXTRACT OF *Pandanus odoratissimus* L.f

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ABSTRACT

In the current wave of antimicrobial resistance against chemotherapeutics drugs, there is need to search for plants that could be resistance-free and affordable. One of the plants that could potentially be used as a medicinal plant is pandan samak (*Pandanus odoratissimus* L. f). The objective of this study is to assess the antifungal activity and to determine the zone of inhibition of extract on some fungal strain. In the present study, the antifungal activity from ethanol extract of leaves *Pandanus odoratissimus* was evaluated for potential antifungal activity against medically important fungal strain. The antifungal activity was determined in the extracts using agar disc diffusion method. The antifungal activities of extract (187,5 mg/ml, 375 mg/ml and 750 mg/ml) of *Pandanus odoratissimus* were tested against three fungal strains, viz. *Aspergillus niger*, *Candida albicans* dan *Penicillium* sp. The result showed various inhibitory effect (7-9 mm). The largest inhibition zone was observed against *Candida albicans* (9 mm) with ethanol extract concentration 187,5 mg/ml, while extract concentration 375 mg/ml showed 8 mm inhibition zone against *Aspergillus niger* and *Penicillium* sp. The highest extract concentration (750 mg/ml) showed the lowest inhibition zone (7 mm) against three fungal strains tested. The phytochemical study showed the presence of alkaloids and tannins, which might be responsible for its good antifungal activity.

Keywords : *Pandanus odoratissimus* , *in vitro* antifungal activity, secondary metabolites

INTRODUCTION

Infectious diseases caused by bacteria, parasites, viruses and fungi are still a major threat to public health, despite the tremendous progress in human medicine. Their impact is particularly large in developing countries due to the relative unavailability of medicines and the emergence of widespread drug resistance (Okeke *et al*, 2005). Microorganisms have developed resistance to many antibiotics in the recent years, and this create clinical problem in the traetment of infectious diseases. Example of some microorganisms that gained resistance to antimicrobials, e.g *Escherichia coli*, *Pesudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* and *Candida albicans*. *Candida albicans* has become resistant to the already limited, toxic and expensive anti-*Candida* agents available in the market. The resistance has increased due to indiscriminate use of commercial antimicrobials drugs commonly used in the treatment of infection diseases. These factors necessitate the search for new ant-fungal agents. (Deborah *et al*, 2006).

Contarry to the synthetic drugs, antimicrobials of plant origin are not associated with side effects and have enormous therapeutic potential to heal many infection diseases (Iwu *et al*, 1999). Plant derived natural products may offer potential lead to new compound, which could act on those fungi.

Previous studies have shown the presence of several substances such as alkaloids, phenols, peptides compound in plant with potentially significant therapeutic application against human pathogen, including bacteria, virus and fungi (El Astal and Aera, 2005)

Medicinal properties of plants are normally dependent on the presence of some phytoconstituents such as alkaloids,

anthraquinones, cardiac glycosides, saponins, tannins and polyphenols which are the bioactive bases responsible for the antimicrobial activity (Ebana *et al*. 1993).

The antimicrobial compounds from plants may inhibit fungal growth by different mechanisms than those presently used antimicrobials and may have a significant clinical value in treatment of resistant microbial strains (Eloff , 1998).

Pandanus odoratissimus L.f (Pandanaceae) is a palm like small tree with fragrant flower found along the coast of Ujung Kulon, West Java, Indonesia. It is common on the sea shore forming a belt of dense, impenetrable vegetation above the high water mark. Leaves are glaucous-green, 2-4 mm long, ensiform, caudate-acuminate, coriaceous, with spines on the margins and on the midrib (Rahayu *et al*, 2012) (**Fig. 1**). Leaves, anthers, as well as roots are medicinally useful. The leaves are said to be valuable in leprosy, small pox, syphilis, scabies, leucoderma, diseases of heart and the brain, and as an aphrodisiac (Kirtikar and Basu, 1995). Chemical component analysis of the root part of *Pandanus odoratissimus* led to the isolation of two phenolic compounds, four lignan type compound, plus a new benzofuran derivative. Among them, pinoresinol and 3,4-bis (4-hidroxy-3methoxy benzyl) tetrahydro furan showed strong antioxidative activities (Ting-Ting and Shang Whang, 1998). In fact, there is lack of information on the distribution of the biological activity in different plant parts, especially on antifungal activities of the leaves. Therefore the present investigation reports the antifungal activities of leaves of *Pandanus odoratissimus* Linn.f.



Figure 1. Habit of *P. odoratissimus*



Figure 2 . Leaf and fruit of *P. odoratissimus*

MATERIALS AND METHODS

Collection of Plant Materials

The wild *Pandanus odoratissimus* sample for this work was collected from its coastal habitat. Fresh materials were taken from local areas of Ujung Kulon, West Java, Indonesia, and identified by the author (SER) in January 2010. The plant parts were carefully cleaned to remove any possible external contamination. They were dried, and deposited at Fakultas Biologi, Universitas Nasional.

Preparation of Plant Extract

Extraction

Leaves of *Pandanus odoratissimus* were subjected to mechanical grinding and powdered by electrical blender. 100 grams of this powder was soaked in 250 ml of ethanol for 48 Hrs. The contents were then filtered through Whatmann filter paper no. 1. The filtrate was

Whatmann filter paper no. 1. The filtrate was dried by using a rotary evaporator at 60°C. The dried extract was stored in sterile glass bottles at -4°C until use.

Phytochemical Screening of the plant extract

Phytochemical screening of active plant extracts was done by following the standard method of Harborne (1998), for the qualitative analysis of various phytochemical studies such as alkaloids, saponins, and tannins which could be responsible for antifungal activity (Results summarized in **Table 1**).

Test Microorganisms and Growth Media.

The following microorganisms

Fungal strains *Aspergillus niger*, *Candida albicans*, dan *Penicillium* sp were chosen based on their clinical and pharmacological importance. The fungal stock cultures were incubated for 24 hours at 37°C on potato dextrose agar (PDA), following refrigeration

storage at 4°C. The yeasts and molds were grown in Sabouraud dextrose agar and PDA media, respectively, at 28°C. The stock cultures were maintained at 4°C.

Antifungal activity

The antifungal studies of ethanolic extract of the leaves were carried out by the disc diffusion method. Antifungal activity was carried out against 24 h cultures of *Aspergillus niger*, *Candida albicans* and *Penicillium* sp. Sabouraud dextrose agar medium was prepared in plates as media for test fungi. Sterilized filter paper discs (Whatman No.1) with diameter 6 mm were impregnated with 20 µl of extract with concentration 187,5 mg/ml, 375 mg/ml and 750 mg/ml respectively. The fungal inoculum was spread evenly into surface of the Sabouraud dextrose agar plates using a sterilized cotton bud before the extract discs were positioned on the inoculated agar surface. Each extract was assayed in duplo. Sterile ethanol served as negative control and chloramphenicol served as positive control. All the plates were incubated for 6 days. The antifungal activity was interpreted from the size of the diameter zone inhibition measured to the nearest millimeter (mm) as observed from the clear zones surrounding the discs.

RESULTS

Preliminary phytochemical screening

The phytochemical screening demonstrated the presence of different types of compounds like alkaloids and tannins, which could be responsible for antifungal activities (Result summarized in **Table 1**). *Pandanus odoratissimus* (leaves) extract exhibited a positive reaction to Mayer's and Dragendorff's test for alkaloids, negative reactions to Foam test for saponin, and positive reaction to Ferric chloride test for tannins.

Table 1. Preliminary phytochemical screening of active plant leaves extract

Plant constituents/ phytochemicals and testing methods	Ethanolic extract
Alkaloids: Mayer's test Dragendorff's test	+ +
Saponins: Foam test	-
Tannins: Ferric chloride's test	+

Antifungal activity

The result of antifungal activity of ethanolic extract of *Pandanus odoratissimus* by disc diffusion method revealed that the extract showed moderate activity against three fungal strain tested, viz. *Aspergillus niger*, *Candida albicans* and *Penicillium* sp. As shown in **Fig. 3**. The result showed various inhibitory effect (7-9 mm) (Figure 3). The largest inhibition zone was observed against *Candida albicans* (9 mm) with ethanol extract concentration 187,5 mg/ml, while extract concentration 375 mg/ml showed 8 mm inhibition zone against *Aspergillus niger* and *Penicillium* sp. The highest extract concentration (750 mg/ml) showed the lowest inhibition zone (7 mm) against three fungal strains tested.

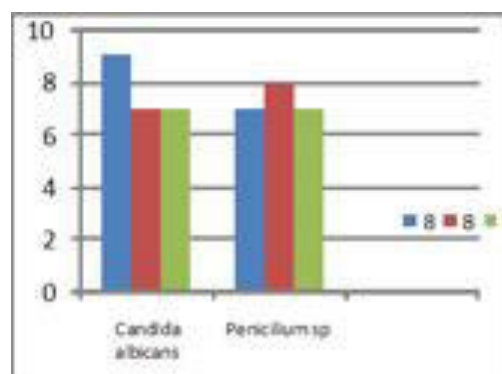


Figure 3. *In vitro* Antifungal Assay

DISCUSSION

Plant are important source for development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antifungal activity assay (Tona *et al.* 1998). Many reports are available on the antiviral, antibacterial; anthelmintic, anti-inflammatory and antifungal (Palombo and Semple, 20010). Some of these observations have helped in identifying the active principle responsible for such activities and in developing the drugs for the therapeutics use in human beings .

The increased frequency of resistance to commonly used antibiotics led to the search for newer, effective, cheap and easily affordable drugs in the management of infectious diseases. Although currently available synthetic drugs are popular, however, herbal medicine continued to be practiced due to richness of certain plants in varieties of secondary metabolites such as alkaloids, flavonoids ,saponin, tannins, and terpenoid which have been reported to have potent antifungal activities (Cowan, 1999).

In the present study, preliminary phytochemical investigation of *P. odoratissimus* leaves revealed the presence of alkaloids and tannins in ethanolic extract (**Table 1**). Alkaloids are potent inhibition of various oxidative processes both *in vitro* and *in vivo*, while tannins evolved in plant as a defense against microbial attack (Bandaranayake, 2002).

The antifungal potency of ethanolic extract was determined by agar disc diffusion method. . The antifungal activities of extract (187,5 mg/ml, 375 mg/ml and 750 mg/ml) of *Pandanus odoratissimus* were tested against three fungal strains, viz. *Aspergillus niger*, *Candida albicans* dan *Penicillium* sp. The result showed various inhibitory effect (7-9 mm) (**Fig. 3**). The largest inhibition zone was observed against *Candida albicans* (9 mm) with ethanol extract concentration 187,5

mg/ml, while extract concentration 375 mg/ml showed 8 mm inhibition zone against *Aspergillus niger* and *Penicillium* sp. The highest extract concentration (750 mg/ml) showed the lowest inhibition zone (7 mm) against three fungal strains tested. The difference rate of inhibition activities appear to be directly related to the qualitative and quantitative diversity of the compound that are being accumulated by the plants as its accumulate secondary chemical, which it will reduce their nutritional quality or make them toxic to potential competitors or parasites (Cotton, 1996). The difference in potency may also due to the stage collection of the sample, sensitivity of the fungal test and also method of extraction. These fungi may also have some kind resistance mechanism, e.g enzymatic inactivation, target sites modification, and decrease intracellular drug accumulation (Schwarz and Noble, 1999).

Fig. 3 illustrate that antifungal activity of *Pandanus odoratissimus* ethanol leaf showed strongest inhibition activity against *Candida albicans* giving 9 mm zone of inhibition. Despite what many researchers have reported that *Candida albicans* are very resistant fungi, this work demonstrate that ethanol leaf extract of *Pandanus odoratissimus* is effective against this pathogenic fungi. It is very clear that these extracts possess compound with antifungal properties that can be used as antifungal agents in new drugs for the therapy against pathogenic microorganisms.

The phytochemical study showed the presence of alkaloids and tannins in the extract, which might be a reason for the good activity of ethanol extract. This extract can be subjected to isolation of the therapeutic antifungal and carry out further pharmacological evaluation.

However, this is a preliminary work and more work is needed to determine the active in these extract which may help in improving management of the different infectious diseases

that are developing resistance to commonly used antibiotics.

CONCLUSION

Based on our results, it can be concluded that ethanol leaf extracts of *Pandanus odoratissimus* possess significant antifungal activity against *Aspergillus niger*, *Candida albicans* and *Penicillium* sp.

The presence of phytoconstituents make the plant useful for treating different ailment and have a potential of providing useful drugs of human use.

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APPLICATION OF ORGANIC FERTILIZER AND NPK TO INCREASE SWEET CORN PRODUCTION IN MALANG EAST JAVA

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ABSTRACT

Fertilization is one of the important factors in improving the productivity of maize. In corn cultivation, required the addition of organic and inorganic fertilizers rationally to obtaining high yield production optimally. Inorganic fertilization that done irrationally lead to the depletion of some nutrients in the soil quickly. Such conditions can lead to a decline in soil fertility The research aimed to obtain the optimal dose of organic fertilizer with NPK fertilizer application. The study was conducted in the village of Malang regency Karangploso Ngenep District from October 12 until December 20, used Split Plot Design with 3 replications. Main plot is organic fertilizer and subplot dosage of NPK fertilizer. Organic fertilizer consists of 4 levels: A0 (without organic fertilizer), A1 (2.5 tons/ha organic fertilizer), A2 (5 tons/ha organic fertilizer), and A3 (7.5 tons/ha organic fertilizer) whereas subplot consisted of: N1 (100 kgs/ha NPK), N2 (200 kgs/ha NPK), N2 (300 kgs/ha NPK) and N4 (400 kgs/ha NPK). Growth and production data were statistically processed by the F test, if there is significance followed by Duncan test (DMRT) at 5% level. Variables measured include plant height, leaf number, cob corn diameter, length of cob corn, number of rows per cob and corn yields. Research shows that the use of organic fertilizers and NPK affect plant height and number of leaves at 60 and 75 HST observations, while at the beginning of the growth of invisible influence. Significant effect on length of cob corn but had no significant effect on the diameter and the number of rows per ear. Fertilization is recommended to 5 tons/ha organic fertilizer and 300 kg/ha of NPK with production of 11.56 tons / ha.

INTRODUCTION

Fertilization is one of the important factors in improving the productivity of maize. In corn cultivation is required the addition of organic and inorganic fertilizers rationally to obtaining high yield production optimally. Inorganic fertilization that done irrationally lead to

the depletion of some nutrients in the soil quickly. Such conditions can lead to a decline in soil fertility (Tisdale et al., 1985; Karama, 1994).

To obtain optimal production of maize required the use of organic and inorganic fertilizers rationally according to the needs of the plant. This is because nearly 100% of agricultural land in Java

in general have very low organic matter content, which is less than 1%. Liebig's law refers to the minimum, any type of fertilizer that is added will be less useful when the content of C-organic is not enhanced (Karama, 1994; Suyamto *et al.*, 2002; Suratmini, 2012; Suyamto, 2010).

Organic matter can improve soil fertility and have an important role in improving the physical properties of the soil. Organic matter can improve soil aggregation, improve aeration and percolation, as well as create a better crumb structure of the soil and easily processed. The application of organic matter have a significant effect on the movement and nutrient leaching (Subowo *et al.*, 1990).

Fertilizer recommendation for optimal production on sweet corn (corn hybrids) are 400 kgs Urea, 125 kgs SP-36 and 100 kgs KCl. This means that the nutrient needs of corn at 180 kgs N, 45 kgs P₂O₅ and 35 kgs K₂O equivalent to 300 kgs/ha of NPK (15:15:15) (Suwono *et al.*, 2009). In this research, organic fertilizer was applied at a dose of up to 5 tons/ha, whereas NPK fertilizer was applied

starting dosage 100 kgs/ha to recommended dosage, 400 kgs/ha. The objective was to obtain a dosage of NPK Top Land 29 and organic fertilizer for production optimally.

MATERIALS AND METHODS

The study was conducted in the village of Ngenep, Karangploso Malang Regency East Java Indonesia from October to December 2012, using a Split Plot design with 3 replications. Organic fertilizer dosage as mainplot whereas NPK Top land 29 as sub plot (Table 1). Organic fertilizer consists of 4 levels: A0 (without organic fertilizer), A1 (2.5 tons/ha organic fertilizer), A2 (5 tons/ha organic fertilizer), and A3 (7.5 tons/ha organic fertilizer) whereas subplot consisted on 4 levels were: N1 (1/4 of dosage recommendation, 100 kgs/ha NPK), N2 (2/4 dosage recommendation, 200 kgs/ha NPK), N3 (3/4 dosage recommendation, 300 kgs/ha NPK) and N4 (dosage recommendation, 400 kgs/ha NPK).

Table 1. The Treatment of Organic Fertilizer and NPK Top Land 29, 2013

Treatment	Treatment			
	Main Plot Organic Fertilizer (tons/ha)	Sub Plot		
		Urea (kgs/ha)		NPK Top Land 29 (kgs/ha)
A0 N1	0	400	100	1/3 dosage recommendation
N2	0	400	200	2/3 dosage recommendation
N3	0	400	300	dosage recommendation
N4	0	400	400	4/3 dosage recommendation
A1 N1	2,5	400	100	1/3 dosage recommendation
N2	2,5	400	200	2/3 dosage recommendation
N3	2,5	400	300	dosage recommendation
N4	2,5	400	400	4/3 dosage recommendation
A2 N1	5	400	100	1/3 dosage recommendation
N2	5	400	200	2/3 dosage recommendation
N3	5	400	300	dosage recommendation
N4	5	400	400	4/3 dosage recommendation
A3 N1	7,5	400	100	1/3 dosage recommendation
N2	7,5	400	200	2/3 dosage recommendation
N3	7,5	400	300	dosage recommendation
N4	7,5	400	400	4/3 dosage recommendation

NPK Fertilizer Top Land 29 has nutrient content of N-total of 10.77%, 11.05% P₂O₅, K₂O 13.56%, 13.19% CaO, 0.29% MgO and 0.02% Zn. Organic fertilizers goat manure are used in this study

The variables were measured on plant height, leaf number, corncob diameter, corncob length, the number of rows per corncob, corncob yield with husk, and corncob yield without husk.

Research implementation in the field:

- The tillage by hoe and then made a raised bed with a seedbeds ("bedengan") size 4 m x 1 m.
- Seeds cultivated in nursery beds for 10 days. At that time high seed approximately 10-12 cm.
- spacing of 70 cms x 20 cms and planted one seed per hole.
- The distance between the plot of 40-50 cm with a channel depth of 40 cm.
- Organic fertilizers applied to the soil before planting
- NPK fertilizer and urea given at 7 days and 25 days after planting, respectively ½ dose of fertilizer in accordance with the treatment.
- Urea was given 400 kgs/ha
- 'Pembumbunan' after the second application of fertilizer, at the age of 25 days after planting (dap) and repeated it in accordance with the conditions in the field.
- Harvest when the plant was 70 days after planting.

Data Analysis

The F-test was conducted to determine plant growth and yield, if there is a significant effect will be continued by Duncan test (DMRT) at α 5%. (Gomez *et.al.*, 1993).

RESULTS AND DISCUSSIONS

Results of soil analysis by PUTS (Rice Soil Test Kit) shows that soil

fertility: N was low, P was moderate and K was low while organic matter was very low. In fields where research is done, the local farmers rarely apply organic materials.

Vegetative components

Observations of plant height components performed 3 times during the plant growth, at age 40, 55, and 70 days after planting. From the analysis of the plant height at age 40 HST has not shown any effect of treatment. On observations of 55 and 70 day after planting there is an interaction effect of organic fertilizer and NPK Land Top 29 (Table 2).

Observations of plant height at age 55 after planting was highest shown in treatment 5 t/ha, 7.5 t/ha and 10 t/ha with the addition of 400 kgs NPK / ha are 140.8 cm 143.7 cm, and 157, 2 cm. Plant height of the lowest in the treatment without the addition of organic fertilizer at application of NPK 100 kgs/ha and 200 kgs/ha.

Table 2. The Interaction Effect of Organic Fertilizer and NPK on the Number of Leaves at 70 dap and Plant Height at 55 and 70 dap, 2013.

No	Treatment	Number of leaves		Plant height (cms)
		70 dap	55 dap	70 dap
1	A0 N1	12.33 a	116,7 g	160.67 d
2	N2	12.50 a	121,3 g	169.83 c
3	N3	12.50 a	127,5 f	169.67 c
4	N4	13.00 a	131,7 ef	174.17 c
5	A1 N1	13.00 a	133,2 def	184.67 b
6	N2	13.33 a	133.0 def	185.50 b
7	N3	13.33 a	139.7 bc	186.50 b
8	N4	13.33 a	140.8 b	186.17 b
9	A2 N1	12.67 a	130.5 ef	184.33 b
10	N2	13.00 a	132.0 ef	184.67 b
11	N3	13.33 a	138.2 bcd	197.83 a
12	N4	13.33 a	157,2 a	197.83 a
13	A3 N1	12.67 a	133,3 def	196.67 a
14	N2	12.83 a	133,8 de	197.33 a
15	N3	13.17 a	134,7 cde	197.67 a
16	N4	13.17 a	143.7 b	197.83 a
CV (%)		6.72	12.37	12.06

Notes : Figures are accompanied by the same alphabet in each column indicate no significant differences in DMRT (at the level of 5%).

Productions are very influenced by vegetative growth factor. With optimal plant growth will expected to produce in an optimal harvest.

Diameter of Corncob, Number of Rows per Corncob, Corncob Length, Corn Yields with Husk and Corn Yields Without Husk

Harvesting was done after the plant was 70 days after planting. Results of data analysis on generative plant observations indicate that the interaction between organic fertilizer and NPK no real effect on the diameter and the number of rows per corncob but significant effect showed on corncob length and corn yield with husk and corn yields without husk (Table 3).

Table 3. Interaction Effect of Organic Fertilizer and NPK Top Land 29 at Corncob length, Corn Yields with Husk and Corn Yield without Husk, 2013

No	Treatment	Corncob Length (cms)	Corn Yields with husk (tons/ha)	Corn Yields without husk (tons/ha)
1	A0 N1	17.4 e	11.67 e	9.14 e
2	N2	18.9 cd	11.70 e	9.16 e
3	N3	19.7 ab	11.71 e	9.17 c
4	N4	19.7 ab	11.71 e	10.17 c
5	A1 N1	19.0 c	11.71 e	9.37 e
6	N2	18.7 d	12.13 d	9.71 d
7	N3	19.5 b	12.16 d	10.00 c
8	N4	19.5 b	12.53 c	10.03 c
9	A2 N1	19.8 a	12.62 bc	11.19 b
10	N2	19.9 a	12.82 c	11.19 b
11	N3	19.9 a	13.97 a	11.56 a
12	N4	19.9 a	13.97 a	11.58 a
13	A3 N1	19.9 a	13.98 a	11.58 a
14	N2	19.9 a	13.98 a	11.58 a
15	N3	20.0 a	13.98 a	11.58 a
16	N4	20.0 a	13.99 a	11.59 a
<i>CV (%)</i>		8.6	7.2	9.5

Notes : Figures are accompanied by the same alphabet in each column indicate no significant differences in DMRT (at the level of 5%).

The highest cob length on the treatment of 2.5 tonnes / ha with the application of organic fertilizer 400 kg / ha NPK although not significantly different by treatment with 5 ton / ha and 7.5 t / ha at all dose levels of NPK treatment, but dose N4 has higher tendency cob length, while the lowest in the treatment without organic fertilizer at all dose levels of NPK treatment. This is in accordance with the opinion reported by Sukristiyonubowo (2007) in rice plants where the combination of NPK and organic fertilizer can increase yield components and yield.

The Financial Analysis Of Sweet Corn Crop Production

Farmers usually sell his sweet corn production to middlemen in the form of sweet corn with husk intended to reduce the risk of yield loss due to mechanical damage and avoid excessive moisture loss during transport and storage. Sales sweet corn to consumers have two (2) types are sold sweet corn with and without husk . Sales of sweet corn with klobot especially for corn grilled whereas sweet corn without husk for household consumption as a vegetable. In the study, in general, farmers sell sweet corn without husk.

Sweet corn farming financial analysis was taking into account the price of corn without klobot (Table 4).

Table 4. Financial Analysis on Sweet Corn, 2013

Treatment	Cost rent fields (IDR/ha)	Cost of labor and production facilities (IDR/ha)	Total Biaya Produksi (IDR/ha)	Corn Yields (kgs/ha)	Price of corn without husk (IDR/kgs)	Benefit (IDR)	R/C
A0 N1	2.000.000	11,720,000	13.720.000	9.140	4.000	15700000	1.34
N2	2.000.000	11,670,000	11.670.000	9.160	4.000	15810000	1.35
N3	2.000.000	11,870,000	11.870.000	9.170	4.000	15640000	1.32
N4	2.000.000	12,070,000	12.070.000	10.170	4.000	16040000	1.33
A1 N1	2.000.000	12,720,000	12.720.000	9.370	4.000	15390000	1.21
N2	2.000.000	12,920,000	12.920.000	9.710	4.000	16210000	1.26
N3	2.000.000	13,120,000	13.120.000	10.000	4.000	16880000	1.26
N4	2.000.000	13,320,000	13.320.000	10.030	4.000	16770000	1.36
A2 N1	2.000.000	14,220,000	16.220.000	11.190	4.000	19350000	1.31
N2	2.000.000	14,520,000	16.520.000	11.190	4.000	19050000	1.31
N3	2.000.000	14,620,000	16.470.000	11.560	4.000	20060000	1.37
N4	2.000.000	14,470,000	16.470.000	11.580	4.000	20270000	1.40
A3 N1	2.000.000	15,220,000	17.220.000	11.580	4.000	19520000	1.28
N2	2.000.000	15,420,000	17.420.000	11.580	4.000	19320000	1.25
N3	2.000.000	15,620,000	17.620.000	11.580	4.000	19120000	1.22
N4	2.000.000	15,820,000	15.820.000	11.590	4.000	18950000	1.20

Notes: Sweet corn farming financial analysis for scale farm farming per hectare; price of Rp 4.000/kgs.

Finally, base on economy analysis showed that sweet corn farming using a 5 ton / ha organic fertilizer plus urea 400 kgs/ha and NPK Top Land 29 at 400 kgs/ha (4/3 dosage recommendation) is most benefit , its showed from the R/C ratio whose value is > 1, which is 1.40, corn yield reached 11.56 tons/ha.

CONCLUSIONS

1. Results of research showed that there are significant interactions between organic and NPK fertilizer on plant height at the observation variables 55 and 70 dap, cob length, and the yields of corn and no real effect on the number of leaves, the cob diameter and number of rows per cob.
2. This research suggest that sweet corn farming system using 5 tons/ha organic fertilizer plus NPK Top Land 29 400 kgs/ha (4/3 dose recommendation), corn yield reached 11.56 tons/ha. R/C ratio of 1,40.

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APPLICATION OF ORGANIC FERTILIZER AND NPK TO INCREASE SWEET CORN PRODUCTION IN MALANG EAST JAVA

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ABSTRACT

Fertilization is one of the important factors in improving the productivity of maize. In corn cultivation, required the addition of organic and inorganic fertilizers rationally to obtaining high yield production optimally. Inorganic fertilization that done irrationally lead to the depletion of some nutrients in the soil quickly. Such conditions can lead to a decline in soil fertility The research aimed to obtain the optimal dose of organic fertilizer with NPK fertilizer application. The study was conducted in the village of Malang regency Karangploso Ngenep District from October 12 until December 20, used Split Plot Design with 3 replications. Main plot is organic fertilizer and subplot dosage of NPK fertilizer. Organic fertilizer consists of 4 levels: A0 (without organic fertilizer), A1 (2.5 tons/ha organic fertilizer), A2 (5 tons/ha organic fertilizer), and A3 (7.5 tons/ha organic fertilizer) whereas subplot consisted of: N1 (100 kgs/ha NPK), N2 (200 kgs/ha NPK), N2 (300 kgs/ha NPK) and N4 (400 kgs/ha NPK). Growth and production data were statistically processed by the F test, if there is significance followed by Duncan test (DMRT) at 5% level. Variables measured include plant height, leaf number, cob corn diameter, length of cob corn, number of rows per cob and corn yields. Research shows that the use of organic fertilizers and NPK affect plant height and number of leaves at 60 and 75 HST observations, while at the beginning of the growth of invisible influence. Significant effect on length of cob corn but had no significant effect on the diameter and the number of rows per ear. Fertilization is recommended to 5 tons/ha organic fertilizer and 300 kg/ha of NPK with production of 11.56 tons / ha.

INTRODUCTION

Fertilization is one of the important factors in improving the productivity of maize. In corn cultivation is required the addition of organic and inorganic fertilizers rationally to obtaining high yield production optimally. Inorganic fertilization that done irrationally lead to

the depletion of some nutrients in the soil quickly. Such conditions can lead to a decline in soil fertility (Tisdale et al., 1985; Karama, 1994).

To obtain optimal production of maize required the use of organic and inorganic fertilizers rationally according to the needs of the plant. This is because nearly 100% of agricultural land in Java

in general have very low organic matter content, which is less than 1%. Liebig's law refers to the minimum, any type of fertilizer that is added will be less useful when the content of C-organic is not enhanced (Karama, 1994; Suyamto *et al.*, 2002; Suratmini, 2012; Suyamto, 2010).

Organic matter can improve soil fertility and have an important role in improving the physical properties of the soil. Organic matter can improve soil aggregation, improve aeration and percolation, as well as create a better crumb structure of the soil and easily processed. The application of organic matter have a significant effect on the movement and nutrient leaching (Subowo *et al.*, 1990).

Fertilizer recommendation for optimal production on sweet corn (corn hybrids) are 400 kgs Urea, 125 kgs SP-36 and 100 kgs KCl. This means that the nutrient needs of corn at 180 kgs N, 45 kgs P₂O₅ and 35 kgs K₂O equivalent to 300 kgs/ha of NPK (15:15:15) (Suwono *et al.*, 2009). In this research, organic fertilizer was applied at a dose of up to 5 tons/ha, whereas NPK fertilizer was applied

starting dosage 100 kgs/ha to recommended dosage, 400 kgs/ha. The objective was to obtain a dosage of NPK Top Land 29 and organic fertilizer for production optimally.

MATERIALS AND METHODS

The study was conducted in the village of Ngenep, Karangploso Malang Regency East Java Indonesia from October to December 2012, using a Split Plot design with 3 replications. Organic fertilizer dosage as mainplot whereas NPK Top land 29 as sub plot (Table 1). Organic fertilizer consists of 4 levels: A0 (without organic fertilizer), A1 (2.5 tons/ha organic fertilizer), A2 (5 tons/ha organic fertilizer), and A3 (7.5 tons/ha organic fertilizer) whereas subplot consisted on 4 levels were: N1 (1/4 of dosage recommendation, 100 kgs/ha NPK), N2 (2/4 dosage recommendation, 200 kgs/ha NPK), N3 (3/4 dosage recommendation, 300 kgs/ha NPK) and N4 (dosage recommendation, 400 kgs/ha NPK).

Table 1. The Treatment of Organic Fertilizer and NPK Top Land 29, 2013

Treatment	Treatment			
	Main Plot Organic Fertilizer (tons/ha)	Sub Plot		
		Urea (kgs/ha)		NPK Top Land 29 (kgs/ha)
A0 N1	0	400	100	1/3 dosage recommendation
N2	0	400	200	2/3 dosage recommendation
N3	0	400	300	dosage recommendation
N4	0	400	400	4/3 dosage recommendation
A1 N1	2,5	400	100	1/3 dosage recommendation
N2	2,5	400	200	2/3 dosage recommendation
N3	2,5	400	300	dosage recommendation
N4	2,5	400	400	4/3 dosage recommendation
A2 N1	5	400	100	1/3 dosage recommendation
N2	5	400	200	2/3 dosage recommendation
N3	5	400	300	dosage recommendation
N4	5	400	400	4/3 dosage recommendation
A3 N1	7,5	400	100	1/3 dosage recommendation
N2	7,5	400	200	2/3 dosage recommendation
N3	7,5	400	300	dosage recommendation
N4	7,5	400	400	4/3 dosage recommendation

NPK Fertilizer Top Land 29 has nutrient content of N-total of 10.77%, 11.05% P₂O₅, K₂O 13.56%, 13.19% CaO, 0.29% MgO and 0.02% Zn. Organic fertilizers goat manure are used in this study

The variables were measured on plant height, leaf number, corncob diameter, corncob length, the number of rows per corncob, corncob yield with husk, and corncob yield without husk.

Research implementation in the field:

- The tillage by hoe and then made a raised bed with a seedbeds ("bedengan") size 4 m x 1 m.
- Seeds cultivated in nursery beds for 10 days. At that time high seed approximately 10-12 cm.
- spacing of 70 cms x 20 cms and planted one seed per hole.
- The distance between the plot of 40-50 cm with a channel depth of 40 cm.
- Organic fertilizers applied to the soil before planting
- NPK fertilizer and urea given at 7 days and 25 days after planting, respectively ½ dose of fertilizer in accordance with the treatment.
- Urea was given 400 kgs/ha
- 'Pembumbunan' after the second application of fertilizer, at the age of 25 days after planting (dap) and repeated it in accordance with the conditions in the field.
- Harvest when the plant was 70 days after planting.

Data Analysis

The F-test was conducted to determine plant growth and yield, if there is a significant effect will be continued by Duncan test (DMRT) at α 5%. (Gomez *et.al.*, 1993).

RESULTS AND DISCUSSIONS

Results of soil analysis by PUTS (Rice Soil Test Kit) shows that soil

fertility: N was low, P was moderate and K was low while organic matter was very low. In fields where research is done, the local farmers rarely apply organic materials.

Vegetative components

Observations of plant height components performed 3 times during the plant growth, at age 40, 55, and 70 days after planting. From the analysis of the plant height at age 40 HST has not shown any effect of treatment. On observations of 55 and 70 day after planting there is an interaction effect of organic fertilizer and NPK Land Top 29 (Table 2).

Observations of plant height at age 55 after planting was highest shown in treatment 5 t/ha, 7.5 t/ha and 10 t/ha with the addition of 400 kgs NPK / ha are 140.8 cm 143.7 cm, and 157, 2 cm. Plant height of the lowest in the treatment without the addition of organic fertilizer at application of NPK 100 kgs/ha and 200 kgs/ha.

Table 2. The Interaction Effect of Organic Fertilizer and NPK on the Number of Leaves at 70 dap and Plant Height at 55 and 70 dap, 2013.

No	Treatment	Number of leaves		Plant height (cms)
		70 dap	55 dap	70 dap
1	A0 N1	12.33 a	116,7 g	160.67 d
2	N2	12.50 a	121,3 g	169.83 c
3	N3	12.50 a	127,5 f	169.67 c
4	N4	13.00 a	131,7 ef	174.17 c
5	A1 N1	13.00 a	133,2 def	184.67 b
6	N2	13.33 a	133.0 def	185.50 b
7	N3	13.33 a	139.7 bc	186.50 b
8	N4	13.33 a	140.8 b	186.17 b
9	A2 N1	12.67 a	130.5 ef	184.33 b
10	N2	13.00 a	132.0 ef	184.67 b
11	N3	13.33 a	138.2 bcd	197.83 a
12	N4	13.33 a	157,2 a	197.83 a
13	A3 N1	12.67 a	133,3 def	196.67 a
14	N2	12.83 a	133,8 de	197.33 a
15	N3	13.17 a	134,7 cde	197.67 a
16	N4	13.17 a	143.7 b	197.83 a
CV (%)		6.72	12.37	12.06

Notes : Figures are accompanied by the same alphabet in each column indicate no significant differences in DMRT (at the level of 5%).

Productions are very influenced by vegetative growth factor. With optimal plant growth will expected to produce in an optimal harvest.

Diameter of Corncob, Number of Rows per Corncob, Corncob Length, Corn Yields with Husk and Corn Yields Without Husk

Harvesting was done after the plant was 70 days after planting. Results of data analysis on generative plant observations indicate that the interaction between organic fertilizer and NPK no real effect on the diameter and the number of rows per corncob but significant effect showed on corncob length and corn yield with husk and corn yields without husk (Table 3).

Table 3. Interaction Effect of Organic Fertilizer and NPK Top Land 29 at Corncob length, Corn Yields with Husk and Corn Yield without Husk, 2013

No	Treatment	Corncob Length (cms)	Corn Yields with husk (tons/ha)	Corn Yields without husk (tons/ha)
1	A0 N1	17.4 e	11.67 e	9.14 e
2	N2	18.9 cd	11.70 e	9.16 e
3	N3	19.7 ab	11.71 e	9.17 c
4	N4	19.7 ab	11.71 e	10.17 c
5	A1 N1	19.0 c	11.71 e	9.37 e
6	N2	18.7 d	12.13 d	9.71 d
7	N3	19.5 b	12.16 d	10.00 c
8	N4	19.5 b	12.53 c	10.03 c
9	A2 N1	19.8 a	12.62 bc	11.19 b
10	N2	19.9 a	12.82 c	11.19 b
11	N3	19.9 a	13.97 a	11.56 a
12	N4	19.9 a	13.97 a	11.58 a
13	A3 N1	19.9 a	13.98 a	11.58 a
14	N2	19.9 a	13.98 a	11.58 a
15	N3	20.0 a	13.98 a	11.58 a
16	N4	20.0 a	13.99 a	11.59 a
<i>CV (%)</i>		8.6	7.2	9.5

Notes : Figures are accompanied by the same alphabet in each column indicate no significant differences in DMRT (at the level of 5%).

The highest cob length on the treatment of 2.5 tonnes / ha with the application of organic fertilizer 400 kg / ha NPK although not significantly different by treatment with 5 ton / ha and 7.5 t / ha at all dose levels of NPK treatment, but dose N4 has higher tendency cob length, while the lowest in the treatment without organic fertilizer at all dose levels of NPK treatment. This is in accordance with the opinion reported by Sukristiyonubowo (2007) in rice plants where the combination of NPK and organic fertilizer can increase yield components and yield.

The Financial Analysis Of Sweet Corn Crop Production

Farmers usually sell his sweet corn production to middlemen in the form of sweet corn with husk intended to reduce the risk of yield loss due to mechanical damage and avoid excessive moisture loss during transport and storage. Sales sweet corn to consumers have two (2) types are sold sweet corn with and without husk . Sales of sweet corn with klobot especially for corn grilled whereas sweet corn without husk for household consumption as a vegetable. In the study, in general, farmers sell sweet corn without husk.

Sweet corn farming financial analysis was taking into account the price of corn without klobot (Table 4).

Table 4. Financial Analysis on Sweet Corn, 2013

Treatment	Cost rent fields (IDR/ha)	Cost of labor and production facilities (IDR/ha)	Total Biaya Produksi (IDR/ha)	Corn Yields (kgs/ha)	Price of corn without husk (IDR/kgs)	Benefit (IDR)	R/C
A0 N1	2.000.000	11,720,000	13.720.000	9.140	4.000	15700000	1.34
N2	2.000.000	11,670,000	11.670.000	9.160	4.000	15810000	1.35
N3	2.000.000	11,870,000	11.870.000	9.170	4.000	15640000	1.32
N4	2.000.000	12,070,000	12.070.000	10.170	4.000	16040000	1.33
A1 N1	2.000.000	12,720,000	12.720.000	9.370	4.000	15390000	1.21
N2	2.000.000	12,920,000	12.920.000	9.710	4.000	16210000	1.26
N3	2.000.000	13,120,000	13.120.000	10.000	4.000	16880000	1.26
N4	2.000.000	13,320,000	13.320.000	10.030	4.000	16770000	1.36
A2 N1	2.000.000	14,220,000	16.220.000	11.190	4.000	19350000	1.31
N2	2.000.000	14,520,000	16.520.000	11.190	4.000	19050000	1.31
N3	2.000.000	14,620,000	16.470.000	11.560	4.000	20060000	1.37
N4	2.000.000	14,470,000	16.470.000	11.580	4.000	20270000	1.40
A3 N1	2.000.000	15,220,000	17.220.000	11.580	4.000	19520000	1.28
N2	2.000.000	15,420,000	17.420.000	11.580	4.000	19320000	1.25
N3	2.000.000	15,620,000	17.620.000	11.580	4.000	19120000	1.22
N4	2.000.000	15,820,000	15.820.000	11.590	4.000	18950000	1.20

Notes: Sweet corn farming financial analysis for scale farm farming per hectare; price of Rp 4.000/kgs.

Finally, base on economy analysis showed that sweet corn farming using a 5 ton / ha organic fertilizer plus urea 400 kgs/ha and NPK Top Land 29 at 400 kgs/ha (4/3 dosage recommendation) is most benefit , its showed from the R/C ratio whose value is > 1, which is 1.40, corn yield reached 11.56 tons/ha.

CONCLUSIONS

1. Results of research showed that there are significant interactions between organic and NPK fertilizer on plant height at the observation variables 55 and 70 dap, cob length, and the yields of corn and no real effect on the number of leaves, the cob diameter and number of rows per cob.
2. This research suggest that sweet corn farming system using 5 tons/ha organic fertilizer plus NPK Top Land 29 400 kgs/ha (4/3 dose recommendation), corn yield reached 11.56 tons/ha. R/C ratio of 1,40.

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STUDY OF LIQUID ORGANIC FERTILIZER FROM URINE OF RABBIT AND UREA ON GROWTH AND PRODUCTION OF MUSTARD (*Brassica juncea* L)

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ABSTRACT

Assessment carried out in Practice Land National Animal Husbandry Training Center Batu City, East Java from February till March 2013. Assessment using a Randomized Block Design, with 7 treatment was repeated four times, using urea fertilizer and Organic Liquid Fertilizer (POC) Rabbit. Objectives of the assessment were to: (a). Determine the effect of urea fertilizer and POC rabbits on growth and yield of mustard plants; (b) To determine the optimal dose of POC with results equivalent to the use of urea fertilizer. Use of Urea respectively 5 g, 10 g, 15 g, and the use of urine to the composition of 100 ml, 200 ml and 300 ml: A (control, without fertilization), B (Urea 5 gram / polybag), C (Urea 10 grams / polybag), D (Urea 15 g / polybag), E (POC rabbit 100 ml / polybag), F (POC rabbit 200 ml / polybag), G (POC rabbit 300 ml / polybag). Mustard plant used as an plant indicator. Planting in polybags of 40 x 20 cm with planting in soil and compost with a ratio of 1:1. Soil and POC rabbit were analyzed in Department of Chemistry Soil Brawijaya University of Malang. Variables measured were: plant height (cm) and number of leaves (were observed every two weeks till harvest), plant canopy, root- length (cm) and yield (grams). Data were analyzed with F-test, If there are differences between the treatment done further test Duncan's Multiple Range Test ($\alpha = 5\%$). Study showed that the highest fresh weight not significantly different in the treatment of urea fertilizer in treatment B (1003.3 grams), C (1006.7 grams), D (1008.3 grams), while treatment with POC produce wet weight lower and not significantly different for all treatment that is E (616.7 grams), F (620 grams), G (621.7 grams). The results of the financial analysis on treatment B urea (urea 5 grams/polybag) R/C ratio is the highest (3.68), while the analysis of the use of POC in E treatment produced R/C the highest (2,19).

Keywords: *mustard, urea, POC Rabbit, Batu city*

INTRODUCTION

Farming system by using chemical fertilizer continuously can cause the degradation of the quality of soil so that organic fertilizer is needed to recover the farming farm. According to the government regulation of Republic of Indonesia No.70 /Permentan/SR.140/10/2011, organic fertilizer derives from dead vegetations, organic or inorganic waste and waste of cattle which fermented in both of solid or liquid process and added microbe also mineral during the process of fermentation. Lingga (1995) stated that the finest structure of soil can support the growth of root to penetrate the soil through the pore of land to absorb the water, nutrients and minerals in the soil. Rabbit's urine as organic fertilizer contains N 4%, P₂O₅ 2.8%, K₂O 1,2% are relative higher than cow's urine fertilizer(N 1.21%, P₂O₅ 0.65%, K₂O 1.6%) and goat's urine fertilizer contains are N 1.47%, P₂O₅ 0.05%, K₂O 1.96% (Balittanah, 2006).

Some results of researches showed that the using of rabbit's urine as organic fertilizer has proved good crops of vegetables, cabbages, string beans, red beans, mustards, potatoes and strawberries. Noor et. al (1996) stated that using rabbit's waste in assorted vegetables in South Sulawesi could increase the productivity of baby corns 2,1 %, cabbages 11,8%, string beans 12,5%, red beans 22,7% and potatoes 5,5%. Research of Mappanganro et. al (2011) at strawberries showed that the using of rabbit's urine as organic fertilizer could give the best productivity and also during the growing period among organic liquid fertilizer of cattle, goat, and chicken waste. Vimala et al (2010) stated that the organic farming system on mustards could produce 8-15 tons/hectare fresh weigh.

Mustard plants can grow well on low land and upland, 5 – 1200 m above sea level. And

the temperature 4-35 C, the precipitation 300-350 mm during the plant growing period, the minimum depth of the soil 25 cms, texture of the soil is good, the pH of soil 5,2-8,2 with maximum pH 6.0- 7.0 (Jaenuddin et. al. , 2000). Mustard caisim could be harvested after 30 – 35 days after seeding with potential of productivity 20-25 ton/hectare. The leaves are oval, thick rather round shape, least fibrous, straight, and the color of the leaves is green and the stalk is light green.

MATERIALS AND METHODS

Assessment carried out in Practice Land National Animal Husbandry Training Center Batu City, East Java from February until March 2013. Assessment using a Randomized Block Design, with 7 treatment was repeated four times, using urea fertilizer and Organic Liquid Fertilizer (POC) Rabbit. Objectives of the assessment were to: (a). Determine the effect of urea fertilizer and POC rabbits on growth and yield of mustard plants; (b) To determine the optimal dose of POC with results equivalent to the use of urea fertilizer. Use of Urea respectively 5 g, 10 g, 15 g, and the use of urine to the composition of 100 ml, 200 ml and 300 ml: A (control, without fertilization), B (Urea 5 gram/ polybag), C (Urea 10 gram/polybag, D (Urea 15 gram/polybag), E (POC 100 ml/polybag), F (POC 200 ml/polybag), G (POC 300 ml/polybag). Mustard plant used as an plant indicator. Planting in polybag of 40 x 20 cm with planting in soil and compost with a ratio of 1:1. Soil and POC rabbit were analyzed in Department of Chemistry Soil Brawijaya University of Malang. Variables measured were: plant height (cm) and number of leaves (were observed every two weeks till harvest), plant canopy, root- length (cm) and yield (grams) (**Table 1.**)

Table 1. Treatment of POC and urea fertilizer

No	Treatment	Urea		POC	
		(gram/polybag)		(ml/polybag)	
		hst, DAP			
		7	14	7	14
1	A	0	0	0	0
2	B	2.5	2.5	0	0
3	C	5.0	5.0	0	0
4	D	7.5	7.5	0	0
5	E	0	0	50	50
6	F	0	0	100	100
7	G	0	0	150	150

Organic liquid fertilizer was made from rabbit's urine fermentation, called POC, contains pH 6,9 C-Organic 1.19 % and total of N 12,33%. Macro nutrient P, K, Ca and Mg each consists of 0.04 %, 0.06%, 0.55%, 0.26 % and 0.04 and micro Fe 1,28; Mn 13,20 (Table 2).

Table 2. Analysis Results of POC

No	Component of analysis	Results
1	pH	6,9
2	C Organic	1,19
3	N Total	12,33
4	P	0,04
5	K	0,60
6	Na	0,55
7	Ca	0,26
8	Mg	0,04
9	Fe	1,28
10	Mn	13,20

Resource : Department of chemistry soil of Brawijaya University in Malang, 2013

Variables measured were: plant height (cm) and number of leaves (were observed every two weeks till harvest), plant canopy, root- length (cm) and yield (grams). Data were analyzed with F-test and testing by DMRT/Duncan's Multiple Range Test ($\alpha = 5\%$).(Gomez and Gomez, 1993).

RESULTS

1. Results of soil Analysis

Analysis of soil has done in Soil Chemistry Laboratory of the University of Brawijaya (Table 3) in which a neutral pH (pH 7.3 H₂O; 1N KCl pH 7.0) and high organic C (4.99%), N medium (0.48%), P medium (107.66%) and very high of K (15.3).

Table 3. Results of Soil Analysis, 2013

No	Component of Soil Analysis	Results	Criteria
1	pH H ₂ O	7.3	Neutral
2	pH KCl 1N	7.0	Neural
3	C Organics (%)	4.99	High
4	N Total (%)	0.48	Medium
5	C/N	10	Low
6	P Olsen (mg kg ⁻¹)	107.55	Medium
7	K (cmol (+) kg ⁻¹)	15.3	Very high
8	Na (cmol (+) kg ⁻¹)	8.3	Very high
9	Ca (cmol (+) kg ⁻¹)	16.3	High
10	Mg (cmol (+) kg ⁻¹)	1	Low
11	KTK (cmol (+) kg ⁻¹)	41.7	Very high
12	Texture	Clay	
13	Sand	29	
14	Dust	47	
15	Clay	24	
16	Levels of base saturation	40.9	

Resource : Department of chemistry soil of Brawijaya University in Malang, 2013

2. Agronomic Component

The effect of treatment on height of plants and the number of leaves were observed for 4 times, 14 DAP, 21 DAP, 28 DAP ,35 DAP respectively after planting,(Table 4).

Table 4. The results of data analysis of plants height

Treatment	Plant height (DAP, cm)			
	14	21	28	35
A	7,83 a	17,10 ab	13,03 a	20,1 b
B	7,33 a	19,90 a	18,5 a	27,3 a
C	7,27a	17,20 ab	22,0 a	27,6 a
D	7,00 a	18,00 ab	22,9 a	27,6 a
E	7,90 a	17,00 ab	22,0 a	24,9 ab
F	7,30 a	15,00 b	18,5 a	25,1 ab
G	7,20 a	17,20 ab	20,9 a	25,3 ab
CV (%)	8,9	13,48	32,39	12,15

Note: Figures in the same column followed by the same letter indicates no significant difference at LSD ($\alpha = 5\%$).

Table 5. The results of data analysis on number of leaves

Treatment	Number of leaves			
	14	21	28	35
A	5,5 a	11,0 b	12,2 a	15,2 a
B	6,0 a	13,0 a	15,0 a	17,8 a
C	5,7 a	12,0 ab	15,2 a	18,0 a
D	5,2 a	12,0 ab	15,3 a	19,3 a
E	6,2 a	10,8 b	15,3 a	18,2 a
F	6,2 a	9,5 c	15,2 a	18,3 a
G	5,5 a	9,7 c	15,2 a	18,5 a
CV (%)	13,31	5,65	18,19	14,14

Note: Figures in the same column followed by the same letter indicates no significant difference at LSD ($\alpha = 5\%$).

The observations were length of canopy, width of canopy, length of root and fresh weight at harvest (Table 6). The effect of dosage POC at height of plants variable was more lower than urea fertilizer treatment. The effect of dosage POC on 35 DAP showed that the treatments of E was 24,9 cm, F 25,1 cm and G 25.3 cm. While the effect of dosage urea fertilizer showed that the treatments of B was 27,3 cm, C 27,6 cm, and D 27,6 cm too. These treatments were more higher than control.

Table 6. The results of data analysis toward length of canopy, width of canopy, length of root and fresh weight at harvest

Treatment	Length of canopies (cm)	Width of canopies (cm)	Length of roots (cm)	Fresh weight
A	22,0 ab	19,0 ab	20,5 b	550,0 b
B	29,0 a	26,8 a	28,7 a	1003,3 a
C	18,5 b	17,2 b	23,6 ab	1006,7 a
D	27,7 ab	24,3 ab	23,2 ab	1008,3 a
E	28,2 ab	23,9 ab	28,6 a	616,7 b
F	18,3 b	16,5 b	28,1 a	620,0 b
G	23,0 ab	17,5 b	27,7 ab	621,7 b
CV	22,43	20,66	15,12	21,22

Note: The figures in the same column which are followed the same letters show that there are no significant different at LSD ($\alpha=5$)

Measuring of fresh weight carried out at harvest after 42 days after seeding. The fresh weight of plants measured to know the productivity of mustard plant.

3. Farming system Analysis

Results of farming system analysis shown in Table 7. Its shown that treatment B with the lowest use of urea dosage 5 grams reached R/C ratio maximum of 3.68. This is due to the use of factor inputs used lower compared with treatment of C and D. This means that every 1000 IDR, - the costs incurred would result in additional revenue of 3,680 IDR, -. For the analysis of the use of organic fertilizer obtained that the treatment E reached the highest R/C was 2.19. R/C ratio E was higher than the treatments of F and G were caused F and G treatment factor requiring input costs of production more higher than E treatment.

Table 7. Mustard Farming Financial Analysis

A 1	Description				Treatment						
	Input Production	Amount	Price	Total	A	B	C	D	E	F	G
X 1,000 IDR											
	Seed POC	6500	0.2	1,300	1,300	1,300	1,300	1,300	1,300	1,300	1,300
	A (control)										
	B	32.5	1.8	58.5		58,500					
	C	65	1.8	117			117				
	D	97.5	1.8	175.5				175.5			
	E	650	20	13,000					13,000		
	F	1300	20	26,000						26,000	
	G	1950	20	39,000							39,000
	Green Manure	500	0.3	150	150	150	150	150	150	150	150
	Total				1,450	1,508.5	1,567	1,625.5	14,450	27,450	40,450
2	Labor										
	Land Preparation	10	25	250	250	250	250	250	250	250	250
	Seedling	5	25	125	125	125	125	125	125	125	125
	Making seedlingbed and planting hole	10	25	250	250	250	250	250	250	250	250
	Planting	10	25	250	250	250	250	250	250	250	250
	Harvest	15	25	375	375	375	375	375	375	375	375
	Total			1,250	1,250	1,250	1,250	1,250	1,250	1,250	1,250
3	Tools										
	Sprayer	3	300	900	900	900	900	900	900	900	900
	Bucket	5	30	150	150	150	150	150	150	150	150
	Basket	10	50	500	500	500	500	500	500	500	500
	Total			1,550	1,550	1,550	1,550	1,550	1,550	1,550	1,550
4	Land lease			1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
	Total			5,250	5,308.5	5,367	5,425.5	18,250	31,250	44,250	
B	Input				10,725	19,558.5	19,617	19,656	40,040	40,300	40,365
C	Benefit				5,475	14,250	14,250	14,230.5	21,790	9,050	(3,885)
	B/C				1.04	2.68	2.66	2.62	1.19	0.29	(0.09)
	R/C				2.04	3.68	3.66	3.62	2.19	1.29	0.91

DISCUSSION

In this research the Soil texture is clay. Islami and Utomo (1995), stated that soil texture is very dominant to provide nutrients for plants. Soil texture has many aspects such as solubility of nutrients, air circulation, specific surface, weight of volume of the soil and compressibility of soil. Clay is very weak to absorb water. Commonly, the texture is sticky during in wet condition and it becomes broken into more small fraction during in dry condition.

C-organic showed 4.99%, in high classification. Organic fertilizer has ability to recover the physical characteristic of soil (Harjowigeno, 1987). Nitrogen content 0.48% is in medium category. P-Olsen content 107,55 % in medium category, too. K content 15.3% in very high category, this K form as an positive ion. Callium is absorbed by root of plants as cation K+. Plants need macro nutrient (Nitrogen, Phosphor and Callium) for growing. Lacking one of nutrient is able to hamper the supply nutrient of other nutrient.

CEC growing media included in the category is as high as 41.7%. The condition of high CEC in planting media is expected to increase the ability of soil to hold nutrients that are not easily washed away by water (leaching) and become available to plants. This is according to Harjowigeno (1987) that the CEC is a chemical component that closely related to soil fertility. Soil with a high CEC is able to absorb and provide nutrients better than soil with a lower CEC.

The Effects Of Treatment On Agronomic Component

Mustard are leafy vegetables that very response to nitrogen fertilizer, especially shown in plant height and number of leaves. According Palimbungan *et.al.* (2006), the liquid fertilizer in an amount corresponding

needs to support optimal plant growth which led to enlargement and elongation of the cells so plant will growing fast.

The observations to the number of the leaves showed no different effect on 14 DAP, 28 DAP, and 35 DAP. Harjowigeno (2003) stated that the organic fertilizer has the low nutrient contents and it effects to the plants were slow. So that the effect of dosage POC at height of plants variable was more lower than urea fertilizer treatment.

The effect of dosage POC on 35 DAP showed that the treatments of E treatment was 24.9 cm, F 25.1 cm and G 25.3 cm. While the effect of dosage urea fertilizer showed that the treatments of B was 27.3 cm, C 27.6 cm, and D 27.6 cm too. These treatments were more higher than control.

Results of analysis of variance showed that treatment dosage of urea was significant on fresh weight and more higher than treatment with POC. However, the weight of fresh mustard was not influenced by the length of the canopy, width of canopy, and length of roots. The analysis showed that all of the treatment of urea fertilizer were significant different and have more higher fresh weight than POC. All of the urea fertilizer treatment were not significant The treatment B (1003.3 grams), C (1006.7 grams), and D (1008.3 grams). POC treatment produce E (616.7 g), F (620 grams), and G (621.7 grams). This is an indication that the dosage of POC needs to be increased so that it can produce the same mustard plants treated with urea fertilizer.

4. Farming system Analysis

POC that used at mustard in this research, the level of it dosage can't enough produce fresh weight as good as plants fertilized by urea. The used of urea fertilizer 5 gm/polybag able to increase productivity with the results of the R/C of 2.04. The use of POC as much as

100 ml/polybag or 650 liters per hectare gave the results the highest of R/C was at 2.19 .

To increase the productivity of field mustard, farmers are faced a problem of the use of capital and the good technology. In the face of these options use a combination of capital such as seed, fertilizer and pharmaceuticals in addition to the right of labor will be the basis to implement these options. Choice to use a combination of labor, seed, fertilizer, pharmaceuticals optimal, will get maximum results. In other words, an exactly combination of production inputs can create more efficient manner (Soekartawi, 2002).

In the fact, the problem of the use of production factors consist in the farming system, which is the main problem faced by farmers beside production factors is skill . As it is known that income has a direct relationship with the farming system production, while the farming system production is produced determined by an individual's expertise in managing the use of factors of production in favor of farming such as land, labor, capital and management. According Soekartawi (2002) , farming system is essentially the company, then a farmer before managing their farming system will considering costs and revenues , by allocating available resources effectively and efficiently , to obtained high profits at any given time. Said to be effective if farmers can allocate their resources as well as possible, and to be efficient when generating output more higher than input production

CONCLUSION

1. Dosage of POC needs to be increased so that it can produce the same mustard plants treated with urea fertilizer.
2. The highest fresh weight is not significantly different in the treatments of urea fertilizer in B treatments (1003.3 grams), C (1006.7 grams) D (1008.3 grams)

3. Treatments with POC produce fresh weight more lower than urea treatment and not significantly difference for all of POC treatments in E (616.7 gram), F (620 gram), G (621.7 gram)
4. The results of financial analysis at B treatment (urea 5 grams/polybag) obtained the highest R/C ratio (3.68) while in POC of the E treatment (100 ml/ polybag produce) obtained the highest R/C ratio (2.19).

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SOCIO-ECONOMIC ASPECT OF VEGETABLE PRODUCTION SYSTEM IN EAST JAVA: THE CASE OF VEGETABLE FARMERS IN KEDIRI AND BLITAR

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ABSTRACT

East Java is a major vegetable producing region that contributes more than 12% of the total national production. High contribution vegetables from this province include chili, shallots, cabbage, potatoes, tomatoes, eggplant, and leeks. High vegetable production is expected to increase farmers' income, resulting in increased production and consumption of vegetables will help people's lives better. This study aimed to observe in depth some aspects of the socio-economic and prospects of vegetable development in East Java. The study was conducted from February to December 2012 in Kediri and Blitar. Data collection was conducted through FGD and survey method. A total of 50 vegetable farmers was surveyed. The results illustrate the most productivity of vegetables at the study site is still below the level of potential. Thus there are still opportunities to be able to increase the production of vegetables in the area. In both studied locations there are a lot of big farmers that rented a land and use hired labor to work on that. From a gender perspective, it seems that there is a great difference between one location to the other, but in general both men and women share the work almost equally. In terms of technology, most farmers apply both fertilizers and crop protection chemicals, where on average both fertilizers and crop protection chemical contribute 40-50 % of the production cost. Improve genetic resources of seed and technologies can increase the vegetable production system. Farmers' access to appropriate information related to the technical aspects should also be provided. Another important effort is the need for information and training for farmers and traders, especially in the case of post-harvest vegetables.

Keywords: socio-economic aspect, vegetable production system, east java

INTRODUCTION

East Java is a province that provides a significant contribution in the production of vegetables in Indonesia with a total contribution of 12,05%. Some of the vegetables with the highest national contribution are shallot (28%), chili (18.6%), cabbage (11.7%) and spring onion/leek (11.3%). This area also produces a variety of other vegetables such as garlic, string beans, eggplant, tomatoes and carrots (Agricultural Statistic Database, 2012). Trend of production of some major vegetable East Java continues to increase during the period 2004-2007 (**Table 1**). Kediri and Blitar are the two districts which are the main vegetable production for large and small chili, tomato and leek (Secretariat Director General of Horticulture, 2010).

Table 1. Production of some major vegetables in East Java 2004-2007 (Ton)

No	Commodities	2004	2005	2006	2007
1	Shallot	224,971	239,530	253,760	228,084
2	Cabbage	150,304	138,999	162,891	171,597
3	Small chili	139,844	158,888	132,148	140,557
4	Potato	105,256	94,787	87,928	95,952
5	Large chili	78,650	63,028	64,924	73,777
6	Spring onion	61,097	66,988	57,775	71,484
7	Tomato	54,826	46,645	43,391	81,851

Source: East Java Province Agricultural Office, 2009

In East Java, vegetables produced in small lands and labor-intensive, thus offering opportunities for small farmers to diversify production and generate employment in order to increase revenue for all actors in the value chain. Diversification of vegetables produce can give benefit for poor farmers and farm workers who do not have land to increase production and employment (Mariyono and Bhattarai, 2010). It is also empowering the poor by increasing their access to decision-making processes, and reduce their vulnerability to shocks through asset accumulation (Mariyono *et al.*, 2010).

RESEARCH METHODOLOGY

This study aimed to observe in depth some aspects of the socio-economic of vegetable production system (especially chilli, tomato and shallot) in the central production of East Java. The Study was conducted from February to December 2012 in the district of Kediri and Blitar, as well as some other vegetables in the central areas of East Java.

Initial data collection was done through FGD (Focus Group Discussions). The use of this method makes it possible to explore the qualitative aspects of agricultural sectors. Further, interviews based on the questionnaires, and information provided by the farmers/traders was directly recorded. The total respondents interviewed were 50 farmers and 20 trades of vegetables. The data was obtained and analyzed descriptively

RESULTS AND DISCUSSIONS

The Circumstances of Vegetable Production System in Studied Areas

The largest vegetable planting area in Kediri in the period of 2006 to 2010 was small chili as annually planted 2,682 ha, followed by a large chili 958 ha, 634 ha of shallot, 529 ha of green beans and 373 ha of tomatoes (Kediri Agricultural Office, 2010). Where are in Blitar at 2008, vegetable production area respectively were small chili (5,899 ha), large chili (598 ha), long yard beans (515 ha), eggplant (114 ha) and tomatoes (128 ha) (Blitar Agricultural Office, 2009).

In general, the farming practice in both locations was almost similar. Farmers started in vegetable farming by preparing the land using plows and rakes before making arrangements beds and plastic mulch. The farmers prepared for the nursery of chilis, tomatoes and cabbage, but not for carrots, squash and beans, as well as

some farmers used local seed varieties and some used hybrid. Weeding and spraying were done manually using a hand sprayer, in addition, harvesting also done by hand. The frequency of spraying depends on the season and crops. Farmers sold their products to the market directly or through intermediaries. It depends on the type of vegetables which they were produced.

Normally, farmers applied a complete fertilizer, including N, P, K and micro-nutrients. Some farmers also applied organic fertilizer. In general, farmers were gradually giving fertilizer 3-4 stages, ie, basal, weeks 1 and 3. Related to nutrient management, farmers observed that there was some indication of plant nutrient deficiency. They have been making a diagnosis, but they do not know the outcome.

The pests and diseases are the majors limiting factors when other productivity has enough inputs had been applied. In Kediri, Anthracnose and Gemini viruses were the main diseases on chili, since developing the disease, the failure reach 100%. Another important disease virus and late blight on tomatoes and sponge gourd (*gambas*), which led to losses of up to 100% and 90% respectively of these vegetables. Pests and other diseases that appeared to be moderate and some farmers had managed to control it. Most farmers used synthetic pesticides, and sometimes combined with botanical pesticides. In Blitar, anthracnose was also a major disease on chili, and late blight is the most destructive disease of tomatoes. Meanwhile, fruit flies could lead to loss of yield up to 100%, is the most important pests on chili. On cabbage, diamond back moth and leaf feeders are the most damaging pests. Potential yield loss due to pests experienced farmers can reach 100%.

For harvest and post-harvest activities, farmers typically harvested in the morning. They use

some kind of bag container to accommodate and reduce physical damage. They put vegetable crops in the shade and dried place and sold as soon as possible to maintain quality. Sorting and grading of chili and tomato, based on color and healthy fruit.

Gross Margin and Socio-economic Analysis

Based on the farm production data taken from the two studied areas, most of big chili farm use hired labor. Hired labor could cost up to 35% of total production cost. Three out of our five respondents use 100% of hiring labor to manage their big chili farm. This is particularly in Kediri where there are a lot of big farmers that rented a land and use hired labor to work on that. From a gender perspective, it seems that there is a great difference between one location to the other, with only 16 % of the total labor are women reported in one farm and 67 % in other farm. But in general both men and women share the work almost equally. In terms of technology, most farmers apply both fertilizers and crop protection chemicals. In an intensive farm fertilizer and crop protection chemicals could contribute up to 60 % of production cost, while in average both fertilizers and crop protection chemical contribute 40 % of the production cost (**Table 2**).

For profitability of farming, return on land of big chill ranged between 35 to 113 million rupiah per ha with 79 million per ha is the average number. On average, net return on land is approximately about 40 % of total revenue. For the family, the return on their one day laborer is very high, 0.5 to 2 million per day. The average on our respondent is 1.3 million per day. Return to total labor is between 125,000 to 300,000 which are still higher than the labor cost of the location that ranged between 50,000 to 80,000 per day (**Table 2**).

Table 2. Big chill economic and social indicators

	Min	Max	Average
Economic indicators			
Yield (tons/ha)	10.0	32.1	17.6
Price (IDR/kg)	6,000	15,000	8,300
Revenue (million IDR/ha)	60	209	137
Cultivation and harvesting cost (million IDR/ha)	25	96	58
Net income (million IDR/ha)	35	113	79
Return on family labor (000 IDR/ workday)	518	2,096	1,307
Return on total labor (000 IDR/ workday)	125	296	211
Labor, environment and gender			
% Women labor	16%	67%	41%
% family labor	0%	24%	8%
% of fertilizer cost	9%	42%	24%
% of agro protection chemical cost	12%	22%	18%

Source: Field Survey, 2012

Small chili farmers use a higher percentage of family labor, although hired labor still more dominant contributor to the labor cost. Approximately family labor contributes 40 % of total labor use in small chili farming. In terms of technology, most farmers apply both fertilizers and crop protection chemicals, although the cost of fertilizers and crop protection chemicals are not significant. Combined together, the total cost of fertilizers and crop protection chemicals are around 20 % of total production cost (Table 3). This is supported by observation on Blitar's farmers that they only use a limited type of crop protection chemicals. Input shops in Blitar also typically only have limited choices of fertilizers and agro protection chemicals.

It seems that in general both men and women share the work almost equally. Women contribute 55 % of total labor cost in small chili. In term of profitability, return on land of small chili ranged between 1 million loss to 20 million profit per ha with 10 million per ha is the average number. On average, net return on land is approximately about 50 % of total revenue. For the family, the return on their one day work is highly varied, ranging from 22 thousand losses to 1 million per day. Similarly on total labor, the return on total labor is between 10,000 losses to 200 thousand rupiah

per day. The average number of return on family labor and total labor are 250 thousand and 60 thousand respectively (Table 3).

Table 3. Small chili economic and social indicators

	Min	Max	Average
Economic indicators			
Yield (tons/ha)	0.4	7.0	2.2
Price (IDR/kg)	7,000	25,000	11,000
Revenue (million IDR/ha)	3	60	20
Cultivation and harvesting cost (million IDR/ha)	2	22	10
Net income (million IDR/ha)	(1)	20	10
Return on family labor (000 IDR/ workday)	(22)	013	248
Return on total labor (000 IDR/ workday)	(10)	226	57
Labor, environment and gender			
% Women labor	34%	74%	55%
% family labor	22%	100%	40%
% of fertilizer cost	1%	34%	14%
% of agro protection chemical cost	0%	27%	9%

Source: Field Survey, 2012

In term of profitability, return on land of Tomato ranged between 4 to 120 million profit per ha with 40 million per ha is the average number. On average, net return on land is approximately about 50 % of total revenue. The study indicates that some tomato farms use 100 % of family labor, some use 100 % of hiring labor and others in between. In general, in tomato farming only around 40 % use women labor and only 35 % use own family labor. In terms of technology, most farmers apply both fertilizers and crop protection chemicals, of which contribute up to 40 % of total production cost. For the family, the return on their one day work is highly varied, ranging from 60 thousand to 340 thousand per day (Table 4).

Table 4. Tomato economic and social indicators

	Min	Max	Average
Economic indicators			
Yield (tons/ha)	24.0	120.0	57.3
Price (IDR/kg)	500	4,000	1,750
Revenue (million IDR/ha)	24	160	78
Cultivation and harvesting cost (million IDR/ha)	17	56	40
Net income (million IDR/ha)	4	123	38
Return on family labor (000 IDR/ workday)	58	344	190
Return on total labor (000 IDR/ workday)	6	344	108
Labor, environment and gender			
% Women labor	25%	64%	43%
% family labor	0%	100%	34%
% of fertilizer cost	16%	22%	21%
% of agro protection chemical cost	18%	20%	17%

Source: Field Survey, 2012

Approximately family labor contributes only 20 % of total labor use in shallot farming. It is common that farmers hired a group of people from his/her area and migrate temporarily to the next district to farm shallot. In terms of gender, it seems that in general both men and women share the workload almost equally. In terms of technology, most farmers apply both fertilizers and crop protection chemicals, although the cost of fertilizers and crop protection chemicals are not significant. Combined together, the total cost of fertilizers and crop protection chemicals are around 30 % of total production cost. However in some cases, the cost of chemicals can contribute up to 50 % of total production cost. It seems that shallot is a high return high risk commodity. Return on land of shallot ranged between 7 million loss to 185 million profit per ha with 78 million per ha is the average number. On average, net return on land is approximately about 45 % of total revenue. For the family, the return on their one day work is varied, ranging from 350 thousand losses to 800 thousand rupiah per day (Table 5).

Table 5. Shallot economic and social indicators

	Min	Max	Average
Economic indicators			
Yield (tons/ha)	2.1	17.1	10.8
Price (IDR/kg)	4,161	15,000	7,383
Revenue (million IDR/ha)	17	257	81
Cultivation and harvesting cost (million IDR/ha)	13	77	39
Net income (million IDR/ha)	(7)	185	42
Return on family labor (000 IDR/ workday)	(355)	839	331
Return on total labor (000 IDR/ workday)	(27)	407	145
Labor, environment and gender			
% Women labor	23%	95%	48%
% family labor	0%	83%	20%
% of fertilizer cost	4%	15%	8%
% of agro protection chemical cost	1%	48%	19%

Source: Field Survey, 2012

CONCLUSION

Productivity of vegetables at the study site is still below the level of potential. Thus there are still opportunities to be able to increase the production of vegetables in the area. From a gender perspective, in general both men and women share the work almost equally. In terms of technology, most farmers apply both fertilizers and crop protection chemicals, where on average both fertilizers and crop protection chemical contribute 40-50 % of the production cost. Improve genetic resources of seed and technologies can increase the vegetable production system. Farmers' access to appropriate information related to the technical aspects should also be provided. Another important effort is the need for information and training for farmers and traders, especially in the case of post-harvest vegetables.

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CHARACTERISTIC OF MANGO DIVERSITY IN EAST JAVA: CASE STUDY IN TIRON, KEDIRI

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ABSTRACT

In rural areas mangoes contributes to farmers' additional income, improved nutrition along with ecosystems and environmental sustainability. Those are mostly grown in either home gardens or farms. However, the genetic diversity of mango is being threatened by specialization of production system in only few cultivars or varieties. The study was carried out to assess genetic diversity relating to the conservation and utilization of the diversity in Tiron village, Banyakan Sub-district, Kediri. Survey during November-December 2010 was conducted by focus group discussions (FGD) of key expert farmers. Participatory four cell analysis method was used to assess on farm mango diversity. The study revealed that four species of *Mangifera* and twenty four local landraces of *M.indica* were identified by the community in Tiron, Kediri, and East Java. Three endangered species (*M foetida*, *M. Laijiwa*, and *M.indica*) and two rare species (*M. odorata*, and *M. indica*) were grown in the home garden by farmers in Tiron, Kediri. Besides, four common varieties (Podang Urang, Podang Lumut, Golek, and Gadung), five endangered varieties (Madu, Lanang, Santok Kapur, Santok Buto, and Kopyor), and fifteen rare varieties of *Mangifera indica* were also found to be grown by farmers. The species/variety trees were generally found to be distributed evenly in the community, and not single farmer has more species/variety trees in his/her home garden or farm compared to other farmers. It was further observed that fruits of some species/varieties have low commercial value; but they are a good source of nutrition for farmers' family.

Keywords: mango, diversity, genetic resources, Kediri

INTRODUCTION

Indonesia is one among the diversity centers of the inter and intra-specific of *Mangifera* in Southeast Asia (Mukherjee, 1972). There are about 23 species in Kalimantan and 250 varieties available in Sumatra, Kalimantan, Java, and West Nusa Tenggara islands, though only about 57 varieties are being cultivated (Sumiarsi, et.al., 2006; Uji, 2007).

In rural areas, mangoes contribute to farmers' additional income, improved nutrition along with ecosystems and environmental sustainability. Those are mostly grown in either home gardens or farms. However, the genetic diversity of mango is being threatened by specialization of production system in only few cultivars or varieties; firstly changes in land use or deforestation, and secondly potential competition from introduction of different profitable species (e.g. forest timber trees in Java).

Therefore, for the recognition of the importance and the need of conservation and utilization of tropical fruit tree species (TFT) diversity; Bioversity International has collaborated with Indonesia Centre for Horticulture Research and Development (ICHORD) and BPTP East Java to implement project entitled 'Conservation and Sustainable Use of Cultivated and Wild Tropical Fruit Diversity: Promoting Sustainable Livelihoods, Food Security and Ecosystem Services'.

The aim of this study is to measure the diversity of mango trees and to understand the rationale of farmer's decision in cultivating as common, rare and endangered categories and use information from farmers' communities to maintain and use such diversity.

METHODOLOGY

Activities were carried out to assess genetic diversity relating to the conservation and utilization of the diversity in Tiron village, Banyakan Sub-district, Kediri. The methods used in this study were: (1) Focus Group Discussions (FGD) with Farmers Groups (including 15 men and 10 women) and participatory four cell analysis were carried out to identify common, unique, endangered and rare mango species and varieties; to document the reasons why the varieties are found in various dynamic state in the community and to identify the level and type of interventions needed for the conservation of mango varieties in a community (Sthapit et al., 2006; Simon de Boef and Thijssen; 2007). (2) Interviews were conducted with key expert farmers and the representatives of the local agricultural extension services. (3) Field visits (transect walk) and diversity fairs were carried out to validate the results of Four-Cell Diversity Analysis.

RESULTS AND DISCUSSIONS

Descriptions of Studied location

Tiron is a village in Banyakan sub-district, Kediri district, in the East Java province. It is one of the mango (*Mangifera indica*) production villages in Central East-Java, at 6048'47 S, 1070 36'52 E. Among different varieties of mangoes grown here, Podang Urang is the most popular variety. Other varieties of mangoes such as Podang Lumut, Gadung, Madu, Golek, etc. are also grown here. Most of the mango trees are grown in the home gardens. Besides, these varieties are also grown in a forest managed jointly by the community and local forestry services of Kediri.

Soil type is rocky Red brown Mediterranean. The climate of Tiron is humid with 6-months

period of summer and rainy seasons respectively. The summers are hot (30 - 34 °C maximum) with relatively cool in the rainy season (minimum 22 - 23 °C). Average annual rainfall is 2250 mm. The rainy season is from November up to April. Soils are deep alluvium. Mango is one of the major income sources of the village, with mangoes just been sold to the nearest towns. However, no private/individual nursery found here that supply saplings to the farmers. Farmers earn their main income of mangoes, corn, cassava, tuber crops such yams, and rain-fed paddy-rice for home consumption and sales

Diversity of Mango in Studied area

Four Cell Analysis (FCAs) revealed four species (*M. indica*, *M. odorata*, *M. foetida*, and *M. lalijiwa*) and twenty four local races of *M. indica* were identified in East Java, Evennes measurement is 0.74 in Tiron, The species/variety trees are generally distributed evenly in the community, therefore no single farmer has more species/variety trees in his/her home garden or farm compared to other farmers.

The species/varieties presented in the cells of many HHs - many trees; many HHs- few trees; and few HHs – many trees, should get more attention from the local stakeholders for possible commercial market linkages and additional value (Fig. 1), whereas the species/varieties in the cell of few HHs and few trees especially shall be conserved and utilized by the research institutes for research purpose e.g. rootstock, tolerance to drought, floods, pest diseases, and for health attributes.

Grouping status of species/varieties based on the FCA method from each site presented in Table 1. *M. indica* is a common species (many HH, many trees), whereas *M. foetida*, *M.*

indica and *M. lalijiwa* are endangered species (many HH, few trees) in Tiron, Kediri. The endangered *M. indica* are mostly non-commercial varieties. In addition, there are also fifteen rare varieties of *M. indica*. (few HH, few trees).

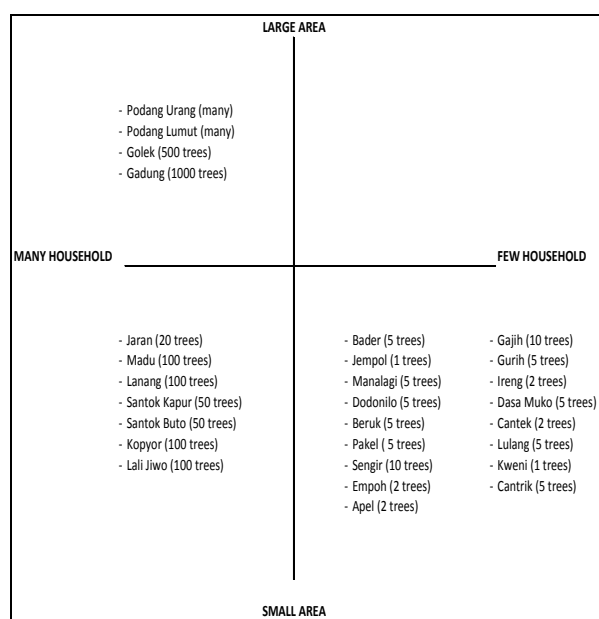


Figure 1. Four Cell Analysis configuration in Tiron, Kediri

Table 1. Four Cell Analysis (FCA) Results.

Status	FCA Results (*)	Tiron	
		Species	Var, <i>M. indica</i>
Unique sp./var.	FHH, MT	--	---
Common sp./var.	MHH, MT	1	4
Endagered sp./var.	MHH, FT	3	5
Rare sp./var.	FHH, FT	2	15

*)FHH, MT = few HH, many trees; MHH,MT=many HH, many trees; MHH,FT = many HH, few trees; FHH, FT = few HH, few trees

Table 2. Overview of Mango Diversity, Tiron, Kediri

No	Local name	Scientific name	FCA Results	Uses	Agronomic, Morphological or Market Traits	Other interesting features / characteristics
1.	Podang Urang	Var. of <i>M. indica</i>	Many HH, many trees	Table fruit and processed	Soft, sweet, reddish yellow flesh colour	High market value ; red peel colour specific location
2.	Podang lumut	Var. of <i>M. indica</i>	Many HH, many trees	Table fruit	Soft, a bit sour, Yellow flesh colour	Yellowish green peel colour
3.	Golek	Var. of <i>M. indica</i>	Many HH, many trees	Table fruit	Yellow flesh colour	Common commercial variety
4.	Gadung	Var. of <i>M. indica</i>	Many HH, many trees	Table fruit	Yellow flesh colour	Common commercial variety
5.	Jaran	<i>M. foetida</i>	Many HH, few trees	Table fruit	Big fruit, a bit sour taste	Drought tolerance
6.	Madu	Var. of <i>M. indica</i>	Many HH, few trees	Table fruit	Yellow flesh colour	Also common variety for rootstock
7.	Lanang	Var. of <i>M. indica</i>	Many HH, few trees	Pickling type	A bit sour fruit taste	Almost no seedless
8.	Santok Kapur	Var. of <i>M. indica</i>	Many HH, few trees	Table fruit	Good root system	Good keeping quality
9.	Santok Buto	Var. of <i>M. indica</i>	Many HH, few trees	Table fruit	Big fruit, small seed	Good keeping quality
10.	Kopyor	<i>M. indica</i>	Many HH, few trees	Sucking type	Fibrous flesh, good root system	Use for juice
11.	Lali Jiwo	<i>M. lalijiwa</i>	Many HH, few trees	Table fruit	Sweet mature fruit	Common commercial variety
12.	Bader	Var. of <i>M. indica</i>	Few HH, few trees	Table fruit, pickles	Sweet fruit taste	Small size fruit as local fish
13.	Jempol	Var. of <i>M. indica</i>	Few HH, few trees	Pickles	Small fruit as thumb size	
14.	Manalagi	Var. of <i>M. indica</i>	Few HH, few trees	Table fruit	Sweet young fruit; manalagi means more and more	Common commercial variety
15.	Dodonilo	Var. of <i>M. indica</i>	Few HH, few trees	Table fruit, juice	Sweet and fragrant fruit	Smooth flesh fiber
16.	Beruk	Var. of <i>M. indica</i>	Few HH, few trees	Table fruit	NA	Fibrous flesh
17.	Pakel	Var. of <i>M. odorata</i>	Few HH, few trees	Table fruit, salad	Sweet fruit taste	Very sour un-ripe fruits
18.	Sengir	Var. of <i>M. indica</i>	Few HH, few trees	Table fruit	Turpentine like taste	
19.	Empok	Var. of <i>M. indica</i>	Few HH, few trees	Table fruit	Big fruit, sour	Very juicy
20.	Apel	Var. of <i>M. indica</i>	Few HH, few trees	Table fruit	Fruit size and shape looks like apple; reddish green skin fruit colour	Also use as ornamental trees
21.	Gajih	Var. of <i>M. indica</i>	Few HH, few trees	Juice	Yellow flesh colour, sour	
22.	Gurih	Var. of <i>M. indica</i>	Few HH, few trees	Table fruit	Yellow flesh colour, delicious	
23.	Ireng	Var. of <i>M. indica</i>	Few HH, few trees	Table fruit	Small and round fruit shape; sweet, grayish black fruit skin colour	
24.	Dasa Muko	Var. of <i>M. indica</i>	Few HH, few trees	Table fruit	Long, big fruit	
25.	Cantek	Var. of <i>M. indica</i>	Few HH, few trees	Table fruit	Sweet fruit taste; bent fruit tip	
26.	Lulang	Var. of <i>M. indica</i>	Few HH, few trees	Table fruit	Oval-shaped fruit; rich of Vit. C	
27.	Kweni	<i>M. odorata</i>	Few HH, few trees	Juice, table fruit	Fragrant fruit	Fibrous flesh
28.	Cantrik	Var. of <i>M. indica</i>	Few HH, few trees	Table fruit	Sweet fruit taste	

Reasons for Diversity of Mango Trees

The 54 interviewed households in Tiron, who represent about 15% of all the families with fruit trees in the community of Kaligayam, Tiron, have 20.5 trees on average per household. On an average 2.8 varieties are maintained per household and in total 4 different species and 24 varieties of *M. indica* is known within the community. Indigenous varieties such as Jaran, Lanang, Santok Kapur, Santok Buto Bader, Jempol, Dodonilo, Beruk, Pakel, Empok, Ireng, Dasamuko, Cantek, Lulang, Cantrik, are combined with commercial varieties like Podang Urang, Podang Lumut, Golek, Gadung, Madu, and Manalagi. See Table 2 for an overview of the main characteristics of the diversity found in the community.

Domestic markets prefer more to flavour, and sweet flesh taste, beside reddish yellow skin colour and fruit size (200-250 grams fruit weight). *M. indica* var. Podang Urang is the widely used commercial variety in Kediri, East Java. The rest of the species and varieties are mostly used for family consumption, either as fresh table fruits or processed for juice, pickles, hot spices mixture, and vegetable/soup. Besides, mango trees provide pleasant environmental benefits as shade trees around the house. Overviews of status based on FCA results, and market traits are presented in Table 2.

CONCLUSIONS

Four species of *Mangifera* and twenty four local races of *M. Indica* was identified by the community in Tiron. Among these species and local races, endangered species *M. foetida*, *M.lalijiwa*, and fifteen rare varieties of *M. indica* were found.

Market value is the main reason for farmers to grow more species/varieties. The fruits of some species/varieties have low commercial value, but they are a good source of nutrition for the local farmers' family. Besides, they also provide environmental benefits as shade trees and soil protection.

ACKNOWLEDGMENT

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Effect of Trees Architectural model and Total Litter at Three Different Types of Agroforestry Management for The Value of Infiltration and Run Off

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ABSTRACT

The study was conducted at three different land uses, i.e. multicultural agroforestry, annual monoculture, perennial monoculture. The location was conducted in the Sumberkunci village, Pakis Aji District, at Malang regency. This study aimed to determine differences in the rate of infiltration and sedimentation. Infiltration rate was measurements done at 2 points in each type of land use. It's measured by infiltrometer. Several parameters measured were plant species on land, tree canopy architecture models, and the amount of litter on the plot of land. Results of measurements, obtained the infiltration value in a multicultural land is 64 cm / hour (highest); run off 0.2 g / l (the lowest), the annual monoculture 40.5 cm / hour (the lowest); run off 3.1 g / l (the highest), and the perennial monoculture land is 56.5 cm / hour; run off of 0.9 g / lt. Inventory result of land showed that the plants on multicultural agroforestry more varied than else. So, the crown cover are wider, and soil surface conditions are more humid because many dropping litter. Index Important Value showed that multicultural land dominated by Waru Gunung /Hibiscus ,Bambu Jawa/ Bamboo , ubi kayu/ Cassava and kopi/ Coffea . They have a form of architecture in such a way and the amount of litter which is high enough to affect both the value of infiltration and run off in the land.

Keywords: Infiltration, Run off, agroforestry, architecture models, litter

INTRODUCTION

Infiltration is the process by which water on the ground surface enters the soil. Infiltration rate in soil science is a measure of the rate at which soil is able to absorb rainfall or irrigation. It is measured in inches per hour or millimeters per hour. If the precipitation rate exceeds the infiltration rate, runoff will usually occur unless there is some physical barrier. The rate of infiltration can be measured using an infiltrometer ^[1].

The soil texture and structure, vegetation types and cover, water content of the soil, soil temperature, and rainfall intensity all play a role in controlling infiltration rate and capacity. The process of infiltration can continue only if there is room available for additional water at the soil surface. The maximum rate that water can enter a soil in a given condition is the infiltration capacity. Water that does not get absorbed into the soil, or rise back into the atmosphere as water vapor, will run off surfaces collecting in varied locations ^[2].

Runoff is any water that flows across the surface of the land. The main source of runoff is precipitation. The water will run off the surface if evaporation does not take place. The amount and type of vegetation on a site will affect runoff. Conservation tillage or reduced-tillage cropping systems leave ample vegetation or crop residue that slows the movement of runoff water ^[3].

Different impact of vegetation type due to the system of water management was caused by the differences architecture model belonged to each kind of tree habitus which presented the morphology as a series phase of tree growing ^[4]. Architecture model of certain tree would influence translocation of rainfall into stem flow, through fall, infiltration, and surface runoff. It was related with the function of vegetation in decreasing erosion rate ^[5]. Result showed that tree architecture was correlated with the partitioning components value of rainfall. The rainfall partitioning were included

through fall, stem flow, interception, runoff, and infiltration ^[6]. Important role of vegetations in regulating hydrological cycle of an ecosystem also were showed by their leaf litter production. The top layer of leaf litter that is not decomposed protects the soil from the pounding action of rain; without this the soil can become far less permeable. Function change from forest to coffee plantation would decrease the input of litter and it would change of micro climate condition because of this opened area ^[7].

In Indonesian, there are various types of land use, such as agroforestry management. Agroforestry management does not always pair various types of plants that are appropriate for the ecological preservation of land, mostly just concerned with the economic interests of the community. Multiculture Agroforestry is agroforestry system that combines components forestry (or woody plants/woody plants) with the agricultural component (or non-wood plants). Farm based Agroforestry is the cultivation of agricultural crops as the main product which is more dominant than woody plants ^[8]. Many existing agroforestry in Indonesia, including in it the wood plant agroforestry is the cultivation of woody plants as the main product is more dominant than agricultural crop.

The amount and type of vegetation that make up the composition of a plot of land need to know to be able to see its effect on the hydrological conditions of the land, ie the value of its infiltration and runoff. This calculation can be done by analysis of vegetation, so it can see the kind of dominant vegetation and an important influence in the community.

Certain models of tree architecture affect translocation of rainwater into the stemflow, throughfall, the rate of infiltration and surface runoff in an area related to the role of vegetation in reducing erosion rates in the area.

However, there are very few studies on how different architecture vegetation types affect the amount of water infiltration and runoff. This paper would like to discuss the

difference in the value of infiltration and run-off on 3 types of different land uses. Where land use has a number and different types of vegetation, where the vegetation has a different architecture models and the amount of litter dropping is different.

MATERIALS AND METHOD

The research was conducted in September 2012 in the Sumber Kunci village, Pakis Aji district, Malang regency (628 ASL; W: 08°02'21,3; E:112°31'57,1) on dry land with a slope 30-40% that is different land types. They are multicultural agroforestry, perennial monoculture, and annual monoculture.

Each land types selecting 2 plots. The first step is analyze the diversity of vegetation through the method of plot measuring 20 m x 20 m in each plot for the trees (dbh > 10 cm) and 5 m x 5 m for the shrub (dbh > 5 cm). Each data recorded tree species, quantity, species quantity and its diameter at breast height (dbh). Then, the data had been calculated the density (K), frequency (F) and dominance (D). Of the three parameters could be calculated Important Value Index (IVI) of each and Diversity Index (H) to determine the level of diversity^[9].

Research activities continued with the analysis of architecture-dominant trees, calculating infiltration and surface runoff. Infiltration rate was measured with a ring infiltrometer. Operation by pouring water into the iron plate diameter of 50 cm, then recorded every 2 min height of the water that absorb into soil until the influx of water into the soil constant (no additions). Infiltration rate data for each type of lands, are presented in tabel or curve infiltration rate versus time of observation. The data obtained were then calculated to obtain the infiltration rate (cm / min). Infiltration rate is calculated by the formula is as follows^[10] :

$$f = \left[\frac{\Delta h_c}{\Delta t} \right] \times 60 .$$

Note : f = infiltration rate (cm/h)
 Δh_c = changes in water level each time interval (cm)
 Δt = Time interval (h)

Surface runoff was measured by using a simple rainfall simulator, is the rain maker is equipped with zinc plate and and plate glass container below. When the rain maker drain enough water on sloping land, water directly concentrated on the iron plate and into the glass container along with the soil particle entrained in it. The water reservoir is vaporized, so that, the soil particles are left to dry weight measured. It's that soil runoff particles and organic matter which is washed away by rain water.

Observations of tree architectural models made by observing morphological features canopy and branches in general, the pattern of tree growth, the development of main stem, branches and twigs development. Then looked for a model canopy architecture in accordance with the provisions of Halle *et al* (1978)^[5].

RESULTS

The highest infiltration value was found at land type of multicultural agroforestry. The value was 64 cm / hour and the lowest was at the land type of annual monoculture, with infiltration value of 40.5 cm / hour. The value of infiltration of perennial monoculture land was 56.5 cm/hour.

The highest run off value was found at the land type of perennial monoculture the value was 3.1 g/lt and the lowest was at at the land type of multicultural agroforestry with run off of 0.2 g/lt. The value of run off of perennial monoculture land was 0.9 g/lt.

Data have been obtained from measurements of activity in the field, then processed using the quadratic formula plot method for calculating the density (individuals/ha), frequency, and dominance (m²/ha), and

important value index (IVI). The results obtained can be seen in the table below:

Thick of Leaf Litter, Infiltration Value, and Run Off Value an Each Land Use Type

Table 1. Analysis Results of the Highest IVI Value of Trees, Habitus, and Shrubs Levels ,

No	Land Use Type	Varietas of Tree	Varietas of Shrub	Vegetation with the Highest IVI/ value of IVI	Thick of Leaf Litter (cm)	Infiltration Value (cm/hour)	Run off Value (g/lt)
1	Multi culture Agroforestry	Sengon laut, waru gunung, kopi, pisang, alpukat, durian, dadap, bambu petung, and bambu jawa	Lamtoro, keladi, laos , mahoni, nilam, ubi kayu, kopi, waru gunung, sengon laut, dadap , pisang, and alpukat	Bambu Jawa (51,6), Waru gunung (43,6), Ubi kayu (43) and kopi (43)	4	64	0,2
2	Annual monoculture	<i>Ficus ampelas</i> , nangka, apokat, aren, bambu jawa	Ubi kayu, sengon laut, waru gunung	Bambu Jawa (125,6)and Ubi kayu (124,1)	1	40,5	3,1
3	Perennial monoculture	Sengon laut	-	Sengon laut (250)	2	56,5	0,9

DISCUSSION

The highest infiltration value was found at the land type of multicultural agroforestry. Result of account the plant diversity showed the plants on multicultural agroforestry more varied than else so the crown cover are wide and soil surface conditions are more humid because many of dropping litter. Leaf litter production of sengon (*Albizia falcataria*), coffee (*Coffea arabica*), and bamboo were high and their decomposition such that it helps the soil pore enlargement. The lowest run off value was found at the land type of multicultural agroforestry. The surface run off was very important for conservation effort because surface run off would take particle of eroded soil which would accelerate process of sedimentation.

Dominance of tree species in an area may affect the amount of rain it falls on the

land. As we look at the results of calculations on multiculture agroforestry, that of the tree species that live in them, Bambu Jawa (*Gigantochloa atter*), Waru Gunung (*hibiscus macrophyllus*) coffee (*Coffea arabica*) and Cassava (*Manihot esculenta*) that have the largest value of IVI, which means that they have a highest density in the habitat and the distribution of species has important ecological position in the community. At perennial monoculture, Sengon (*Albizia falcataria*) that has the largest value of IVI. At annual monoculture, Bambu Jawa (*Gigantochloa atter*) and cassava (*Manihot esculenta*) that have the largest value of IVI

Results of the observation of the architectural model trees showed some differences in the characteristics of each tree. They were Leeuwenberg's model in Ubi kayu (cassava)/ *Manihot esculenta* , Roux 's model in Kopi (coffee)/*Coffea arabica*, Chamberlain's model in Waru Gunung, Troll's model in Sengon/ *Albizia adiantifolia*, Thomlinson's

model in Bamboo . The architecture model was presented as in Figure 1 below.

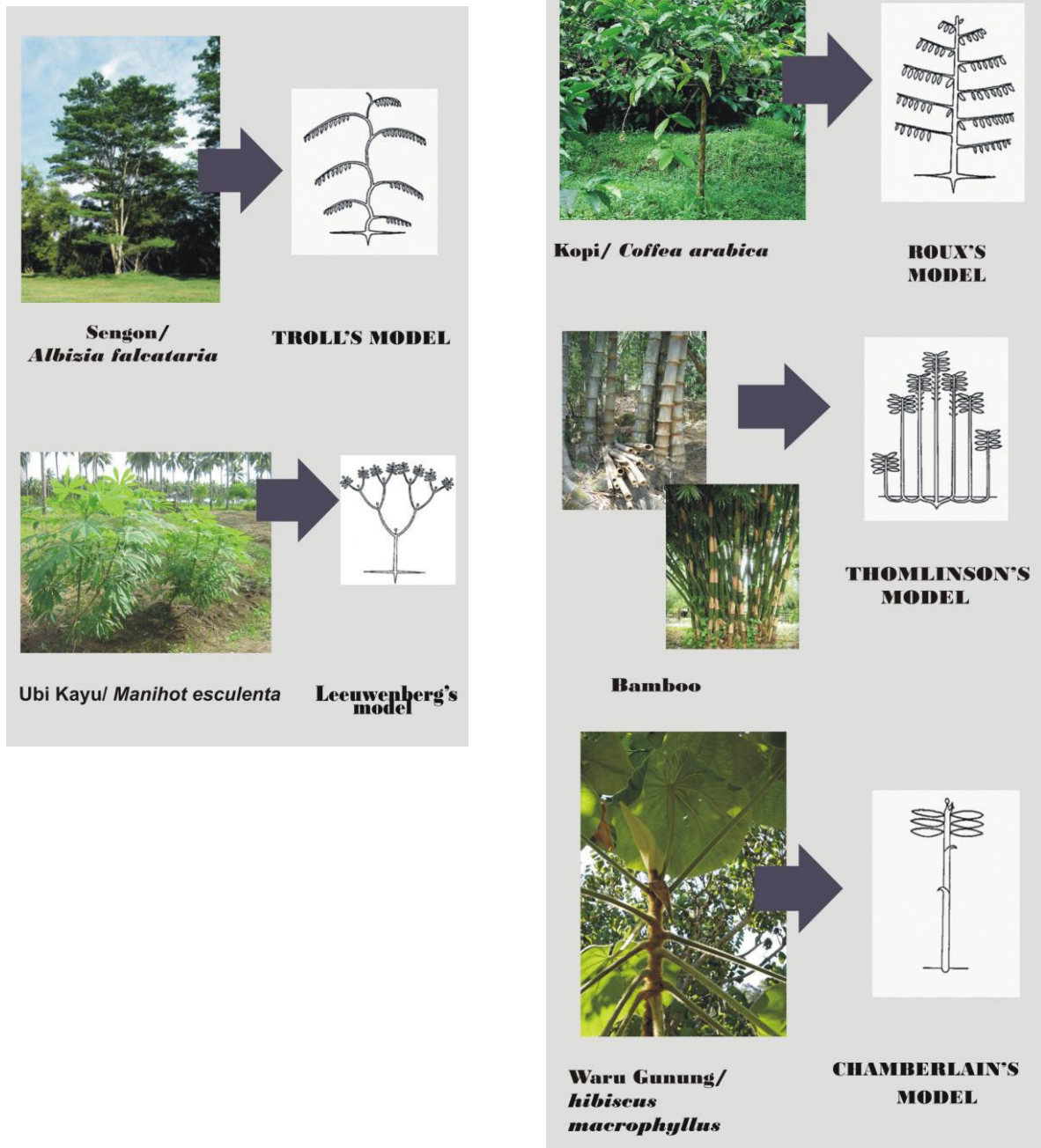


Fig 1. The Architecture's Model of Vegetation that Have the Largest IVI (Index of Value Important)

From the analysis of the architecture of the tree Bambu Jawa, it has Thomlinson's model. Tree with branching that occurs at the bottom of the module, generally below the soil surface (basitoni), continuous growth and axis form pleonanthy or hapaxanthy called Tomlinson's model ^[5].

Bamboo stem density and the number of gaps between the bamboo sticks which allow rain water falling to the ground. A smooth trunk without branches and other obstacles also allows water to flow from the base of the stem to the ground. Besides that, the length and shaped of the leaves hanging down is not a barrier for rain water to drip from the leaves to the soil surface.

Waru gunung tree (*Hibiscus macrophyllus*) has Chamberlain's model. Tree with a branching simpodium (single axis formed from collection in a series of lateral meristem) and monokaulus ie tree with a single trunk that is produced by one or more apical meristem functioning as a circuit. Tree with description of the model is called the chamberlain ^[5]. Hibiscus tree canopy is not too tight, so, a lot of space between the branches is a way for the rain fall to the ground. Waru also has a long-stemmed leaves, circular or round shape egg or heart shape with flat edge, dangling limply down, causing a lot of water flow from the canopy that fell to the ground.

The thick and tight canopy closure, throughfall that occurred will be smaller ^[11]. Throughfall of kopi (*coffea Arabica*) is low, it caused of kopi (*coffea Arabica*) crown covering a more widely area (about 80%). The leaves of kopi (*coffea Arabica*) are spread almost evenly across all branches, the arrangement leaves tightly. It causes the rain that falls is not much that a throughfall. But the rain water still dropping and passing through the pores around the leaves and stems gap. The location of the branch on the Roux, are symmetrical and evenly distributed along the stem to the top of the tree. This resulted in canopy pores become larger so much rain that penetrates to the forest floor ^[5].

Kopi (*Coffea arabica*) is a tree with Roux's model, which is one of the architectural models of trees with stems branching characteristics. Meristem stem's growth is monopodial (erect stem produced by extension of a terminal meristem) and orthotropic, show continuous growth. Branching are plagiotropik and usually continuous. Spiral arrangement of leaves on the stem but usually branched shows a clear line ^[5].

Manihot esculenta has Leeuwenberg's Model. Tree architecture with vegetative axis equivalent, homogeneous, orthotropic, akrotoni and branching consists of two or more branches, called Leeuwenberg models. Because it is a shrub, *Manihot esculenta* not have a large diameter shaft and a rough surface, so that the rainfall to the ground just past the canopy resistance. Editorial *Manihot esculenta* is not too tight even though its diameter is quite wide, because the shape of its leaves so many gaps so that's skipped a lot of rain. Planting the annual meeting of the monoculture causing rain water dropping is not too hard to pound the ground.

Sengon (*Albizia falcataria*) is a tree with Troll's model, with bud growth and axes all plagiotropic, architecture built a continuous superposition⁵. Plagiotropic leaf arranged as above with a sympodial trunk (produced by extension of lateral meristems). In the troll's model, plagiotropik branching (horizontal) causes the canopy diameter is wide. This of course causes canopy also greater extents. Similarly, the branching lateral meristem growth estimated contributed to the development plagiotropik trunk diameter so that a trunk diameter of troll's is great, because of a large trunk supports the existence of a highly branched tree and extends to the side.

Sengon (*Albizia falcataria*) trunks are smooth and high cause rain water fell to the ground with a big impact. Rain blows directly on ground causing some crushed aggregate, so the macro pore space is reduced. Along with the loss of top soil, occurred blockage of the pores in the layer below it, which in turn emerged as

the top layer. This resulted in infiltration decreased and increased surface runoff ^[12].

The presence of litter on the soil surface also affects the value of participating infiltration and run-off. In the rainy season litter on the soil surface played an important role in increase the amount of water that goes into the ground, reducing the amount and rate of surface runoff on sloping lands. Diversity of litter quality and quantity will determine the closing level surface soil by litter. Litter quality is the speed of weathering litter (decomposition). In multiculture agroforestry, a lot of vegetation causes the quantity of leaf litter more high than monoculture agroforestry. There were Bamboo and coffea that have a lot of leaf litter. This leaf litter's vegetations are a slowly decomposed group. Decomposed more slowly in the presence of litter ground becomes longer ^[13], so, its role as the protector of the land surface into better. The survey results in Sumberjaya, Lampung showed that coffea produces a lot of litter; coffee plantations multistory (age > 10 years) produce litter approximately 1.8 tonnes/ha, the shade coffee plantations approximately 1.2 tonnes / ha, and on coffee plantations monoculture around 1.2 tonnes/ha ^[7]. Therefore, annual monoculture was consists of a lot off *Manihot esculenta* that produce a few of litter. It causes the surface of soil was open more quickly. In perennial monoculture, sengon (*Albizia falcataria*) produce a lot off litter that is a quickly decomposed group. Legume species have weathering and time value ratio C / N lower than non-legumes. The plant Sengon (*Albizia falcataria*) has C / N ratio is low this can be caused by leaf anatomy, especially the thickness of the cell wall sengon (*Albizia falcataria*) ^[14]. Litter is categorized quickly obsolete when the ratio of C: N <25, lignin content <15% and polyphenols <3% ^[15]. The length of time sengon (*Albizia falcataria*) leaves decomposed will cause possibility of loss of land due to erosion is big ^[7].

CONCLUSION

The amount of land sloping soil macropores and the amount of water that can enter the soil is an important factor that must be maintained to reduce the amount of runoff surface and soil loss through erosion.

Ideally, in a garden should be able to produce a litter various time decomposition, so the need for a protective ground of the run-off and to supply of nutrients and mulch can be fulfilled. The model of multiculture agroforestry is a gardens model close to the ideal, is better than annual and perennial monoculture. Multiculture agroforestry is also a good combination of different types of trees that have architectural form different, thus ensuring the distribution of rain water can seep into the ground either

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ADSORPTION STUDY OF NICKEL FROM AQUEOUS SOLUTION BY COAL FLY ASH

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ABSTRACT

The presence of nickel in water can cause serious problem for environment and human health. Many important attempts to remove nickel as one of toxic metals from water are carried out. Among many separation methods, adsorption is considered as an interesting one. The use of coal fly ash as adsorbent was investigated in this study. The objectives of this study were to determine nickel removal by coal fly ash, nickel adsorption capacity of coal fly ash and to evaluate adsorption kinetics of nickel adsorption by coal fly ash. Preliminary adsorption study was carried out using nickel concentration 100 mg/L. To determine adsorption capacity, experiments using nickel concentration in the range 1-100 mg/L were carried out. To evaluate adsorption kinetics, a set of kinetics experiment using time duration 3 hours was carried out. The results obtained showed that coal fly ash had capability to remove nickel from aqueous solution, nickel adsorption capacity by coal fly ash could reach the value 0,3 mg/g. Adsorption kinetics data plotted to pseudo second-order rate equation.

Keywords: nickel, adsorption, coal fly ash, adsorption capacity, adsorption kinetics

INTRODUCTION

Metals pollutants are needed to be controlled before discharging to the aquatic system. These pollutants are known for their toxicity to the organism. Among these pollutants, nickel is considered as metal having potential to cause nasal and lung cancer (Lu et al., 2005).

There are various methods for metals pollutants control from aqueous solution. The most used method is precipitation. This method can be divided in to hydroxide, sulfide, and chelating precipitation (Fu and Wang, 2011). The problem encountered from precipitation is sludge generation that needs further treatment. The promising method is membrane filtration. Ultrafiltration, reverse osmosis, nanofiltration, and electrodialysis are the methods in the group of membrane filtration. The problem found from membrane filtration is the high cost of membrane. The interesting method that gets many attentions at this time is adsorption. Adsorption is a mass transfer process, metals ions are transferred to the surface of adsorbent material. Adsorption is relatively easy to operate and used for low concentration of metals.

The use of coal fly ash is interesting for metals adsorption. This material is a waste from coal combustion. In the country where the use of coal is dominant for power generation, the use of coal fly ash for heavy metals adsorption is considered as a good solution for waste management and waste treatment. Coal fly ash is used as adsorbents for cleaning flue gas, toxic metals from wastewater, inorganic components from wastewater, organic compounds from

wastewater, and dyes from wastewater (Ahmaruzzaman, 2010). There are many reports about metals removal (Zn, Cu, Pb, Cd) using coal fly ash (Weng and Huang, 2004; Erol et al., 2005; Balsamo et al., 2011).

The objectives of this study are to determinate nickel removal by coal fly ash, nickel adsorption capacity of coal fly ash, and to evaluate adsorption kinetics of nickel adsorption by coal fly ash.

MATERIALS AND METHODS

The sample of coal fly ash was obtained from thermal power plant Paiton, East Java. The coal fly ash was prepared by following to the previous work (Kuncoro and Fahmi, 2013). Coal fly ash was heated in the oven at 120°C for 24 hours. After heating, the coal fly ash sample was sieved in order to get the particle size of 149-250 µm. The quantity of 25 g coal fly ash was added to 100 mL of 0.1 M CH₃COOH then CH₃COOH solution containing coal fly ash was stirred for one hour and stabilized for 16 hours. After stabilization, the solution was filtered by Buchner filter. By filtering, we got coal fly ash. Coal fly ash was ready to use after being heated in the oven at the temperature of 120°C for 24 hours. Coal fly ash treated by acid and followed by base was obtained by adding NaOH to coal fly ash obtained by acid treatment, filtering, and heating for 24 hours at 120°C. The heavy metal solutions were prepared by dilution of metals salts Ni(NO₃)₂ in to demineralized water. The concentrations of heavy metal were prepared according to the need of experiments.

Preliminary adsorption study

A volume of 250 mL nickel solution with the concentration of 100 mg/L was placed in an Erlenmeyer flask. 20 g of coal fly ash was added to nickel solution and then nickel solution containing coal fly ash was stirred for 5 hours. After stirring the solution was stabilized and filtered. The solution filtered was analyzed by AAS (Shimadzu, Japan) to determine the concentration of nickel. The nickel removal is given by a simple formula:

$$R = [(C_0 - C_e) / C_0] \times 100\%$$

C_0 , C_e are initial metal concentration and equilibrium metal concentration, respectively.

Isotherm adsorption study

Six bottles containing nickel solution were used to perform isotherm adsorption study. The concentration of nickel was in the range of 1-100 mg/L. Each bottle was added by 15 g of coal fly ash. The bottles were shaken for 12 hours then solutions were filtered and analyzed by AAS (Shimadzu, Japan) to determine the concentration of nickel. The formula for quantification of nickel adsorbed is given by:

$$q = [(C_0 - C_e) / m] \times V$$

q , m , V are quantity of metal absorbed, mass of adsorbent, and volume of metal solution, respectively.

Isotherm adsorption data were plotted to Langmuir and Freundlich model. The equation for Langmuir model is (Aly and Luca, 2013) :

$$q_e = (Q_0 b C_e) / (1 + b C_e)$$

q_e , Q_0 , b , C_e are maximum metal adsorbed at equilibrium, capacity of adsorbent, constant related to adsorption energy, and equilibrium concentration of metal, respectively.

The equation for Freundlich model is (Aly and Luca, 2013):

$$q_e = K_f + (1/n) \ln C_e$$

q_e , K_f , n , C_e are maximum metal adsorbed at equilibrium, Freundlich constant, Freundlich constant representing adsorption intensity, and equilibrium concentration of metal, respectively.

Kinetics adsorption study

100 mL solution of nickel with the concentration 100 mg/L were placed in 6 bottles. Each of nickel solution was added by 15 g of coal fly ash. The solutions were shaken overnight. After shaking, the solutions were filtered and analyzed by AAS (Shimadzu, Japan) to determine the concentration of nickel for certain time interval (every hour).

Kinetics adsorption data were plotted to pseudo first order, pseudo second order, and intra- particle diffusion kinetic model. The equation for pseudo first order kinetic model is (Balsamo *et al.*, 2011):

$$\ln((q_e - q_t) / q_e) = -k t$$

q_e , q_t , k , t are maximum metal adsorbed, metal adsorbed at t , constant, and time, respectively.

The equation for pseudo second order kinetic model is (Balsamo *et al.*, 2011):

$$t/q_t = (1/(k q_e^2)) + (t/q_e)$$

t , q_t , k , q_e are time, metal adsorbed at t , constant, and maximum metal adsorbed, respectively.

The equation for intra-particle diffusion kinetic model is (Areco and dos Santos Afonso, 2010):

$$q_t = k t^{1/2}$$

q_t , k , t are metal adsorbed at t , constant, and time, respectively.

RESULTS

The results of preliminary nickel adsorption study is presented in **Fig. 1**. Using two types of coal fly ash (treated by acid, treated by acid and followed by base) gave different result of nickel removal. Coal fly ash treated by acid (CH_3COOH) gave 25% removal of nickel while coal fly ash treated by acid (CH_3COOH) and followed by base (NaOH) gave only 2% of nickel removal. For further experiments, coal fly ash treated by acid (CH_3COOH) was used.

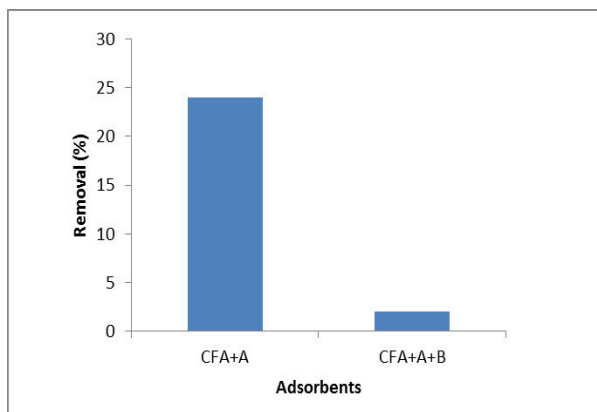


Figure. 1. Nickel adsorption on coal fly ash

(CFA+A = coal fly ash treated by acid, CFA+A+B = coal fly ash treated by acid and followed by base)

Quantification of nickel adsorbed by coal fly ash was determined by carried out a series of isotherm adsorption experiment. Isotherm adsorption data is presented in **Fig. 2**. Data consist of nickel equilibrium concentration and quantity of nickel adsorbed on coal fly ash. For nickel equilibrium concentration of 50 mg/L, adsorption capacity of coal fly ash was 0.3 mg/g. For nickel equilibrium concentration less than 50 mg/L, the values of adsorption capacity were less than 0.3 mg/L.

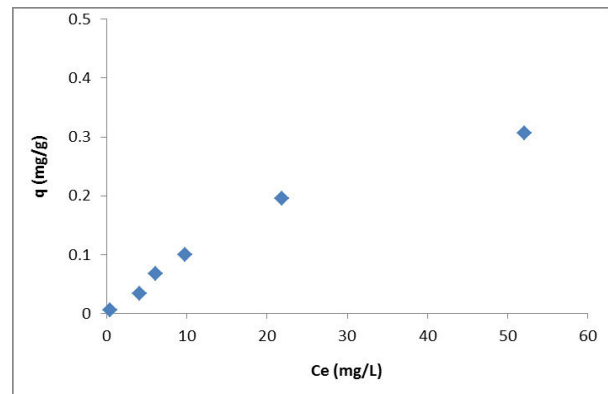


Figure. 2. Isotherm adsorption of nickel on coal fly ash

Isotherm adsorption data was plotted to Langmuir and Freundlich model, the results are presented in **Fig. 3** and **Fig. 4**. **Fig. 3** presents a graph of C_e versus C_e/q_e while **Fig. 4** presents a graph of $\ln C_e$ versus $\ln q_e$.

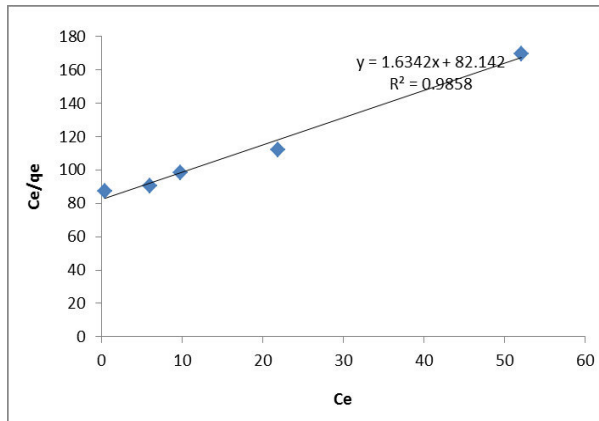


Figure. 3. Langmuir plot for nickel adsorption on coal fly ash

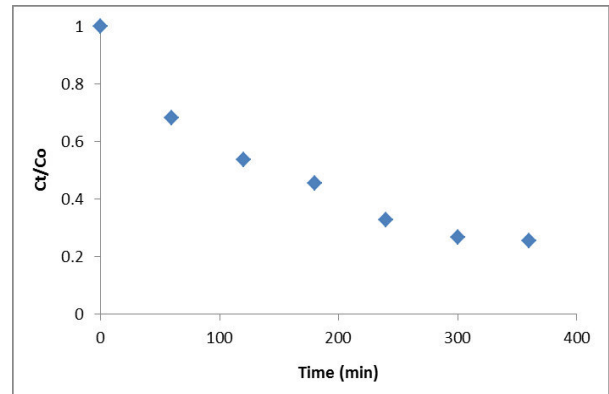


Figure. 5. Kinetics of nickel adsorption by coal fly ash

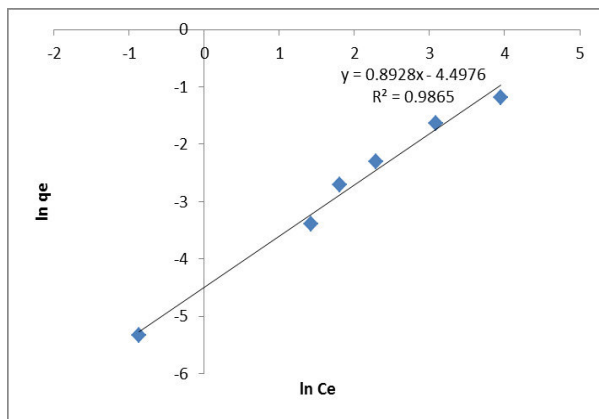


Figure. 4. Freundlich plot for nickel adsorption on coal fly ash

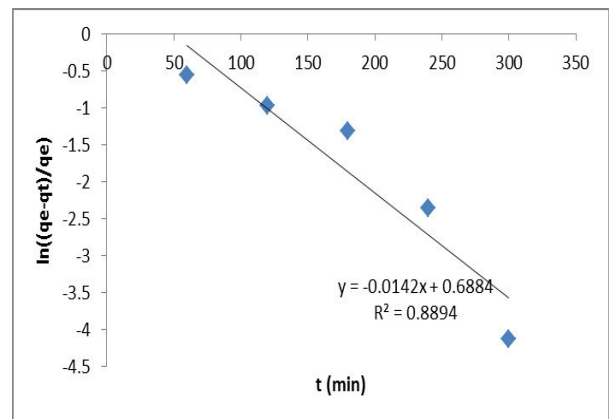


Figure 6. Pseudo first order kinetic plot for nickel adsorption on coal fly ash

The rate of nickel removal in adsorption by coal fly ash was determined by carried out a series of kinetics experiment. **Fig. 5** presents data of kinetics of nickel adsorption by coal fly ash. The nickel adsorption reached equilibrium after 300 minutes. The 50% of nickel removal was reached after 120 minutes.

Kinetics adsorption data was plotted to pseudo first order, pseudo second order, intra-particle diffusion kinetic model (**Fig. 6**, **Fig.7**, and **Fig. 8**). **Fig. 6** presents a graph of t versus $\ln((q_e - q_t)/q_e)$. **Fig. 7** presents a graph of t versus t/q_t . **Fig. 8** presents a graph of $t^{1/2}$ versus q_t .

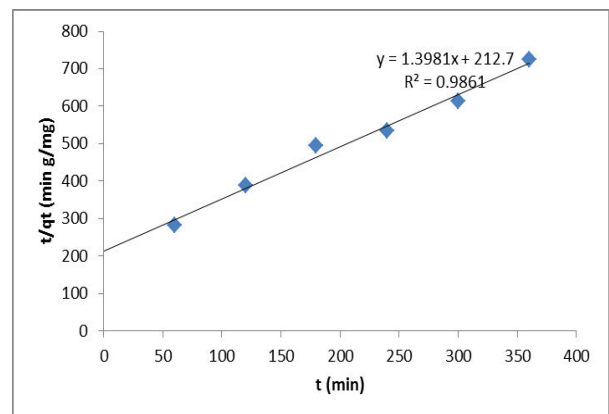


Figure. 7. Pseudo second order kinetic plot for nickel adsorption on coal fly ash

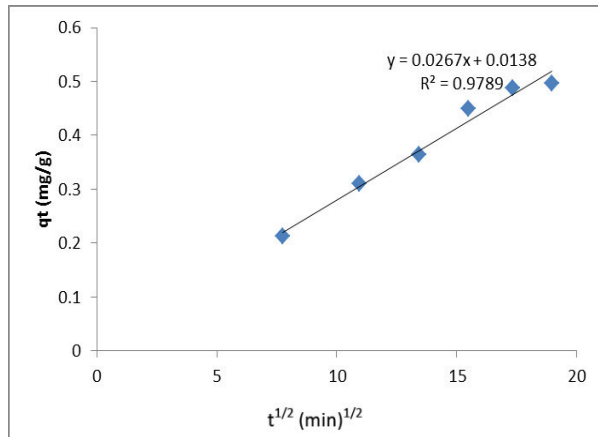


Figure 8. Intra-particle diffusion kinetic plot for nickel adsorption on coal fly ash

DISCUSSION

Preliminary adsorption study showed that coal fly ash treated by acid (CH_3COOH) had higher nickel adsorption than coal fly ash treated by acid (CH_3COOH) and followed by base (NaOH) (**Fig. 1**). This result was similar with the result of Pb adsorption by coal fly ash treated by acid and base. Nickel adsorption by coal fly ash treated by base was low (Munoz and Aller, 2011). Acid treatment ameliorate characteristics of coal fly ash to have interaction with metal.

Maximum adsorption capacity of coal fly ash for nickel removal in this study was 0.3 mg/g (**Fig. 2**). This result might be explained by acid treatment given to coal fly ash (Munoz and Aller, 2011). Various values were found for maximum capacity of coal fly ash for heavy metals removal. The maximum adsorption capacity of coal fly ash for Pb was 40 $\mu\text{g/g}$ (Munoz and Aller, 2012). For Cd removal, the maximum adsorption capacity was 0.5 mg/g (Balsamo

et al., 2011). The variation of maximum capacity adsorption depends on the source of coal and treatment given to coal fly ash. The chemical components of coal fly ash (alumina, silica, ferric oxide, calcium oxides, magnesium oxide and carbon) play important role for heavy metals – coal fly ash interaction (Ahmaruzzaman, 2010). Coal fly ash treated by different treatment gave different value of maximum adsorption capacity: raw coal fly ash in small size gave higher maximum adsorption capacity than coal fly ash treated by acid and followed by base (Balsamo *et al.*, 2011).

Langmuir and Freundlich model were applied to the isotherm adsorption data of nickel adsorption on coal fly ash (**Fig. 3 and Fig. 4**). Both models were suitable for isotherm data in this study. The values of correlation coefficient were high: 0.9858 and 0.9865 for Langmuir and Freundlich, respectively. According to Langmuir model, monolayer is formed during adsorption while Freundlich model developed based on heterogeneous surface.

Pseudo first order kinetic model was applied to the kinetics data of nickel adsorption on coal fly ash (**Fig. 6**). Correlation factor using this model was 0.8894.

Pseudo second order kinetic model was applied to the kinetics data (**Fig. 7**). Correlation factor using this model was 0.9861. This high correlation factor indicated that nickel adsorption on coal fly ash followed pseudo second order kinetic model. Pseudo second order kinetic model was the most applied model to heavy metals adsorption (Cu, Zn, Cd, Pb) (Areco and dos Santos Afonso, 2010; Munoz and Aller, 2011).

The last kinetic model applied to data kinetics was intra-particle kinetic model (**Fig. 8**). The value of correlation factor was 0.9789. This value was lower than the value obtained by pseudo second order kinetic model. It was also found in adsorption of copper, zinc, cadmium and lead that correlation factor of intra-particle kinetic model was lower than the value obtained by pseudo second order kinetic model (Areco and dos Santos Afonso, 2010).

CONCLUSION

In this study, coal fly ash treated by acid (CH_3COOH) gave better removal than coal fly ash treated by acid (CH_3COOH) and followed by base (NaOH). Maximum adsorption capacity of coal fly ash for nickel removal was 0.3 mg/g, the data of isotherm adsorption followed both Langmuir and Freundlich model. Kinetics data of nickel adsorption followed pseudo second order kinetic model.

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PLANKTON COMMUNITIES IN BENING RESERVOIR MADIUN

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ABSTRACT

Plankton often used as an indicator of water quality. The aim of this study was to investigate the plankton communities in relation to the physical and chemical characteristics of Bening reservoir Madiun during the dry season (March-July 2013). Plankton and surface water samples were collected from the six stations monthly during dry season (March-July 2013). Field sampling procedures were done at the reservoir, followed with identification and data analysis at laboratory using standard method. There are 16 genus of phytoplankton and 14 genus zooplankton which were distributed unevenly. *Synedra* was the most abundant of the overall phytoplankton samples. The most abundant zooplankton found during this investigation were *Cyclops* in March samples and *Nauplius* in April, May, June and July samples. The low abundance found in this study for the plankton communities might explained by the presence of planktivorous fishes and most probably low light penetration (low transparency).

Keywords: dry season, transparency, *Synedra*, *Cyclop*, *Nauplius*

INTRODUCTION

The productivity of any aquatic water body depends on the amount of plankton present in the water body. Adesalu and Nwankwo (2010) identified phytoplankton as one of the useful indicators of aquatic environmental quality because they act as early warning signals thereby provoking appropriate remediation. Growth and distribution of plankton (phytoplankton) depend on the carrying capacity of the environment and on the nutrients concentration both intracellular and extracellular (Davies et al. 2009). According to Suthers and Rissik (2009), the major limiting nutrients for phytoplankton are nitrogen in form of ammonium (NH_4^+),

nitrite (NO_2^-) and phosphate (PO_4^-). Nitrogen tends to be the limiting nutrients in marine systems, while phosphate in the limiting nutrient in the freshwater systems (Suthers and Rissik 2009). These two nutrients are needed for cell membranes and for proteins such as enzymes. Ezra and Nwankwo (2001) observed that changes in plankton population in Gubi Reservoir were influenced by physico-chemical parameters. Physico-chemical parameters also affect plankton distribution and species diversity (Astirin 2000; Sugianti 2006; Pirzan and Pong-Masak 2008; Qingyun 2008; Adesalu 2012).

Plankton distribution and abundance are affected by season (Ezra

and Nwankwo 2001; Davies 2009). Davies (2009) reported that in Minichinda Stream, Niger Delta, Nigeria, the wet season phytoplankton (6180 counts/ml) and zooplankton (1924 counts/ml) were higher than the dry season phytoplankton (4480 counts/ml) and zooplankton (1426 counts/ml). Diversity and composition of algae in the Niger Delta water bodies varies seasonally with peak in dry season (Yakubu et al. 2000). Seasonal variations affect the physico-chemical variables thus causing variation in abundance and diversity of plankton. Variation in some of the physical and chemical parameters such as rainfall, temperature, salinity, nitrate-nitrogen, phosphate-phosphorus, sulphate, biological oxygen demand and chemical oxygen demand have been reported to influence phytoplankton abundance (Adesalu et al. 2010). There is no information on the seasonal abundance and distribution of phytoplankton and zooplankton as well as the water quality of this reservoir. The present study attempts to provide such vital information for future references. Bening reservoir is one of reservoir in the Brantas River that was built in 1978 and completed in 1982. This reservoir is located at Petung, Pajaran Village, Saradan district, Madiun, East Java. Purposes of built this reservoir is to provide irrigation water for 9,120 ha (Nganjuk District, Gondang District and Rejoso District), flood control, tourism and aquaculture (Jasa Tirta 2012).

The aim of this study was to investigate the plankton communities in relation to the physical and chemical characteristics of Bening reservoir Madiun during the dry season (Maret-Juli 2013). The study was carried out in March-July 2013. Study site is located at Bening Reservoir, Petung, Pajaran village, Saradan district,

Madiun, East Java. This reservoir has a vast pool 5,7 km with an effective volume 23,9 million m³ in 2007. Type of reservoir is homogeneous pile with a volume of pile 800.000 m³. Jasa Tirta (2012) reported that the low water level at elevation 96,40 m, with a high water elevation 108,60 m and 109,40 m flood water level.

MATERIAL AND METHODS

Plankton and surface water samples were collected from the six stations monthly during dry season (Maret-Juli 2013). Plankton samples were collected with 20 and 62- μ m plankton nets for phytoplankton and zooplankton, respectively (Edmondson and Winburg 1971). Phytoplankton samples were preserved in Lugol's solution while zooplankton samples were preserved in 4% formalin. The species were identified using *Fresh Water Biology* (Edmonson 1959), *The Plankton of South Vietnam* (Shirota 1966), dan *The Marine and Freshwater Plankton* (Davis et al. 1955). Enumeration was done by using binocular microscope.

Surface water temperature was measured in-situ using mercury in glass thermometer while pH was determined using pH meter. Water transparency (vertical visibility) was estimated using a standard secchi disc of 20 cm diameter while concentration of dissolved oxygen (DO) was determined using DO meter. Water samples were collected with a bottle and placed in a cooler box before being brought to the laboratory for analysis NH₄⁺, NO₃⁻, and PO₄³⁻ according to the standard methods.

RESULT AND DISCUSSION

The results of the physico-chemical parameters analyzed in this study are presented in **Table 1**.

Table 1. Variation in physico-chemical parameters in Bening reservoir Madiun

Parameter	March 2013	April 2013	May 2013	June 2013	July 2013	Average	SD
Transparency (cm)	68	56.7	67.3	61	63	63.20	4.66
Suhu Udara ($^{\circ}$ C)	30	32.4	30.67	29.6	30.67	30.67	1.07
Surface water temperature ($^{\circ}$ C)	31.3	32.6	31.47	31.2	30.9	31.49	0.65
DO	8	10.33	9.37	9	11.27	9.59	1.25
pH	8.4	7.86	8.47	8.41	8.36	8.30	0.25
NO ₃ (mg/L)	0.603	0.5725	0.633	0.512	0.542	0.57	0.05
PO ₄ (mg/L)	0.1045	0.109	0.10	0.009	0.059	0.08	0.04
NH ₃ (mg/L)	0.245	0.35	0.14	0.21	0.28	0.25	0.08

The few water quality parameters that were analysed in the studied reservoir indicate a general level that is acceptable in comparison to the natural levels in freshwater (Sinkala et al. 2002). Transparency of water was low in the study area, pH was more or less alkaline, ranging from 7.86 to 8.47. These values are in agreement with the pH of most natural

waters that ranges between 6.0 and 8.5 (Chapman, 1992). Phosphate and nitrate are in the normal and acceptable ranges and did not show any significant differences during the study period. NO₃ concentrations were generally high. This agrees with other studies in reservoirs (Pirzan and Pong-Masak 2008).

Table 2. Composition and abundance of the plankton in Bening Reservoir Madiun during the dry season

N0.	Genus	March 2013	April 2013	May 2013	June 2013	July 2013	Average
	PHYTOPLANKTON						
1	Ceratium	140	85	80	50	30	77
2	Closterium	170	85	85	45	0	77
3	Cocconeis	0	45	25	45	90	41
4	Cymbella	30	20	20	15	10	19
5	Denticula	40	45	35	35	50	41
6	Euglena	40	50	35	40	60	45
7	Fragilaria	150	135	105	100	120	122
8	Gyrosigma	80	90	65	70	100	81
9	Melosira	70	50	45	35	30	46
10	Navicula	370	400	295	310	430	361
11	Nitzschia	250	210	170	150	170	190
12	Peridinium	0	25	15	25	50	23
13	Rhopalodia	60	40	35	25	20	36
14	Staurastrum	130	115	90	85	100	104
15	Surirela	20	35	25	30	50	32
16	Synedra	1040	1395	960	1135	1750	1256
	ZOOPLANKTON						
1	Asplachna	10	20	15	20	30	19
2	Cyclop	1300	1175	915	850	1050	1058
3	Diaptomus	890	775	610	555	660	698

4	Diffugia	30	15	15	10	0	14
5	Eubranchipus	60	85	60	70	110	77
6	Euclanis	0	20	10	20	40	18
7	Filinia	20	40	25	35	60	36
8	Kellicottia	30	60	40	55	90	55
9	Keratella	20	90	50	85	160	81
10	Lecane	150	140	110	105	130	127
11	Nauplius	1120	1155	860	875	1190	1040
12	Notholca	60	75	55	60	90	68
13	Polyarthra	50	40	35	30	30	37
14	Sida	20	40	25	35	60	36

The reservoir plankton identified and counted in March-July 2013 is presented in **Table 2**. This study found that the diversity and abundance of plankton species varied monthly. *Synedra* was the most abundant of the overall phytoplankton samples. *Synedra* are described as having narrow frustules, free-floating or sometimes in colonies. They also can be benthic, meaning they grow on different surfaces. The valves are linear and on some, slightly curved. *Synedras* live in a variety of habitats, so can be found throughout the world. As a species within the algal community, *Synedras* have specific ecological requirements and tolerances. Nitrates, phosphates, and hydrogen ions are three of the major chemicals present in water, and all three have effects on the health of aquatic organisms. Nitrate is the most oxidized form of nitrogen found in the natural world. It is one of the most water-soluble anions known. Plants need nitrogen as a nutrient, and most plants prefer nitrate to ammonia. *Synedras* are forms of algae, so they also need a supply of nitrate. NO_3 concentrations in Bening reservoir were generally high.

The most abundant zooplankton found during this investigation were *Cyclops* in March samples and *Nauplius* in April, May, June and July samples. *Cyclops* is one of the most common genus of

freshwater copepods while *nauplius* is the earliest free-living stage in the development of most crustaceans, except in the majority of the Malacostraca.

The lower abundance found in this study for the plankton communities might explained by the presence of planktivorous fishes and most probably low light penetration (low transparency). Though fish abundance was not part of this study, it was noted that fishes were present in the reservoirs. Humans were observed actively fishing on the reservoirs. Planktivorous organisms have preferences for specific food items (Wetzel 1983). Arcifa et al. (1986) concluded that plankton proliferation is greatly affected by the predator-prey relationships in reservoirs.

Based on the present observation, Bening reservoir is not rich in plankton and the nutrient status is high enough to support this plankton population during dry season. It will support fisheries production. According to Townsend et al. (2000) and Miller (2005), plankton communities serve as a base for the food chain that supports the commercial fisheries.

CONCLUSION

This paper investigated the plankton communities in relation to the physical and chemical characteristics of Bening reservoir Madiun during the

dry season (March-July 2013). Water quality throughout the study area was at acceptable levels, and did not significantly differ between time. The diversity and abundance of plankton communities in the study area were influenced by NO₃ concentrations, transparency and predator-prey relationships. Zooplankton species abundance was at a low level, possibly due to the presence of planktivorous fish in the reservoirs.

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MACROINVERTEBRATE DIVERSITY AT SENGGURUH AND SUTAMI RESERVOIRS REGENCY OF MALANG

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ABSTRACT

Sengguruh and Sutami reservoirs are ways of water storage to anticipate of dryness disaster around Malang regency. Water from both reservoirs is used for irrigation, tourism and Hydro Electric Power Plant (HEPP). Activities around the reservoir influence on water quality and also influence the diversity of organism. The purpose of this study to know the diversity of aquatic macroinvertebrate in Sengguruh and Sutami reservoirs.

The research results showed that the macroinvertebrate families in Sengguruh Reservoir consists of Atyidae, Thiaridae, Pleuroceridae II, Tubificidae I, Pleuroceridae III, Tubificidae II, Chironomidae I and Hydropsychidae. Families of macroinvertebrate in Sutami Reservoir are Atyidae, Libellulidae, Chironomidae II, Lestidae, Pleuroceridae II, Caenidae, Thiaridae, Pleuroceridae I, Coenagrionidae, Aeshnidae, Belostomatidae, Mesoveliidae, Gerridae, Ampulariidae, Viviparidae, Hydrobiidae, Tubificidae II and Curculionidae. The macroinvertebrate diversity index in Sengguruh Reservoir is 1,768 and Sutami Reservoir is 2,611, both classified as moderate biota community or the diversity are being abundant.

Keyword: *Diversity, Macroinvertebrate, Sengguruh reservoir, Sutami reservoir*

INTRODUCTION

Lentik waters such as the Sutami Reservoir Regency of Malang, which has a primary function of the operator floods, Hydro Electric Power Plant (HEPP), a provider of irrigation water, tourism and freshwater fisheries. The whole activity can reduce the river in terms of ecological sustainability. Because almost every human activity that is likely to risk mutilate natural ecosystems. Seeing the potential and wealth Sutami Reservoir Regency of Malang, in an effort to defend the sustainability of the water, is to look at productivity levels, with the main

parameters of the oxygen measurement. Because the level of productivity is a measure of the quality of waters (Ana, 2006).

Other problems faced by Sengguruh and Sutami Reservoirs included in the Brantas river flow is environmental damage. According to Perum Jasa Tirta in Faturrohman (2008), problems in the District of River Flow is a form of environmental degradation Brantas watershed upstream part, among them the condition of the water catch Brantas river area watershed upstream of the declining due to illegal logging and

poor land management attention to the concept conservation land.

All forms of activity around the reservoir will affect reservoir conditions, physical condition or ecosystem in the reservoir. According Wardhana (1999), pros and cons of an activity is influenced by the surrounding waters. Often times there are activities that may lower water quality, which in turn will disrupt the lives of water biota. Tarumingkeng (1994) also noted that environmental conditions affect the biological diversity forms and many types of organism or biodiversity, and instead the diversity and abundance of living creatures also determine the range of conditions.

An environmental condition are still good and has not been contaminated, it can be habitation to many organisms from different trophic levels. However in the event of a contamination, it is likely that only certain organisms can tolerate the change of environment. Doing so may cause the amount of pollution tolerant organisms is to be increased and this will of course affect the diversity of types of organisms in the area, which eventually also impact on the balance of the environment. Barus (2004) states that an unpolluted waters will show a balanced number of individuals of all species available. Instead, contaminated waters would have led dissemination and uneven numbers of individuals tend to have certain species that are dominant.

The results of research by Agrista (2005) concluded that macroinvertebrate fauna types that can be used as biological indicators of not contaminated water quality is family Cinygmula. Other studies related to the diversity of macroinvertebrate fauna and water quality of the reservoir by Fauziyyah (2012),

indicating that the macroinvertebrate fauna in the reservoir Wonorejo Tulungagung District find Aeshnidae, Pulmonata, Crustacean, Libellulidae, Hemiptera and Chironomidae which shows that the condition of the waters based on macroinvertebrate fauna specimens have quality indicators light polluted waters.

The purpose of this study to know the diversity of aquatic macroinvertebrate in Sengguruh and Sutami reservoirs. Diversity important for scrutiny because research related to environmental conditions and it will be directional to land conservation.

MATERIALS AND METHODS

The study was carried out in May 2013 on the Sengguruh and Sutami Reservoir, Malang regency. Identification of samples were done in Ecology and Optic Laboratories, State Islamic University of Maulana Malik Ibrahim Malang.

Description of Study Area

Sampling of macroinvertebrate, water and substrate were done in 5 stations each reservoir. For Sengguruh Reservoir, the station I was at the water inlet area of the Brantas river, station II was at the input of the Lesti river area, the station III was located in the central region reservoir (confluence between the Brantas river and the Lesti river), stations IV was on reservoir production area, and station V was the near the protected forest area. For Sutami Reservoir, the station I was in the area where the water comes out, the station II was located on the water inlet area of the Brantas River, Station III was in the tourist area of the reservoir, station IV was closed

to the rice fields, and station V was the near protected forest area.

Sampling Removal

The sample taken at 08:00 until 10:00 a.m. at their predetermined substations. Three substations direct result of accumulated into a station. Macroinvertebrate collection on the surface and water body was done using Surber Net. The use of these tools was done by 3 times. Oscillation performed along 2 meters horizontally with constant speed around 10 cm/sec on each substation. Samples were netted and captured in bottles bearing Surber Net poured into plastic tray filled with water, then filtered with a filter with hole of diameter 0,5 millimeters and the remaining material was sorted by hand. Samples included in the plastic clip then preserved with 70% alcohol and labeled.

RESULTS AND DISCUSSION

The Atyidae family is the most common family that found in Sengguruh

Table 1. Macroinvertebrate that caught in Sengguruh reservoir

No.	Macroinvertebrate		Station					Total
	Order	Family	I	II	III	IV	V	
1.	Decapod	Atyidae	2	4	3	2	3	14
2.	Mesogastropoda	Thiaridae	-	6	-	-	-	6
		Pleuroceridae II	-	2	-	-	-	2
		Pleuroceridae III	-	4	5	-	-	9
3.	Tubificida	Tubificidae I	-	-	3	-	-	3
		Tubificidae II	1	-	2	-	-	3
4.	Diptera	Chironomidae I	2	-	-	-	-	2
5.	Trichoptera	Hydropsychidae	-	-	-	1	-	1
Individual Total (N)			5	16	13	3	3	40
Families Total (S)			3	4	4	2	1	8

A large number of Atyidae Family found at each station in Sengguruh Reservoir showed that the five stations have conditions that allow for the survival of the family Atyidae. The small number

Research Design

This research was quantitative descriptive research type. The sample using exploratory methods, namely observation or direct sampling in the research location. Parameters measured in this study, namely Diversity Index (H') of the Shannon-Wiener macroinvertebrate fauna in Sengguruh and Sutami reservoirs Malang regency.

Data Analysis

Diversity index calculated by the Shannon-Wiener diversity index :

$$H' = - \sum_{i=1}^S pi Ln pi$$

Description of formulas:

H' : diversity index

pi : ni/N

ni : number of individuals of the ith type

S : number of genera

N : total number of individuals

reservoir. While the Hydropsychidae family is the least.

of family was found to indicate that the limited availability of food in the habitat. Not finding of macroinvertebrate families in station observations show that the station did not have the conditions that support life for families of

macroinvertebrates were not found them. It can be caused by the unavailability of food, as well as abiotic factors that do not support them to breed. According to Suin (1997), physical and chemical factors were almost evenly distributed within the habitat and availability of food for the organisms that live in it will determine the organisms that normally live in groups or random.

Macroinvertebrate in Sutami Reservoir is more abundance than

Table 2. Macroinvertebrate that caught in Sutami Reservoir

No.	Macroinvertebrate		Station					Total
	Order	Family	I	II	III	IV	V	
1.	Decapod	Atyidae	2	-	-	1	2	5
2.	Odonata	Libellulidae	-	1	1	-	-	2
		Coenagrionidae	1	-	-	-	-	1
		Lestidae	-	1	4	-	1	6
		Aeshnidae	-	-	-	1	-	1
3.	Hemiptera	Belostomatidae	-	1	-	-	-	1
		Mesoveliidae	1	-	-	-	-	1
		Gerridae	-	1	-	-	-	1
4.	Mesogastropoda	Ampuliriidae	-	-	1	-	-	1
		Viviparidae	-	-	1	-	-	1
		Thiaridae	-	-	-	3	-	3
		Pleuroceridae I	-	-	-	3	-	3
		Pleuroceridae II	-	-	-	4	-	4
		Hydrobiidae	-	-	1	-	-	1
5.	Tubificida	Tubificidae II	1	-	-	-	-	1
6.	Diptera	Chironomidae II	1	-	1	-	-	2
7.	Ephemeroptera	Caenidae	2	3	1	-	1	7
8.	Coleoptera	Curculionidae	1	-	-	-	-	1
Individual Total (N)			9	7	10	12	4	42
Families Total (S)			7	3	4	3	3	18

Macroinvertebrate accumulation diversity index values in Sengguruh and Sutami Reservoirs can be seen that the diversity of aquatic macroinvertebrates in Sengguruh Reservoir (1,768) is lower than the Sutami reservoir (2,611). The high diversity of macroinvertebrates in the water compared to the Sengguruh and Sutami reservoirs can be caused from a

Sengguruh Reservoir. The family total in Sengguruh Reservoir is 8, while in Sutami Reservoir is 18. The Caenidae, Lestidae and Atyidae families have total individu higher than other family.

variety of aspects, including biotic factors such as the availability of food for aquatic macroinvertebrates in the more supportive. In addition, abiotic environmental factors in Sutami reservoir more support for the development of existing macroinvertebrates in these waters. This causes some aquatic macroinvertebrates prefer Sutami reservoir as habitat.

Table 3. Comparison of Diversity Index (H') at Sengguruh and Sutami Reservoirs

No.	Station	H'		Classification (Fachrul, 2007)
		Sengguruh Reservoir	Sutami Reservoir	
1.	I	1,055	1,889	$H' > 3$: High Diversity $1 < H' < 3$: Medium Diversity $H' < 1$: Low Diversity
2.	II	1,321	1,475	
3.	III	1,332	1,748	
4.	IV	0,367	1,266	
5.	V	0	1,040	
	Cumulative	1,768	2,611	

Diversity of macroinvertebrates is highest in Sengguruh Reservoir at Station III (middle of the reservoir area which is a meeting between the Brantas River and Lesti River) and lowest in Station V (near the protected forest area). The high diversity at Station III allegedly because of the many ingredients both organic and inorganic pollutants. Organic material can be derived from waste and impurities that enter the waters along with the flow of water from houses and factories in the stream Brantas River or Lesti River. Doing so may lead to the availability of food for macroinvertebrates are tolerant to environmental III Station. The low diversity in Station V allegedly because the station is rarely found in the activity, so that causes water conditions tend to be fixed and do not allow for other macroinvertebrate life.

Highest diversity of macroinvertebrates Sutami reservoir occurred at Station I (the area where the water comes out) and the lowest occurred in Station V (near protected forests). High diversity that occurs in the station I because suspected contaminants at the base of the reservoir suspended. It can provide enough food for macroinvertebrates are found in this station. While the low diversity index at Station V presumably because they are rarely found that human activities can

trigger a great variety of macroinvertebrates. Thus, diversity is relatively fixed station.

According to Odum (1993), species diversity is affected by the division or distribution of individuals of each species, although many species of a community but spread unevenly causing the low value of diversity. Based on the criteria in **Table 3**, it can be seen that in general the state of Sengguruh and Sutami reservoirs have a level of diversity or community stability being. It is based on the value of the macroinvertebrate diversity indices cumulatively, both in the Sengguruh and Sutami reservoirs still lies between 1 and 3. However, if viewed per station Sengguruh Reservoir, for the first station (water input area of the Brantas River) $H' = 1,055$, Station II (local input from the Lesti River) $H' = 1,321$ and a third station (central region reservoir which is a meeting between the Brantas River and Lesti River) $H' = 1,332$ levels were in the diversity or community stability biota being. While the IV Station (regional expenditure reservoir) $H' = 0,367$ and Station V (near the protected forest area) $H' = 0$ belonging to communities biota unstable or low diversity.

Macroinvertebrate diversity in Sutami Reservoir if monitored per station, it can be seen that the Station I (the area where the water comes out) $H' = 1,889$,

Station II (water input area of the Brantas River) $H' = 1,475$, Station III (tourist area reservoirs) $H' = 1,748$, Station IV (near the rice field area) $H' = 1,266$ and Station V (near the protected forest area) $H' = 1,040$ including biota community stability or moderate diversity. Although classified as a place that shows human activities became more abundant, pollution levels Sutami lower than Sengguruh Reservoir. It can be caused due Sutami Reservoir wider than Reservoir Sengguruh can be quickly parse incoming waste into the reservoir.

The big difference in the results of the determination of the level of pollution through bioindicators of quality and macroinvertebrate diversity indexes allegedly due to abiotic factors that differ between the two waters, so little macroinvertebrates found. Some families were found to indicate that they are tolerant to abiotic environmental waters. In addition, the difference between the amounts of pollution levels by using bioindicators of macroinvertebrate diversity indexes also influences the results of the different conclusions. Limitations researchers can also be a factor at least macroinvertebrate are found, such as the difficult terrain and the limitations of research tools. Wardhana (2006) states that the use of macroinvertebrate as bio indicators in practice it is very difficult to include all members of the population as a sample of aquatic biota. Various constraints such as the availability of time, effort, cost, terrain, and vast study area are obstacles that are often encountered. Therefore macroinvertebrate sampling as samples to monitor the water quality of the sampled only a small fraction of the population there.

CONCLUSION

The research results showed that the macroinvertebrates in Sengguruh reservoir consists of 14 Atyidae, 6 Thiaridae, 2 Pleuroceridae II, 2 Tubificidae II, 9 Pleuroceridae III, 3 Tubificidae I, 2 Chironomidae I and a Hydropsychidae. In Sutami reservoir consists of 5 Atyidae, 2 Chironomidae, 2 Libellulidae II, 6 Lestidae, 4 Pleuroceridae II, 7 Caenidae, 3 Thiaridae, 3 Pleuroceridae I, and Coenagrionidae, Aeshnidae, Belostomatidae, Mesoveliidae, Gerridae, an Ampulariidae, Viviparidae, Hydrobiidae, Tubificidae II, Curculionidae. The macroinvertebrate diversity index in Sengguruh reservoir (1,768) and Sutami reservoir (2,611), both classified as moderate biota community or the diversity are being abundant.

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CHARACTERIZATION OF EXTERNAL MORPHOLOGY ON VARIOUS SEEDS IN PURWODADI BOTANIC GARDEN

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ABSTRACT

Seed is a form of mini plants (embryos), endosperm and other food reserves, with protection that consist skin seed and in the certain seeds contain additional structure. Basic knowledge about seed characterization is very important to be able deal with various problems in the field of seed technology. Seed characterization is needed on the process of species identification. The aim of this study was to know external morphology character of various seeds in Purwodadi Botanic Garden, which can be used for basic identification of certain seeds. Several parameters were observed in external morphology seeds characterization include size (length and width), diameter or thickness, weight, color, surface, shape, dispersion mode, pattern and type of germination, and storage character. Characterization of external morphology in 48 species of selected seeds in Purwodadi Botanic Garden showed that there are variations in size, diameter, weight and shape of selected seeds. Seed color ranged between white, brown, and black up to red. Surface tends to smooth, some are rough and coarse. Many seeds spread by wind, animals, water and explosion; mostly included in orthodox seeds with epigeal type of germination and simultaneously germination patterns. Detailed explanation of external morphology character on selected seeds can be seen in this paper.

Keywords

character, morphology, Purwodadi Botanic Garden, seed

INTRODUCTION

Seed is a form of mini plants (embryos), endosperm and other food reserves, with protection that consist skin seed and in the certain seeds contain additional structure. They are still in a state of unfettered development (Sutopo, 2002; Justice and Bass, 2002). Seed is one part of the plant that serves as a unit of deployment (dispersal units) and plant propagation naturally. Seeds are the most sophisticated means of propagation in the plant kingdom, and the most complex structure that a plant produces. Seeds range in size from dust-like orchid seeds that can contain up to 1 million seeds in one gram, to the giant Coco de Mer or *Lodoicea maldivica* (Poles, 2009).

Basic knowledge about seed characterization is very important to be able deal with various problems in the field of seed technology, such as hard seed germination (Mugnisjah *et al.*, 1994). Seed characterization is needed on the process of species identification of certain seed, one of them is external morphology characterization. That is characterize of physical seed, such as shape, weight, diameter, color, surface or the other. Characters most frequently used for taxonomic purposes are seed surface, seed shape and presence or absence of a shiny surface (Degano *et al.*, 1997). Knowledge of morphological characteristics of seeds is important to activities designed to maintain biodiversity and for understanding and describing germinative processes. Study of the characteristics of seeds and seedlings can provide information on necessary to identify species in the field and among seed samples (Abud *et al.*, 2012).

Purwodadi Botanic Garden as an ex-situ conservation organization have an unit of seeds collection, that have an assignment of conserve seed lowland dry. One of them is characterize of physical seeds and germinated. With a high diversity of seeds in Purwodadi Botanic

Garden, it is necessary to characterize of seeds to support plant conservation efforts.

Aim of this study is to know external morphology character of various seeds in Purwodadi Botanic Garden, which can be used for basic identification of certain seeds.

MATERIALS AND METHODS

This research was carried out at August 2012 until April 2013 in laboratory of seed and seedbed of Purwodadi Botanic Garden, Pasuruan, East Java. The tools used were digital scales, ruler, graph paper, digital camera, digital caliper, seedbed and stationery. Materials used are various seed selected (based on the result of seed monitoring and collecting at August 2012 until April 2013) and sand for seed sowing.

Several parameters were observed in external morphology seeds characterization include size (length and width; cm), diameter or thickness (cm), weight (g), color, surface, shape, dispersion mode, pattern and type of germination, and storage character. Immediately after harvesting, four replicates of 100 seeds each were weighed and three replicates of 40 seeds each were measured to estimate the average seed weight and seed size (length; width and thickness) (Yang *et al.*, 2008).

Size of seeds observed using graph paper, especially for small-sized seed. Diameter or thickness was measured using digital caliper. Weight of seed was measured by digital scale. Observation of seed surface differentiated into smooth, glabrous, wrinkled, ribbed, hairy,

winged, pulpy, coarse and rough. Seed shape differentiated into symmetrical globular, obconical, reniform, oblong, obpyriform, napiform, ovoid, conical, campanulate, ellipsoid, globose, plateriform, pyriform, heart-shaped, semi-circle, 3-dimension, irregular and rhomboid. Dispersion mode divided into spread with wind, water or animal. Germination pattern divided into simultaneously and gradually, while germination type divided into epigeal, hypogeal and semi hypogeal. To determine pattern and type of germination, seed sowing in seedbed. Storage character of seed divided by orthodox, recalcitrant and intermediate seed.

RESULTS

Table 1. Characterization of external morphology on various seeds in Purwodadi Botanic Garden (Murray, 1987; Kyndt et al., 2009; Lowry et al., 1995; Scutres, 2009; Lee, 1984; Samansiri and Weerakoon, 2008; Navie, 2010)

Characterization of external morphology												
No	Species	Family	Size (cm)	Thick (cm)	Weight (gr)	Colour	Surface	Shape	Dispersion mode	Germination Pattern	Germination Type	Storage character
1	<i>Acacia cathecu</i>	Mim.	1,01x0,85	0,14	0,08	Brown, center of light brown	Smooth	Plateriform, globose	Animal	Simultaneously	Hypogeal	Orthodox
2	<i>Acacia oraria</i>	Mim.	0,4x0,36	0,22	0,03	Black	Smooth	Globose-irregular	Animal	Gradually	Epigeal	Orthodox
3	<i>Adansonia digitata</i>	Bomb.	1,29x1,06	0,76	0,54	Brown	Coarse	Reniform	Animal	Gradually	Epigeal	Orthodox
4	<i>Adenanthera pavonina</i>	Mim.	0,9x0,82	0,63	0,28	Red	Smooth, shine	Globose	Animal	Gradually	Epigeal	Orthodox
5	<i>Albizia acle</i>	Mim.	0,84x0,49	0,14	0,05	Light brown, center of fawn-colored	Smooth	Globose-oblong	Wind	Gradually	Epigeal	Orthodox
6	<i>Albizia lebbeck</i>	Mim.	0,91x0,65	0,21	0,11	Light brown	Smooth	Ovoid	Wind	Gradually	Epigeal	Orthodox
7	<i>Annona muricata</i>	Annon.	1,56x0,86	0,6	0,32	Dark brown - black	Smooth, shine	Oblong	Animal	Gradually	Epigeal	Orthodox
8	<i>Antidesma bunius</i>	Euph.	0,65x0,44	0,35	0,03	Early pink, if kept turn into dark brown	Rough, wrinkled	Ovoid	Animal	Gradually	Epigeal	Orthodox
9	<i>Argyrea hookeri</i>	Convul.	0,77x0,53	0,54	0,17	Fawn-colored	Coarse	Oblong	Animal	Gradually	Epigeal	Orthodox
10	<i>Arctobotrys uncinatus</i>	Annon.	1,9x1,3	0,72	1,06	Brown	Rough	Oblong	Animal	Gradually	Epigeal	Orthodox
11	<i>Asclepias curassavica</i>	Asclep.	0,61x0,28	0,05		Dark brown	Smooth	Oblong	Explosion	Simultaneously	Epigeal	Orthodox
12	<i>Bauhinia acuminata</i>	Caes.	0,74x0,51	0,35	0,11	Brown	Smooth	Ovoid	Explosion	Simultaneously	Epigeal	Orthodox
13	<i>Bauhinia purpurea var.</i>	Caes.	1,42x1,22	0,25	0,3	Dark brown	Smooth, ribbed	Plateriform	Explosion	Gradually	Semi hypogeal	Orthodox

14	<i>Bauhinia purpurea</i> <i>variegata</i> var. <i>variegata</i>	Caes.	1,4x1,17	0,22	0,19	Dark brown	Rough, wrinkled	Plateriform	Explosion	Simultaneously	Semi - hypogaeal	Orthodox
15	<i>Bauhinia winitii</i>	Caes.	1,36x1	0,47	0,5	Light – dark brown	Smooth, shine	Oblong- conical	Explosion	Simultaneously	Semi - hypogaeal	Orthodox
16	<i>Bouea oppositifolia</i>	Anac.	2,04x1,04	0,61	0,58	Orange – yellowish red	Hairy	Oblong	Animal	Simultaneously	Hypogaeal	Recalcitrant
17	<i>Buchanania arborescens</i>	Anac.	1,06x0,92	0,61	0,24	Gray	Coarse	Globose	Animal	Gradually	Epigeal	Orthodox
18	<i>Caesalpinia gilliesii</i>	Caes.	0,74x0,56	0,18	0,04	Blackish brown, end tawny	Smooth	Globose	Water	Simultaneously	Epigeal	Orthodox
19	<i>Caesalpinia pulcherrima</i>	Caes.	0,96x0,72	0,32	0,12	Brownish green	Smooth	Pyriform	Water	Simultaneously	Epigeal	Orthodox
20	<i>Caesalpinia sappan</i>	Caes.	1,6x0,85	0,56	0,52	Cream	Smooth	Oblong- pyriform- ovoid	Water	Simultaneously	Epigeal	Orthodox
21	<i>Calliandra haematocephala</i>	Papil.	0,84x0,54	0,26	0,1	Light brown, dark brown shade	Smooth, shine	Ellipsoid	Animal	Simultaneously	Epigeal	Orthodox
22	<i>Calophyllum inophyllum</i>	Clus.	2,88x2,46	2,35	5,74	Fawn-colored	Rough until smooth	Globose	Animal	Gradually	Hypogaeal	Orthodox
23	<i>Cassia surratensis</i>	Caes.	0,66x0,39	0,13	0,02	Brown, center of light brown	Smooth	Oblong	Explosion	Gradually	Epigeal	Orthodox
24	<i>Clausena lansium</i>	Rut.	1,5x0,89	0,47	0,44	Light green, end of brown fibrous	Smooth	Pyriform	Animal (bird)	Simultaneously	Epigeal	Recalcitrant
25	<i>Clitoria ternatea</i>	Papil.	0,6x0,34	0,26	0,05	Black	Smooth	Oblong	Animal	Gradually	Epigeal	Orthodox
26	<i>Coix lacryma-jobi</i>	Poac.	0,98x0,74	0,66	0,17	White milk - grey	Smooth, shine	Ovoid	Water	Simultaneously	Hypogaeal	Orthodox
27	<i>Crescentia mirabilis</i>	Bign.	0,77x0,52	0,22	0,02	Dark brown – brownish black	Smooth	Heart-shaped	Animal	Simultaneously	Epigeal	Orthodox
28	<i>Diospyros forbesii</i>	Eben.	0,87x0,44	0,29	0,09	Black	Rough until smooth	Semicircle	Animal	Gradually	Epigeal	Recalcitrant
29	<i>Diospyros javanica</i>	Eben.	1,14x0,56	0,37		Golden brown	Rough	Semicircle	Animal	Gradually	Epigeal	Recalcitrant
30	<i>Emblica officinalis</i>	Euph.	0,57x0,29	0,23	0,02	Golden brown – dark brown	Smooth	3-dimension	Explosion	Gradually	Epigeal	Orthodox
31	<i>Euodia ridleyi</i>	Rut.	0,28x0,16	0,16		Black	Smooth, shine	Pyriform	Animal	Simultaneously	Epigeal	Recalcitrant
32	<i>Gleditsia fera</i>	Caes.	2,52x0,57	0,38	0,14	Brownish green	Smooth	Oblong-ovoid	Animal	Gradually	Epigeal	Orthodox

33	<i>Glochiton obscurum</i>	Phyll.	0,38x0,27	0,25	0,009	Red - orange	Smooth	3-dimension	Animal	Gradually	Epigeal	Orthodox
34	<i>Glycosmis pentaphylla</i>	Rut.	0,98x0,81	0,78	0,42	Dark green, shades of yellow fibres brownish, yellowish green edge	Rough	Conical	Animal	Gradually	Epigeal	Recalcitrant
35	<i>Protium javanicum</i>	Burs.	0,9x0,56	0,379	0,064	Cream	Rough until smooth	Oblong	Animal	Gradually	Epigeal	Orthodox
36	<i>Pterocarpus indicus</i>	Papil.	7x3,8	0,41	0,3	Brown	Coarse, wrinkled, winged	Irregular	Wind	Gradually	Epigeal	Orthodox
37	<i>Randia fitzalanii</i>	Rub.	0,74x0,74	0,21	0,08	Brown, if kept turn into black	Smooth	Campanulate-pyriform-globose	Animal	Gradually	Epigeal	Orthodox
38	<i>Sansevieria trifasciata</i> var. <i>laurentii</i>	Drac.	0,72x0,52	0,52	0,15	Cream	Coarse	Reniform	Explosion	Gradually	Hypogaeal	Recalcitrant
39	<i>Santalum album</i>	Santal.	0,7x0,68	0,65	0,15	Yellow – light brown	Coarse	Symmetrical globular	Animal	Simultaneously	Epigeal	Orthodox
40	<i>Sapindus rarak</i>	Sapind.	1,4x1,29	1,26	1,4	Black	Smooth	Symmetrical globular	Animal	Simultaneously	Epigeal	Orthodox
41	<i>Senna alata</i>	Caes.	0,69x0,51	0,16	0,03	Blackish brown	Smooth	Campanulate	Animal	Gradually	Epigeal	Orthodox
42	<i>Senna bicapsularis</i>	Caes.	0,55x0,26	0,21	0,02	Dark brown	Smooth	3-dimension	Animal	Gradually	Epigeal	Orthodox
43	<i>Senna reticulata</i>	Caes.	0,58x0,18	0,12	0,03	Brown, center of green	Smooth	Oblong	Animal	Gradually	Epigeal	Orthodox
44	<i>Senna siamea</i>	Caes.	0,8x0,62	0,08	0,03	Brown	Smooth	Globose, plateriform	Animal	Gradually	Epigeal	Orthodox
45	<i>Tabebuia donnell-smithii</i>	Bign.	0,58x1,94	0,06		Brown, winged transparent	Smooth, winged	Reniform	Wind	Gradually	Hypogaeal	Orthodox
46	<i>Triphasia trifolia</i>	Rut.	0,83x0,61	0,49	0,12	Dark green with fibrous ends	Smooth	Ovoid	Animal	Gradually	Hypogaeal	Recalcitrant
47	<i>Trivalvaria macrophylla</i>	Annon.	0,93x0,68	0,69	0,26	Brown	Rough	Ovoid	Animal	Gradually	Epigeal	Orthodox
48	<i>Uvaria purpurea</i>	Annon.	0,92x0,58	0,026	0,114	Brown	Smooth	Ovoid	Animal	Simultaneously	Epigeal	Orthodox

DISCUSSION

Table 1 showed that there are quite a lot of variation in external morphological seeds character of 48 species were observed. The variations include size, diameter, weight, shape, color, surface, seed dispersal, type and pattern of seed germination.

Certain characters can lead to certain species of family, for example in group winged seeds tend to lead to the species from family Papilionaceae, Meliaceae or Bignoniaceae (e.g. *Pterocarpus indicus*, *Tabebuia donnell-smithii*, *Swietenia mahagoni*, etc.). But these characters are not an absolute requirement in identifying the species of seeds.

CONCLUSION

Characterization of external morphology in 48 species of selected seeds in Purwodadi Botanic Garden showed that there are variations in size, diameter, weight and shape of selected seeds. Seed color ranged between white, brown, and black up to red. Surface tends to smooth, some are rough and coarse. Many seeds spread by wind, animals, water and explosion; mostly included in orthodox seeds with epigeal type of germination and simultaneously germination patterns.

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QUALITY OF TREES DIVERSITY GROWING AROUND WATER SPRINGS AT SOUTHERN PART OF PASURUAN DISTRICT, EAST JAVA

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ABSTRACT

The aim of this research was to know the quality of trees diversity growing around water springs at southern part of Pasuruan District, East Java. Data was collected in 22 sampling sites for vegetation analysis using Mueller-Dombois's method in order to calculate density, frequency, dominancy and Important Value Index. The lists of trees were then compiled into one integrated list and used as reference for determining the status of endemism. Based on the trees natural distribution, which the information was obtained from several literature, the observed trees species was divided into three groups i.e (1) trees species spreads widely, included in Southeast Asia region or Malesia phytoregion, be categorized as endemic; (2) trees species that originate from outside the Southeast Asia region, be categorized as exotic; and (3) trees species that is not known certainty distribution, be categorized as uncertain known distribution yet. Degree of endemism was determined following the Barthlott's *et.al* method. Result indicated that at least 39 families, 77 genera which consisted of 104 species of trees grown surrounding water springs with 6.37 of diversity index. From the list of observed trees around the spring was known that 86.5 % be categorized as endemic, 11.5% be categorized as exotic and the remaining 2% was not certain status its endemism yet. Based on some criteria, the diversity trees around the spring was relatively good.

Key words: Quality, Trees diversity, Water springs, Pasuruan

INTRODUCTION

Biological diversity or the shorter biodiversity is the variety and variability among living organisms and the ecological complexes in which they occur (Gibbons *et al*, 1987). It is very commonly used as a synonym of species diversity, in particular of 'species richness', which is the number of species in a site or habitat (Groombridge & Jenkins, 2002). However, some other aspects of biodiversity

of an area can be identified by means of quality criteria such as: 1. Taxon richness. 2. Abundance structure. 3. Taxonomic, phylogenetic and character diversity. 4 Range sizes and degree of endemism. 5. Share of allodiversity. 6. Ecosystem Functions.and 7. Actual and potential economic value (Barthlott 1999). Management of biodiversity also requires measurement, and measures of diversity only become possible when a quantitative value can be ascribed to them and

these values can be compared. It is thus necessary to try and disentangle some of the separate elements of which biodiversity is composed (Groombridge & Jenkins, 2002). In this study we will try to assess the trees diversity on the habitats around the water springs based on only several criteria namely: number of taxa, densities, important value, diversity index, strata of tree height and degree of endemism. Thus the aim of this research was to know the quality of trees diversity growing around water springs at southern part of Pasuruan District, East Java, Indonesia.

MATERIALS AND METHODS

Data was collected in 22 sampling sites for vegetation analysis using Mueller-Dombois's method in order to calculate density, frequency, dominance, Important Value Index and estimated of tree height as note in (Soerianegara and Indrawan, 1983). Each sampling site covers approximately one ha square shape. The lists of trees were then analyzed to obtain information tree diversity on each sampling site and also compiled into one integrated list used as reference for determining the status of endemism. Based on the trees natural distribution, which the information was obtained from several literature, (Ashton, 1982; Backer and Bakhuizen, 1963; Backer and Bakhuizen, 1965; Backer and Bakhuizen, 1968; Berg & Corner, 2005; Keng, 1969; Narko *et al.*, 2012; Pratiwi, 2008; Sastrapradja, 1984; Soerianegara and Lemmens, 1994), the observed trees species was divided into three groups i.e (1) trees species spreads widely, included in Southeast Asia region or Malesia phytoregion, was categorized as endemic; (2) trees species that originate from outside the Southeast Asia region, be categorized as exotic; and (3) trees species that is not known certainty distribution, be categorized as uncertain known distribution yet. Degree of

endemism was determined following the Barthlott's *et.al* method.

RESULTS

Result of combined data from 22 sampling sites indicated that at least 39 families, 77 genera which consisted of 104 species of trees grown surrounding water springs with 6.37 of diversity index. The average density was 63 trees per ha. while percentage of estimation result of tree height strata, recorded as follows: strata A: 3.03%, strata B: 23.2%; strata C: 55.7% and strata D: 18.1% , from 1,385 individual of trees. Five co-dominant species included *Bambusa blumeana*, *Dendrocalamus asper*, *Ficus racemosa*, *Ceiba pentandra* and *Ficus virens*. From the list of observed trees around the spring was known that 86.5 % be categorized as endemic, 11.5% be categorized as exotic and the remaining 2% was not certain status its endemism yet (**Table 1**).

Table 1. The. Description of the Observations Results

No.	Descriptions	Results
1	Families richness	39
2	Genera richness	77
3	Species richness	104
4	Diversity Index (combined)	6.37
5	Abundance of individuals	1,385
6	The average density of trees per ha	63
7	Co-Dominant Species	<i>Bambusa blumeana</i> , <i>Dendrocalamus asper</i> , <i>Ficus racemosa</i> , <i>Ceiba pentandra</i> and <i>Ficus virens</i>
8	Proportion of stratification (A, B, C, D) %	3.03; 23.2; 55.7; 18.1
9	Degrees of endemism (Endemic, exotic, uncertain) %	86.5; 11.5; 2

The data on each sampling plot which was more detailed be included as follows:

a. Number of taxa

Number of taxa which includes families, genera and species of each sampling site were varies. The lowest number was 4 families, 5 genera and 6 species, while the highest number was 19 families, 28 genera, 30 species (**Fig. 1**).

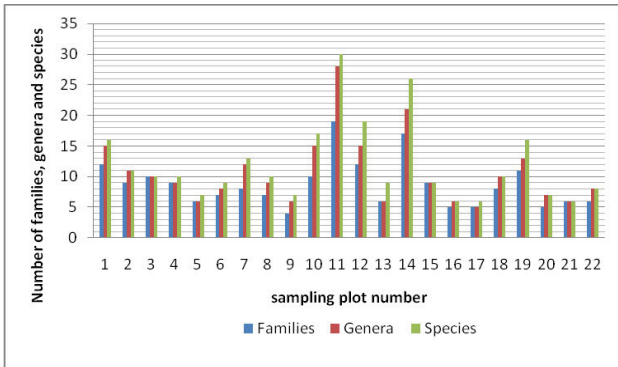


Figure 1. Number of taxa richness per sampling plot.

b. Densities

Tree density was determined by counting the number of individuals per sampling plot. The calculations show that the lowest number was 10 individuals and the highest was 191 individuals. (**Fig. 2**).

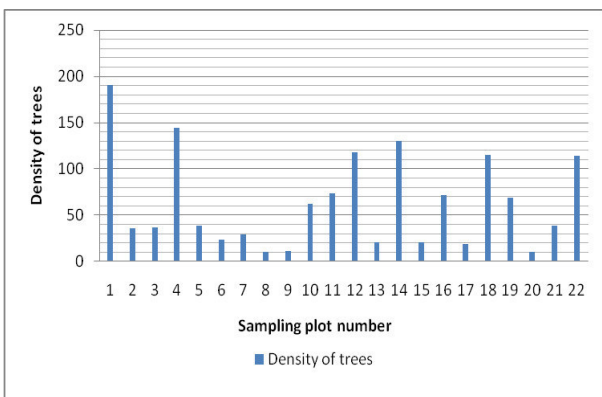


Figure 2. Tree density

c. Important value Index

Important value index describes the amount of influence exerted by a species of the community. Importance value index of a tree species in each sampling plot, was the sum of the relative abundance and relative dominance of each sampling site, while the important value index, of all sampling sites was determined by the sum of relative density, relative dominance and relative frequency (**Fig. 3**).

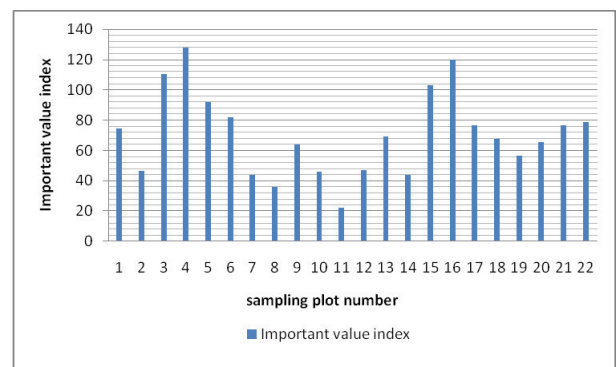


Figure 3. The highest important value index of each sampling site. Tree species name below, corresponds to the number of sampling sites (1-22).

1. *Bambusa blumeana*, 2. *Sterculia foetida*, 3. *Ceiba pentandra*, 4. *Bambusa blumeana*, 5. *Horsfieldia irya*, 6. *Ficus virens*, 7. *Ficus racemosa*, 8. *Ficus sp.* 9. *Ficus benjamina*, 10. *Dendrocalamus asper*, 11. *Dracontomelon dao*, 12. *Dendrocalamus asper*, 13. *Syzygium picnanthum*, 14. *Ficus racemosa* 15. *Ceiba pentandra*, 16. *Bambusa blumeana*, 17. *Bambusa blumeana*, 18. *Ficus racemosa*, 19. *Syzygium javanicum*, 20. *Artocarpus altilis*, 21. *Pinus merkusii*, 22. *Bambusa blumeana*.

d. Diversity index

To calculate the level of species diversity based on the Shannon-Wiener diversity index was used parameters: relative density, relative dominance and relative frequency. The results are listed in **Fig. 4**.

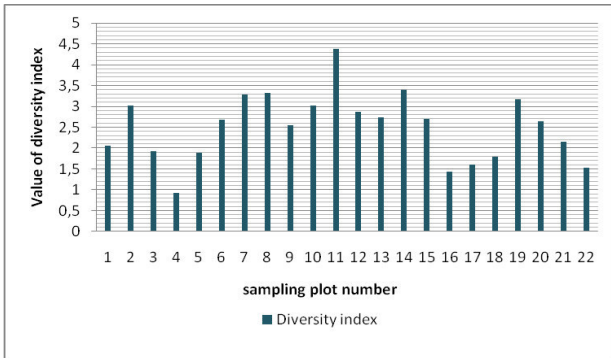


Figure 4. Value of Diversity Index

e. Strata of tree height

Estimate of the height of the tree stratum of each sampling site refers to grouping by Soerianegara and Andry Indrawan (1983) (Figure 5) while the result of percentage of average were: stratum A > 30 m: 3:03%; strata B <30 m> 20 m: 23:18%; strata C > 10 <20 m: 55.67% and strata D <10 m: 18:12% (Figure 6)

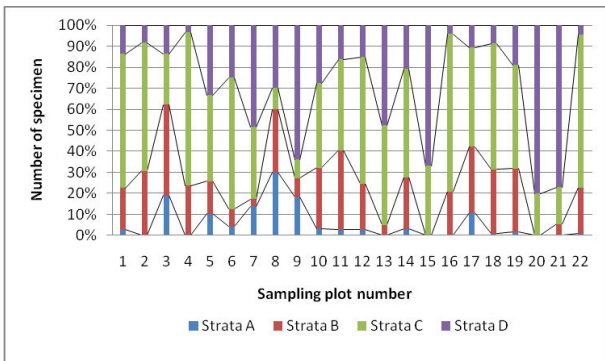


Figure 5. Percentage of tree height estimation strata of each sampling site

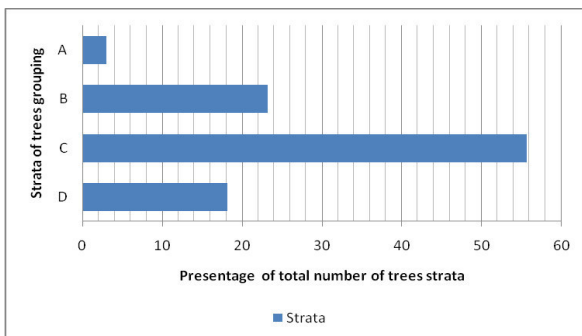


Figure 6. Percentage of total number of trees strata

f. Degree of endemism

Degree of endemism in each sampling site was determined by comparing the abundance of endemic, exotic and uncertain species with total abundance of species, while the degree of endemism throughout the sampling site was determined by comparing the total abundance of endemic species, exotic and uncertain species with total abundance of species throughout the sampling site (Fig. 7 and 8).

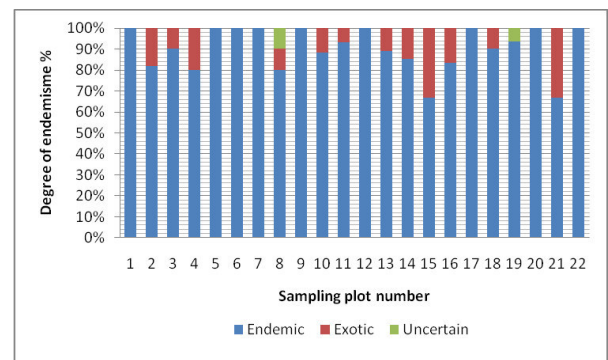


Figure 7. Percentage of Degrees of Endemism on Each Sampling site

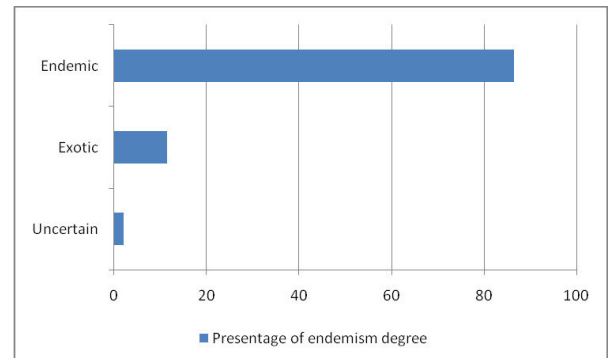


Figure 8. Percentage of Degrees of Endemism on total species

DISCUSSION

Based on some criteria, such as, number of taxa, diversity index, percentage of stratification and degree of endemism, the diversity trees around the spring was relatively good. It means that in general,

although the habitats around the springs which was observed, have been and are being degraded, but we still have the opportunity to think, select and propagate various trees species remaining there, in terms of diversity of tree species, tree species stratification height, and degree of endemism respectively, for rehabilitation or restoration. Some trees, even classified as rare status its existence (Ashton, 2012) that needs to be preserved immediately. On the other hand, other data, namely, tree density and dominance at several sampling sites were not so good. The average density of trees was only 63 trees per ha., while the dominance of the tree species, with indicated by the hight of importance value index, were recorded in several sampling sites, especially sampling site No. 3, 4, 15 and 16. Trees species with the highest of important value index occurred at the sampling site number 3, *Ceiba pentandra*; number 4, *Bambusa blumeana*, number 15, *C. pentandra* and number 16, *B.blumeana*. More over, it is known that water is a product of a very important ecosystem services to humans, and its existence depends on many factors, among other things are the carrying capacity of the physical environment and vegetation diversity cover in the catchment area. Therefore, conservation of plants diversity and ecosystems of degraded areas is very important to be attention (Bruijnzeel, 1990; Marinelli, 2004; Soejono, 2011). To that end, some experts presented the results of research and expressed their opinions. Lieberman and Diana Lieberman (1994) reported the results of his research that the total number of stems ≥ 10 cm dbh, enumerated in 12.4 ha, a mean density is 446.0 individuals ha^{-1} . This information is useful for determining the choice of tree species diversity remaining from those areas to be propagated and then for rehabilitation or restoration to that degraded areas through ecological value consideration, function approach and estimate the number of

seedlings required per unit area. According to Manan, 1992, the best approach to restore diversity or for rehabilitation of degraded land was using the adjacent natural community structure (primary forest) as a vegetation model, especially in complexity, composition, vertical or horizontal stratification, richness, diversity and endemism rate. Cosequently, the result of the succession acceleration by rehabilitation would be optimal as expected and harmoni under natural condition. In general, the more diverse plant species and structure, the better its effect on soil and water conservation. We should also consider ecological suitability in more detailed and autoecology of each species (Soejono *et al.*, 2013). Another thing to consider in the process of restoration is the effect of invasive plants. Some researchers (Djupri, 2004; Hernowo, 1999, Iskandar, 2006; Zuraida, 2011), take an example, the impact of the use of *Acacia nelotica* for greening in Baluran. Planting exotic plant, *Acacia nilotica* in Baluran, expanding rapidly. The plant is then known to be invasive type so its control becomes a a serious problem.

CONCLUSION

At least 39 families, 77 genera which consisted of 104 species of trees grown surrounding water springs with 6.37 of diversity index. The average density was 63 trees per ha. while percentage of estimation result of tree height strata, recorded as follows: strata A: 3.03%, strata B: 23.2%; strata C: 55.7% and strata D: 18.1% , from 1,385 individual of trees. Five co-dominant species included *Bambusa blumeana*, *Dendrocalamus asper*, *Ficus racemosa*, *Ceiba pentandra* and *Ficus virens*. From the list of observed trees around the spring was known that 86.5 % was categorized as endemic, 11.5% was categorized as exotic and the remaining 2% was not certain status its endemism yet.

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BIODIVERSITY OF MACROSCOPIC FUNGI IN THREE DIFFERENT HABITAT IN PANIIS VILLAGE UJUNG KULON WEST JAVA

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ABSTRACT

Paniis village located on the border of the National Park Ujung Kulon Banten. This area has three types of habitat, namely land, swamp and coastal. In general, the land is quite fertile region, composed of primary forests and secondary forests, plantations and a wide expanse of rice fields, which allows the finding of several species of macroscopic fungi. The purpose of this study is to determine the macroscopic fungi species diversity based on different types of habitats, i.e. forests, plantations and coastal areas. Sampling was performed by browsing around and looked for macroscopic fungi paths. Macroscopic fungi samples collected in the study site were observed and recorded by morphologic characters, then grouped based on their benefit or potential usage (food or medicine). The results recorded 70 species, 29 species are in the forest, 20 species in plantations and 21 species on the coast. Diversity indices were moderate, the highest diversity index in the forest with the lowest value of 2.508 and on the coastal with a value of 2.245. Value comparison between the species composition of the highest locations in forests and plantations amounted to be 36.73 % and the lowest among the coastal plantations by 34.15 % . Highest presence frequency was found in the forest, i.e. 51% (*Microsporus xanthopus*), followed by the coastal location of 43.11% (*Shcyzophyllum communae*) and the lowest was 20.32% in the plantation (*Shcyzophyllum communae*) . There are 14 species of macroscopic fungi were potential as food, 32 species potential as medicine, and 6 species potential as food and medicine.

Keywords

Biodiversity, macroscopic fungi, Ujung Kulon.

INTRODUCTION

Tropical forest in Indonesia has a high mushroom diversity due to its environmental factors such as humidity, sufficient water, nutrient resources, pH and temperature that can support fungal growth.

Fungus can be seen and identified easily especially in the places that are humid, such as litter for examples. In its natural environments, fungus can thrive in places that contain carbohydrates, both already degraded or still in larger molecules like cellulose, lignin, and other materials.

In the forest various types of substrate can be overgrown by certain types of fungi. Fungi can live and occupy various types of substrates ranging from soil, water, woods that already withering, litters, animal wastes and so forth (Noverita dan Setia, 2010). One type of fungi usually had certain requirement towards the substrate for itself to grow, nevertheless environment condition should support the growth of the fungi. The difference of substrate and environmental condition for example air and land humidity, temperature, acidity (pH) of soil, light intensity will effect on growth of different types of fungi (Ronald, 2000).

Fungal organism in forest had its retribution for the substrate (environment) they had grown. In the forest fungi had important tasks as decomposer together with other microorganisms such as bacteria, actinomycetes, termite, and so on to degrade any pile material that already accumulate in the woods. Process of decomposition had become great help to balance the ecosystem where the results of degradation organic material can be used by plants and others soil organism (Ronald, 2000). Meanwhile in human's life fungi had its potential too as food stuffs. Few species of fungi that grows in the forest are

edible because it has good nutrients for humans, such as *Pleurotus* sp, *Auricularia* sp, and *Lentinus* sp.

Paniis is an area that lies on the border area of Ujung Kulon National Park, this area had three ecosystem there is coastal, swamp, and land ecosystem. Judging from the region that are supportive, allowing it to had great probability to find variety of macroscopic fungal.

The purposes of this research is to know diversity of macroscopic fungal based on habitat types including forest, plantation and coastal area in Paniis Village Desa Taman Jaya around Ujung Kulon National Park, Banten.

MATERIALS AND METHODS

This research conducted for five days on 16 – 22 May 2012 at Paniis village Desa Taman Jaya around the area of National Park, Ujung Kulon, Banten.

Tools that is used in this research are tabulation data, stationery, wrote plank, paper, label, sample container, soil pH meter, hygrometer, altimeter, thermometer, plastic bag, bunsen burner, beaker glass, inoculation needle, Petri discs, pincette, counter, plastic rope, and digital camera. Materials that is used in this research are alcohol 70%, formalin 4%, cotton, aquadestillata, Potatos Detrosa Agar Medium.

Sampling locations

Sampling sites were chosen in three places, the forest, plantation, and coastal region of Paniis Village Desa Taman Jaya around area of National Park Ujung Kulon Banten, because it has different types of substrate, humidity, and altitude in each habitat that are considered represent the region.

Sampling method

Sampling was performed using *Explorer* method to browse and search for mushrooms that is passed around the tracks. Samples mushroom that were found in research sites were observed and recorded few of its morphological characters such as hood's shape, lamella, and other parts as well and took it's picture. Species that are unknown were put in plastic bag and samples were preserved in a bottle who already had formalin in it for further identification.

Surroundings Parameter

Surroundings parameter includes temperature using thermometer, acidity (pH) of soil using soil pH meter, and humidity using hygrometer. These parameter were taken from morning daytime until sampling time was over. Parameter was recorded in data tabulation sheet.

Identification of Samples

Samples that are stored were identified at laboratory with the help mushroom identification namely "*Simon & Schuster's Guide To Mushrooms*" (Simon and Schusters. 1994). *Introductory mycology*, 4th eds (Alexopoulos at all. 1996), and "*Working with Mycorrhiza in Foresty and Agriculture* (Brundrett and Baugher.1997).

Data analysis

The data was acquired with this analysis :

1. Diversity using Shanon-Winner equation (Magurran, 1987)
2. Species composition between habitat types, using Sorensen similarity index equation (Brower and Zar, 1990)
3. Frequency of encounter of each habitat Using frequency equation.

RESULTS

Environmental Factors

Environmental conditions were noted during the study at three observation area, consist of parameters humidity, temperature, and soil acidity (pH), shown in Table 1.

Table 1. Condition of Physical Environment at each Location area sample

Location	Soil Acidity	Temperature (°C)	Humidity (%)
Forest (A)	6.4 - 7.3	31 – 34	53 – 67
Plantation (B)	6.0 - 7.0	31 – 35	50 – 80
Coastal (C)	5.7 - 7.2	30 – 34	56 – 74

Diversity of Macroscopic Fungi

Diversity index (H ') at three locations displayed in **Fig. 1** below:

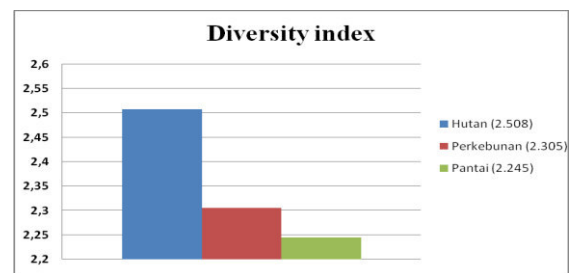


Figure 1. Bar Chart Diversity of macroscopic Fungi at Three Location area sample

Composition of macroscopic fungi at Three Locations

1. Number of species

Number of species of macroscopic fungi were found in three locations as many as 70 species, with the number at each location are: 29 types at forests, 20 species at plantations, and 21 species at shore. The data is displayed in the diagram Fig. 2 below.

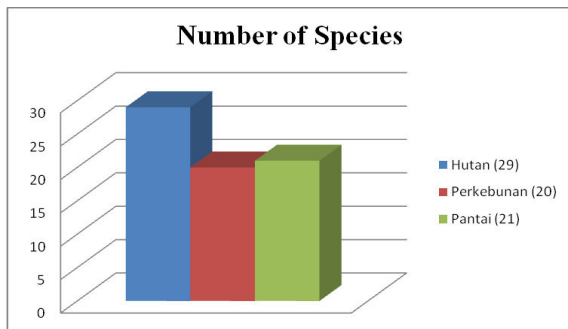


Figure 2. Bar Chart Number Of Species Macroscopic Fungi in Three Locations

2. Similarity Indeks

Indeks Similarity of macroscopic fungi were found in three locations in the data collection at Paniis village of Taman Jaya Paniis around Ujung Kulon National Park shown in Figure 3 below.

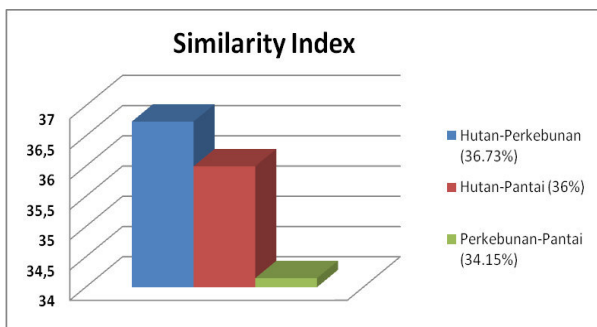


Figure 3. Bar Chart of Similarity Index Between Type Location

Frequency of Attendend

Encounter rates in three locations of macroscopic fungi sampling is calculated based on Frequency of Attendendis the presence of the macroscopic fungi in the three locations. The Frequency of Attendendare presented in Fig. 4 below.

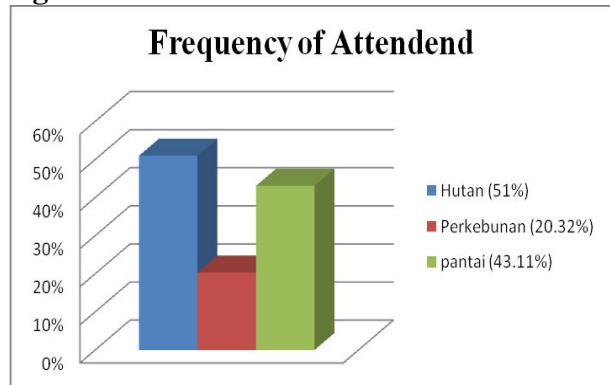


Figure 4. Bar Chart Frequency presence of Macroscopic Fungi

Potential and Benefits

Potential fungus classified using any literature, local guides and information of local people who using macroscopic fungi in daily life. Species that found in three different location is 14 species Potential as a foodstuff, 32 species as a medicine, and 6 species as a both of them

1. Potential Mushroom as Food

The macroscopic fungi which known have used as a foodstuff that found when taking sample in the location is; *Auricularia* sp, *Coprinus* sp, *Lentinus sajor-caju*, *Lentinus tigrinus*, *Lentinus* sp., *Pteurotus ostreatus*, *Schizophyllum commune*, *Termytomyces mammiformis*, *Termitomyces*, *Sarcoscypha speciosa*, *Tremella* sp1., *Tremella* sp2., *Volvariella spesiosa* and *Skleroderma citrinum*. The following images is an example of fungi that found potential as food.



Figure 5. Some examples of potential macroscopic fungal as a Foodstuff found in Paniis Village Ujung Kulon

2. Potential Mushroom as Medicine

The macroscopic fungi which known have medicinal benefits that found when taking sample in the location is *Amauroderma*, *Ganoderma*, *Polyporus*, *Picnoporus*, *Rigidoporus*, *Scleroderma*, and *Trametes*. *Amaroderma parasiticum*, *Auricularia* sp., *Cookeina* sp., *Daldinea concentric*, *Ganoderma lucidum*, *Ganoderma aplanatum*, *Lentinus sajor caju*, *Lentinus tigrinus*, *Lentinus squamolosus*, *Microporus xhantopus*, *Picnoporus cinnabarinus*, *Picnoporus* sp 2. *Picnoporus* sp.3, *Picnoporus* sp.4, *Polyporus elegans*, *Rigidoporus* sp.1, *Rigidoporus* sp2, *Rigidoporus* sp.3., *Rigidoporus* sp.4., *Stearum* sp., *Trametes* sp.1, *Trametes* sp.2., *Trametes* sp 3., *Trametes* sp.8., *Trametes* sp.9., *Trametes* sp.10., *Polyporus arcularius*, and *Pleurotus ostreatus*. The following figure shows an

example of a fungus found potential as medicine;



Figure 6. Some examples of potential fungal as a Medicine found in Paniis Village Ujung Kulon

3. Potential mushroom as food and medicine

The macroscopic fungi which known have food and medicine benefits that found when taking sample in the location is *Auricularia* sp., *Lentinus sajor-caju*, *Lentinus tigrinus*, *Lentinus squamolosus*, *Pleurotus ostreatus*, *Sarcoscypha spesciosa* and *Skleroderma citrinum*.

DISCUSSION

Environmental conditions which recorded at the time of the study at each sampling area (**Table 1**) are generally very supportive for the growth of fungi. According to Chang and Miles

(2004), generally the fungus will grow on range from 4.5 to 8.0 with a pH optimum between 5.5-6.5. Fungal growth temperature ranged between 20-28⁰C, while humidity for mycelial growth of around 50-75%.

The bar chart above (**Fig. 2**) shows that the index of diversity (H') that at A location is greater than B and C location, which amounted to 2,508, whereas B and C location respectively amounted to 2,305, and 2,245. Hal This is due to the condition of the habitat on A location has a factor better environment for fungal growth compared with 2 other habitats (**Table 1**), except that these location crossed the river path that retains moisture and vegetation diversity, so the diversity of fungi are found to be more varied than the location other. Smallest diversity index found at the C location where the condition of this location is adjacent to the beach. salinity influenced to pH, and became one of the environmental factors that inhibit the fungus to grow. At these location also has an open shade, so the presence of the fungus is limited because light penetration is too excessive.

Based on the Shannon-Winner Test, diversity of mushroom on the A, B and C location included in the 2245-2508 categories. This is in accordance with the opinion Maguran (1987) who stated range diversity index value or (H') between 0 to 2.302 is low, (H') between 2.302 to 6.907 were moderate and if the value is high (H') is more than 6,907.

Generally, the amount of macroscopic fungi in each location is determined by habitat conditions and environmental factors. The location itself, when the retrieval data found various fungi growing on various substrates such as soil, litter, dead wood and trees. Number of fungal species that obtained at location A at most as many as 29 species, it was related to habitat conditions that affect the humidity and temperatures that support macroscopic fungigrowth. At B location and C

location has found a lower number of fungi in a location that is equal to 20 and 21 species, this is caused by the presence of substrates that are not shaded by tree so that habitat conditions are not in optimal conditions for port macroscopic fungi growth. Besides that location B and C also has a rather dry soil conditions and high temperatures so that the macroscopic fungi is difficult to grow in these conditions.

Similarity index at A location and B location is worth the highest of 36.73% which means only little different macroscopic fungi were found. This is due to the location of habitat adjacent to the factors that affect the growth of fungi is almost the same, so the macroscopic fungi are found mostly the same. For B location and C location have the value of similarity index by 36%. while the similarity index of the lowest types A and C is the location of the forest and the beach. This can occur due to different external factors, especially the substrate, and the shade that causes the differences in each type of habitat.

Percentage Frequency attendance based of the results obtained ranged from 20-51%. According to Michael (1994), categories of frequency attendance at A location that has a percentage of 51% is called a constant. Type of fungus that is found in here is *Microporus xanthopus*, whereas the frequency of attendance at B location and C has a percentage of 20.32% and 43.11% so that could be called assesori. Types of fungi were found in both locations are *Schizophyllum commune*.

Judging from the potential , pretty much macroscopic fungi found in this study has potential as an ingredient, or as medicine. However, Based on the results in discussions with local guide, there are many people do not know the type of fungus that can be used. Those species are used as food because they

have a thick fruit body, savory taste, soft texture and high nutritional value.

Used as medicine. macroscopic fungi used as ingredient because it contains a potential chemical found on fruit body. one example is *Ganoderma* which can be used as an anti tumor and also can inhibit HIV infection in humans. *Polyporus arcularius* used as medicine in some countries in Asia and Africa and *Auricularia* also used as anti-cholesterol drug (Chang and Miles, 2004).

Some fungi are found with double potential (as Foodstuff and medicine) like *Auricularia*, *Pleurotus*, etc. One example like *Auricularia* can produce mucus when cooked, but it can be used as poison antidote that contained in the cooked ingredients, derived from pesticide residues, detergents, etc. (Suriawiria, 2000).

CONCLUSION

1. pH, humidity, and temperature are the environmental factors that influence the growth of fungus
2. Found as many as 70 species of fungi in three different habitats.
3. Highest diversity index at location A with a value 2.508 while the lowest value at location C with a value 2,245.
4. Comparison of the similarity of the highest percentage of 36.73% at locations A and B, while the smallest was 34.15% at site B and C.
5. Percentage frequency of fungus presence with value 51% in location A (forest area), one example is *Mikrosporopus xanthopus*; location B and C for the plantations and coastal with value of 20.32% and 43.11%. one example is *Shcyzophyllum communae*.
6. There are 14 species of fungi that used as foodstuff, 32 kinds of potential as a medicine, and 6 types of potential as a food stuff and medicine.

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SUSTAINABLE BIODIVERSITY CONSERVATION BASED AGROFORESTRY IN SUB-WATERSHED OF KRUENG SIMPO ACEH PROVINCE

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ABSTRACT

Sub-watershed of Krueng Simpo is located in the downstream watershed of Peusangan which has an area of 31,392 hectares. Lack of understanding of the biophysical biodiversity in watersheds of Krueng Simpo led researchers and policy makers looking for solutions biodiversity issues in the watershed area from the standpoint of conservation. Indonesian statistics in 2013 showed that the Indonesian population growth rate continues to increase as much as 1.34% per year. This resulted in a threat to the existence of biological diversity both upstream and downstream in the watershed mainly due to the conversion of forest land into plantations and other development. This paper specifically addresses natural resources and sustainable biodiversity-based agroforestry in the sub-watershed of Krueng Simpo. Sustainable biodiversity management in Sub-watershed of Krueng Simpo includes two districts, namely Bener Meriah and Bireuen. Lack of understanding and attention to the human activities in the upstream that threatening biodiversity related to the land use changes which resulting ineffectiveness in managing the ecosystem and environmental management. Therefore, agroforestry-based conservation efforts for the sustainability of biodiversity and ecosystem management in Sub-watershed of Krueng Simpo are needed so that biodiversity is not threatened by extinction

Keywords: *Conservation, sustainable biodiversity, agroforestry, watershed.*

INTRODUCTION

The rate of Indonesian population growth which continues to increase by 1.34% per year based on 2013 statistics led the conversion of forest land into plantations, agricultural and settlement development also to increase. Lack of understanding

and concern on the human activities at upstream area that threaten biodiversity related to the land use changes resulting ineffectiveness in managing the ecosystem and overcoming the environmental issues. Biological diversity is a gift of God. As beings who always take advantage of

nature, human is obliged to manage natural resources, habitats, and biodiversity on the earth. Indonesia is the country with the second top biodiversity in the world after Brazil. The spread of biodiversity in Indonesia include the spread of flora and fauna in the entire country, both on land and in the islands of Indonesia, which is useful as food, clothing, medicine and cosmetic sources. Lack of biophysical understanding of biodiversity in river basins in Indonesia, especially in Sub watershed krueng Simpo Aceh province became a problem on the existence of biological diversity from the conservation point of view which resulted a threat to the existence of biological diversity both upstream and downstream of watershed. Krueng Peusangan watershed is located in several districts composed by 12 sub-watersheds and Lake Laut Tawar which is located in the upper watershed. The rainfall in the Krueng Peusangan watershed can be categorized quite high, namely 1848 - 2055 mm / yr. Gayo ethnic live along the river and around the Lake Laut Tawar, which is at upstream of Krueng Peusangan watershed, while the Acehnese ethnic live in the middle and downstream of the watershed of Krueng Peusangan. Most Gayo ethnic plant highland rice, coffee, cocoa and areca both monoculture and mixed plantation

systems, while Acehnese ethnic fish in river and plant irrigated rice, coconut, palm and mixed garden (Khasanah, 2010).

The development of agroforestry systems in Sub watershed Krueng Simpo both upstream and downstream by planting a variety of commodities with high, medium and lower canopy plants can improve conservation of biodiversity so that the environment can be sustained. The aim of this paper is to discuss the natural resources and the sustainable biodiversity-based agroforestry in the sub-watershed of Krueng Simpo.

Vegetative Conservation of Krueng Simpo Sub Watershed

Vegetative Conservation can be conducted by planting a variety of crops or vegetation on land use to preserve biodiversity and prevent erosion. Vegetative conservation in Krueng Simpo Sub watershed based on a research (Fitri, 2012) shows that conservation with vegetative methods can maintain the productivity of the soil through crop rooting system. The vegetation rooting system greatly affects the soil erosion. The planting of various types of plants or vegetation include planting at the hallway through planting annual plants (banana, papaya and chilli) on the alleys between

rows of fenced plants are according to the contour lines, the distance between plant is 5-10 cm, the steeper of slopes, the planting distance is getting narrower. Planting multistrata is conducted through planting plants with high canopy i.e. teak plant, sengon and areca nut while a mixture planting is done through agricultural systems by the development of various types of seasonal plants (soybeans, papaya and bananas) and annual crops (coffee, betel nuts, oil palm and sengon) without a clear sequence of direction and irregular in cultivation. Vegetative conservation conducted in Krueng Simpo Sub watershed in by planting elephant grass, agroforestry of sengon with bananas-based, and cocoa-based banana in combination with elephant grass plantings can increase the sustainable biodiversity.

Agroforestry practices in Krueng Simpo Sub-Watershed

Multistrata system: through a system of agriculture by planting plants with multilevel canopies such as sengon and teak trees. The planting of vegetation with medium canopies, banana, papaya and plants with lower canopies (Chili, corn and soybeans) are conducted in the upstream and downstream of the Krung Simpo sub-watershed. The mixture planting: the agricultural system by

combining various types of seasonal plants in the middle side of Krueng Simpo sub-watershed (corn, bananas and papaya) with annual plants without a clear row direction. **Sylvopastoral:** a grazing field for cattle in between trees. Elephant grass crop plantings in between the caconut, banana and teak trees is conducted in the downstream of Krueng Peusangan Sub-watershed. **Agrosilvikultural:** a mix food crops (soybeans, corn and rice) with the forest trees (teak, sengon), done in the middle and downstream side of Krueng Simpo Sub-watershed where the use of land is to produce agriculture and forestry comodities. **Agrosilvopastoral:** the combination of food crops (corn, rice and soybean), grass fields (elephant grass, Erythrina Variegata and Huntersville) with forest trees (areca nut, cocoa, caconut, sengon and teak trees), usually done in the middle, downstream and upstream of Krueng Simpo Sub-watershed, the land management to produce agricultural and forestry products and keeping farm animals can be done in the same time.

The sustainable biodiversity conservation

Biodiversity conservation is very important to support sustainable development. Changes in closing land at downstream of Krueng Peusangan

watershed, namely in Krueng Simpo Sub watershed according to the results of research (Khasanah,2010) from 1990 to 2010, there was an increase in coverage for coffee agroforestry, mixed plantations, coconut, seasonal plants lands and palm oil agroforestry in the downstream of Sub Watersheds of Krueng Simpo. Beside agroforestry, other vegetation that can preserve biodiversity are also useful in ekohidrologically are a *Hibiscus tiliaceus* or siron (*Hibiscus tiliaceus* L.) and bamboo and jaloh (*Salix tetrasperma* Roxb) planted in the area of Krueng Simpo Sub watershed. They are species ecologically can also reduce the impact abrasion and erosion of riverbanks. Jaloh and siron that have reached a phase of growth with closed canopies have a strong root fibers that are able to grip the ground. The natural Habitat of these species are on the river. However, when the streams with a high intensity occurs, in the area where *Hibiscus tiliaceus* or siron was not big enough, the role of 'bronjong' is very important to protect the collapse of the river so as to preserve the biodiversity and ekohidrological function.

CONCLUSION

Vegetative conservation that can maintain the sustainable biodiversity in Krueng Simpo Sub watersheds can be done by

planting a variety of crops or vegetation on land use. Planting agroforestry systems, teak and sengon coffee annuals/seasonal plants based in the hallways (papaya, corn and bananas), planting, planting multistrata mix.

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Activation Communities on Sustainable River Conservation Based on Macroinvertebrates Knowledge in Brantas Upper Watershed Area

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ABSTRACT

The aims of this study was to analyse activation communities on Sustainable River Conservation based on macroinvertebrates knowledge in upper watershed area: a case study in Batu District, East Java, Indonesia. This study was designed as analytical cross-sectional survey in upper watershed area with 70 resident living in three village of Batu District. Macroinvertebrates were collected to count and determine from March 2012 until January 2013 in dry season. The questionnaire was used as a tool for communities data collection twice in bahasa Indonesia and a view cases in local language, before they are receiving information about bioindicator of macroinvertebrates and two month later after they get information about biondicators. The result of this study shows that in upper watershed area, there are Thirty-six Species namely *Lymnaea rubiginosa*, *Tiara scabra*, *Tarebia granifera*, *Melanoides tuberculata*, *Sulcospira* sp., *Corbicula javanica*, *Leptonema* sp., *Hydropsyche morose*, *Macrostemum* sp., *Ceratopsyche sparna*, *Lype diversa*, *Bibiocephala* sp., *Amphiaschna ampla* (Rambur), *Libellago lineata* (Burmeister), *Rhinocypha fenestrata* (Burmeister), *Agriocnemis femina* (Brauer), *Ischnura senegalensis* (Rambur), *Pseudagrion pruinatum* (Burmeister), *Brachythemis contaminata* (Fabricius), *Crocothemis servilia* (Drury), *Neurothemis terminata* (Rambur), *Orthetrum chrysis* (Selys), *Orthetrum glaucum* (Brauer), *Orthetrum pruinatum* (Burmeister), *Orthetrum sabina* (Drury), *Orthetrum triangulare* (Selys), *Trithemis festiva* (Rambur), *Trepobates* sp., *Limnogonus fossarum*, *Ptilomera hemmingseni*, *Metrobates* sp., *Aquarius paludum paludum*, *Planaria* sp., and *Helobdella* sp., *Lumbricus terrestris* respectively. The Questionnaire result shows in general there is a change of perception and community participation based on Bioindicator of Macroinvertebrates. Specifically that almost all parameters results indicated significant increase after two month they receive information's on Bioindicator of macroinvertebrates ($p < 0,001$); Environmental ethics ($p < 0,001$); Intention for river conservation ($p < 0,001$); The role of the community in environmental stewardship ($p < 0,001$); The role of the community in protection environmental damage ($p < 0,001$); The level of community in participation environmental conservation ($p < 0,001$) and Type of community participation in environmental conservation ($p < 0,001$).

Keywords: Activation communities; Sustainable River Conservation; Bioindicator; macroinvertebrates; Watershed

INTRODUCTION

Brantas river has a resource potential \pm 12 billion m³ of water which is used as raw water by the community in many ways and the river is the largest river in the East Java province, Indonesia. The watershed covered approximately 26.5% area of East Java Province (River Regional Center of Brantas, 2008). Upper Brantas watershed geographically located between 7^o 44'- 8^o 26' N and 122^o 17' - 122^o 57' east longitude with altitude of 690-1200 m above sea level (Statistical Beureau of Batu City, 2011).

Brantas river as a one of large river in Indonesia has an importance role in sustainable development. As a long river that coverage nine regencies and five municipalities with average rainfall 2,000/year, this river used as a source of sustainable energy supply that must be maintained. Keeping the flow of the river, not only by looking at the volume and flow of the water present in these waters, but also by maintaining ecosystems that exist around the river. Preservation of existing ecosystems in the area around the river will keep the existence and function of the river as a whole.

In the recent years many emerging public complaints relating to the condition of the river as a water source for the community. Decrease in water discharge trigger the seizure of the district and the city of Malang (Surya, 2006). Springs that are usually pretty much found in the upstream region and the number of discharges decreased by 30% also lead to reduction of water supply needed by the communities in the watershed area.

At the field, the availability of land which always tends to decrease both the breadth and quality in the long run can disrupt water resource systems. Water supply is also likely to experience shortages in space (spatial) and time (temporal). Unfortunately, public understanding in the river conservation is still very limited, so it has not been able to participate optimally in this activity.

On the other hand, there are many organisms that do not have a backbone that is often referred to as macroinvertebrates living in bottom of the river. These organisms are sedentary life in a relatively long period of time, with a relatively large size to be observed with unaided eye, and are always exposed by the result of human activities conducted in terrestrial areas.

Macroinvertebrates are a group of organisms that are sensitive to changes in the aquatic environment and may reflect a comprehensive manner in the waters changes. These organisms are responding not only on singular changes of waters parameter, but also changes in water conditions are more complex. Changes in these organisms can be seen from the change in the number of existing species and number of individuals concerned.

Dynamics of macroinvertebrates can be used as a lesson for the people to know the real condition of the ecosystem environment. By observing macroinvertebrates, people realize that they do not live alone in the ecosystem, but there are other creatures that live around the people that can provide sustainable environmental quality signals.

The aims of this study was to analyse activation communities on Sustainable River Conservation based on macroinvertebrates knowledge. Macroinvertebrates as bioindicators of river health can be used as guidelines to see the quality of the environment and represents natural conditions as to increase public awareness in the river conservation at upper watershed area. Introduction of macroinvertebrates knowledge in public life would indirectly encourage public awareness of environmental conservation in the headwaters area.

Macronvertebrates generally abundant in the river, have little mobility and spend up to one year in the stream. Existence of aquatic macroinvertebrates in stream is a reflection of the interrelation between physical and chemical factors. Both of these factors greatly affect the

lives of macroinvertebrates. Among the creatures that exist in the waters, macroinvertebrates are organisms that are very good for use as bioindicators (Barbour, 1996a; Barbour, 1996b; Barbour, 1999; Downes B.J., 2000; Benstead, 2003; Ziglio and Maurizio Siligardi, 2006; Holmes, 2008; Pliūraitė, 2009; Wright, 2011).

MATERIALS AND METHODS

Sampling of Macroinvertebrates

To see of the existing condition diversity of Macroinvertebrates in the study area, by observing Macroinvertebrates by using Surber net (fixed sampling area of 30 x 30 cm; 250 µm mesh size) from March 2012 until January 2013 in dry and wet season (Stark et al., 2001; Ode, 2007). Macroinvertebrates collected from the river substratum. The samples were fix in 4% formalin solution (Pliūraitė, 2009). Specimens Macroinvertebrates were then taken to Biology Laboratory Brawijaya University, East Java, Indonesia for identification and determination. Diagnosis of each species Macroinvertebrates was based on the examination of formalin-treated specimens, with the naked eye or under a 10x magnifier and 45x stereomicroscope, with reference to color photos of living organism. Macroinvertebrates were confirmed according to the references of (Birmingham and Luzier, 2005; Ziglio and Maurizio Siligardi, 2006; Mazzacano, 2007; Stark, 2007; Beauchene, 2009; Marwoto, 2011; 2012).

Sampling of Communities Data

The methods of collection data for community in the study area by using questionnaire (Solimun, 2010). Sample data was chosen from 70 resident living in three village upper watershed area of Batu District. Public interest in the conservation of the environment can be improved by increasing public interest in

conservation. Intention to evoke the hang of how the existing conditions in the waters, so that the public understand the conditions of life in it. Public participation will thrive if raised from society itself. Initiation of activities in by the people will be considered as a joint activity, so it will be escorted from the planning, implementation and evaluation of such activities. Socio-economic conditions are expected to colour the public's participation (Abdullaev I., 2009). Socio-economic conditions of these include: age, education, household size, income, land ownership, and house to live. Socio-economic conditions to describe the condition of the people residing in the study area in terms of income, education, and the relative dependence on the environment. Local knowledge as part of the socio-culture has a major role in conservation (Kumar, 2009). Socio-cultural variables include: knowledge of macroinvertebrates bioindicator, access to information, beliefs/norms, status and role of Social, Environmental Ethics. Characteristic attitude of society as a variable participation (Cockerill.,CH., 2006) Under-standing of the knowledge macroinvertebrates bioindicators is an important variable in the socio-cultural conditions. Most of the phases life cycle of macroinvertebrates in stream water, they stay and live in the aquatic environment and are sedentary at a relatively long time. Environmental ethic reflects how the wisdom of indigenous communities in the conservation of the river. Indigenous wisdom is always growing and evolving in a dynamic society. Public awareness of better environmental conditions will be reflected on the ethics of the society. Parameters used in this study to reveal beliefs, public attitudes, access to watershed information, and sharing society in environmental management, including how local communities close relationship with the government in environmental management. Engagement and Empowerment expected to affect the success of sustainable river

conservation. Initial questionnaire was given at the time the community still as it is, which is when they do not understand their knowledge of bioindicators of the environment. The second questionnaire was given after two month the public was introduced to the knowledge of bioindicator Macroinvertebrates. The second questionnaire is also intended as an assessment of how far the community has been to apply their knowledge of bioindicator Macroinvertebrates.

RESULTS

Diversity of Macroinvertebrates as River Bioindicators

To determine the diversity of Macroinvertebrates in the research area, a survey was carried out in the Upper Brantas watershed area for community of Macroinvertebrates. The results showed that there are there are fifteen Familia of Macroinvertebrates (Lymnaeidae, Thiaride, Pachychilidae, Corbiculidae, Hydropsychidae, Psychomyiidae, Baetidae, Blephariceridae, Aeshnidae, Chlorocyphidae, Libellulidae, Gerridae, Planariidae, Glossiphoniidae, and Lumbricidae); into Thirty-one Genus (*Lymnaea*, *Thiara*, *Tarebia*, *Melanooides*, *Sulcospira*, *Corbicula*, *Leptonema*, *Hydropsyche*, *Ceratopsyche*, *Lype*, *Baetis*, *Bibliocephala*, *Amphiaeschna*, *Libellago*, *Rhinocypha*, *Agriocnemis*, *Ischnura*, *Pseudagrion*, *Brachythemis*, *Crocothemis*, *Neurothemis*, *Orthetrum*, *Trithemis*, *Trepobates*, *Limnogonus*, *Ptilomera*, *Metrobates*, *Aquarius*, *Planaria*, *Helobdella*, *Lumbricus*); and consist of Thirty-six Species namely *Lymnaea rubiginosa*, *Tiara scabra*, *Tarebia granifera*, *Melanooides tuberculata*, *Sulcospira* sp., *Corbicula javanica*, *Leptonema* sp., *Hydropsyche morose*, *Macrostemum* sp., *Ceratopsyche sparna*, *Lype diversa*, *Bibliocephala* sp., *Amphiaeschna ampla* (Rambur), *Libellago lineata* (Burmeister), *Rhinocypha fenestrata* (Burmeister),

Agriocnemis femina (Brauer), *Ischnura senegalensis* (Rambur), *Pseudagrion pruinosum* (Burmeister), *Brachythemis contaminata* (Fabricius), *Crocothemis servilia* (Drury), *Neurothemis terminata* (Rambur), *Orthetrum chrysis* (Selys), *Orthetrum glaucum* (Brauer), *Orthetrum pruinosum* (Burmeister), *Orthetrum sabina* (Drury), *Orthetrum triangulare* (Selys), *Trithemis festiva* (Rambur), *Trepobates* sp., *Limnogonus fossarum*, *Ptilomera hemmingseni*, *Metrobates* sp., *Aquarius paludum paludum*, *Planaria* sp., and *Helobdella* sp., *Lumbricus terrestris* respectively as table 1 below.

Table 1. Diversity of Macroinvertebrate were found in the Brantas river watershed

Classis	Ordo	Familia	Genus	Species
Gastropoda	Sommatophora/Pulmonates	Lymnaeidae	<i>Lymnaea</i>	<i>Lymnaea rubiginosa</i>
			<i>Thiara</i>	<i>Tiara scabra</i>
	Coataenioglossa	Thiaridae	<i>Tarebia</i>	<i>Tarebia granifera</i>
			<i>Melanooides</i>	<i>Melanooides tuberculata</i>
	Sorbeoconcha	Pachychilidae	<i>Sulcospira</i>	<i>Sulcospira</i> sp.
Pelecypoda	Veneroidea	Corbiculidae	<i>Corbicula</i>	<i>Corbicula javanica</i>
Insecta	Trichoptera	Hydropsychidae	<i>Leptonema</i>	<i>Leptonema</i> sp.
			<i>Hydropsyche</i>	<i>Hydropsyche morosa</i>
			<i>Ceratopsyche</i>	<i>Macrostemum</i> sp.
			<i>Ceratopsyche</i>	<i>Ceratopsyche sparna</i>
		Psychomyiidae	<i>Lype</i>	<i>Lype diversa</i>
	phemeropte	Baetidae	<i>Baetis</i>	<i>Baetis</i> sp.
	Diptera	Blephariceridae	<i>Bibliocephala</i>	<i>Bibliocephala</i> sp.
	Odonata	Aeshnidae	<i>Amphiaeschna</i>	<i>Amphiaeschna ampla</i> (Rambur)
			<i>Libellago</i>	<i>Libellago lineata</i> (Burmeister)
			<i>Rhinocypha</i>	<i>Rhinocypha fenestrata</i> (Burmeister)
			<i>Agriocnemis</i>	<i>Agriocnemis femina</i> (Brauer)
			<i>Ischnura</i>	<i>Ischnura senegalensis</i> (Rambur)
			<i>Pseudagrion</i>	<i>Pseudagrion pruinosum</i> (Burmeister)
		Libellulidae	<i>Brachythemis</i>	<i>Brachythemis contaminata</i> (Fabricius)
			<i>Crocothemis</i>	<i>Crocothemis servilia</i> (Drury)
			<i>Neurothemis</i>	<i>Neurothemis terminata</i> (Rambur)
			<i>Orthetrum</i>	<i>Orthetrum chrysis</i> (Selys)
				<i>Orthetrum glaucum</i> (Brauer)
				<i>Orthetrum pruinosum</i> (Burmeister)
				<i>Orthetrum sabina</i> (Drury)
			<i>Orthetrum triangulare</i> (Selys)	
			<i>Trithemis</i>	<i>Trithemis festiva</i> (Rambur)
			Hemiptera	Gerridae
<i>Limnogonus</i>	<i>Limnogonus fossarum</i>			
<i>Ptilomera</i>	<i>Ptilomera hemmingseni</i>			
<i>Metrobates</i>	<i>Metrobates</i> sp			
<i>Aquarius</i>	<i>Aquarius paludum paludum</i>			
Turbellaria	Tricladida	Planariidae	<i>Planaria</i>	<i>Planaria</i> sp.
Hirudinea	synchobdella	Glossiphoniidae	<i>Helobdella</i>	<i>Helobdella</i> sp.
Oligochaeta	Lumbricina	Lumbricidae	<i>Lumbricus</i>	<i>Lumbricus terrestris</i>

Utilization of Macroinvertebrates Knowledge as the Activation of the River Conservation in the Watershed Communities

The analysis of Activation Communities on Sustainable River Conservation Based on Macroinvertebrates Knowledge in Brantas Upper Watershed Area shows that in general, community in upper Brantas watershed area do not understand their role in environmental stewardship (14.59 ± 2.9), their role in protection environmental damage (12.41 ± 4.09) and lack of information on Bioindicator of Macroinvertebrates (7.64 ± 0.08). Detailed information is presented in table 2. After they get information bioindicator Macroinvertebrates knowledge, almost all parameters results indicated significant increase in t-test analysis after two month they receive information's on Bioindicator of Macroinvertebrates ($p < 0,001$); Environmental ethics ($p < 0,001$); Intention for river conservation ($p < 0,001$); The role of the community in environmental stewardship ($p < 0,001$); The role of the community in protection environmental damage ($p < 0,001$); The level of community in participation environmental conservation ($p < 0,001$); and Type of community participation in environmental conservation ($p < 0,001$).

Table 2. Relationship Activation Communities on river conservation based on bioindicator Macroinvertebrates Knowledge

Parameters	Average \pm std dev,		p-value of t-test before vs after
	Before	After	
Information on Bioindicator of Macroinvertebrates	7.64 \pm 0.08	11,69 \pm 0.27	p<0.001
Environmental ethics	18.97 \pm 3.48	21.57 \pm 1,77	p<0.001
Intention for river conservation	21.71 \pm 2,87	22.60 \pm 2.03	p<0.001
The role of the community in environmental stewardship	14.59 \pm 2.9	17.99 \pm 1.59	p<0.001
The role of the community in protection environmental damage	12.41 \pm 4.09	16.74 \pm 2.41	p<0.001
The level of community in participation environmental conservation	19.81 \pm 5.01	25.40 \pm 2.57	p<0.001

Type of community participation in environmental conservation	19.94 \pm 4.40	23.69 \pm 1.91	p<0.001
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Note: before and after data indicated value each parameter giving information bioindicator

DISCUSSION

The position and function of the aquatic macroinvertebrates has an important role. Macroinvertebrates are a group of organisms that serve as the primary consumers in the waters, as well as the source of energy for trophic thereon. Macroinvertebrates abundance in the ecosystem is dependent upon the availability of basic foodstuffs these organisms are carbon sources derived from riparian vegetation that exist around the river. Litter from plant and Death Organic Material (DOM) into one of determining the existence of Macroinvertebrates in these waters. On the other hand, human activity that live in terrestrial areas can affect aquatic macroinvertebrates. Garbage of human activity in the form of non-degradable plastic and other materials that can not be parsed by nature in the long term, residual fertilizer containing N and P were high coming into the river, and rest of organophosphates and organochlorin derived from pesticides will greatly affect the existence and macroinvertebrates life.

Variety of forms and ways of macroinvertebrates in response to a pollutant into waters in various ways, such as by changing the composition and number of species of macroinvertebrates itself. Responses of different macroinvertebrates in response to the same pollutants on aquatic organisms due to differences in sensitivity to the pollutant. Macroinvertebrates are very sensitive to pollutants is an organism that would immediately respond to the presence of pollutants in the water, although in very small quantities. Examples of this group are members of the Ordo Ephemeroptera, Plecoptera, and Trichoptera Groups of macro-invertebrates is

medium tolerant to pollution, an organism with the ability to live in different conditions can be found in good or poor quality water, including Ordo Odonata, Tricladida, Basommatophora, and Hemiptera. The later groups of macroinvertebrates that is very tolerant to certain forms of pollution, including Ordo Diptera, Neotaenioglossa, Sorbeoconcha, Veneroidea, Rhynchobdellida, and Lumbricina (Barbour, 1999; Mandaville, 2002).

Functionally in the ecosystem, macroinvertebrates based on the functional feeding classes, which can be grouped in the class **scrapers**, groups of this organism include various herbivores and detritivores that graze periphyton and attached microflora and fauna on mineral or organic surfaces. The second grouped in the class **filterers**. Filterers are the class of collectors. The filterers group are include various suspension feeders consisting of a combination of detritivores, herbivores, and carnivores. The third grouped in the class **shredders**. The class shredders includes a large group of detritivores and herbivores that feed on both live and dead matter in the river. The fourth grouped in the class **gatherers**. The class gatherers include detritivores and herbivores that are deposit feeders in the river, and the later class grouped in the class **collector**. This class is a group of collectors and filterers. Because collecting responses may vary, this class is a group that can tolerate water pollutants (Mandaville, 2002; Lillie, 2003; Tullios, 2006; Mazzacano, 2007).

Utilization of the existence of macroinvertebrates in river waters more widely used by scientists to understand the presence of these organisms in the ecosystem. on the other hand, people who stay and live 24/7 around the river, because of the limitations of their knowledge, still assume that the existence of living beings that exist in the waters are supposed to be as it is. The impact of the lack public knowledge on the environmental condition is the public indifference to environmental conditions around it.

In fact, according to the theory proposed by Ajzen (1991), public awareness of the environment can be improved by enhancing their interest in understanding the surrounding environment. A complete theory of Planned Behavior by Ajzen is presented in **Fig. 1** as follows:

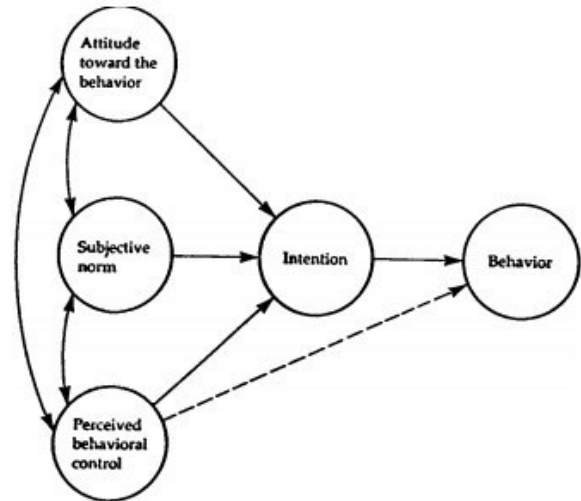


Figure 1. Theory of Planned Behavior (Ajzen, 1991)

The intention is a key factor to change the attitude of society towards the environment initially indifferent to environmental concerns raised from themselves. Public interest generated by the hang of existence, function and role of aquatic macroinvertebrates that exist in people's daily lives and as bioindicators of aquatic environments.

From the results of research conducted on people in the upper Brantas watershed area found support for the theory of Planned Behavior that developed by Ajzen (1991). Activation of society based on increasing knowledge-based macroinvertebrates bio-indicator has shown that an understanding of local communities in the upper watershed of the macroinvertebrates as bioindicators increased significantly after two months of existence and functions of introduced macroinvertebrates in their environment ($p < 0.01$) as shown in table 2 above. Improvement is also seen for other parameters

research such as Environmental ethics ($p < 0,001$); Intention for river conservation ($p < 0,001$); The role of the community in environmental stewardship ($p < 0,001$); The role of the community in protection environmental damage ($p < 0,001$); The level of community in participation environmental conservation ($p < 0,001$); and Type of community participation in environmental conservation ($p < 0,001$). Increased in all parameters of the research going on toward better environmental stewardship, the role and level of community participation, as well as the public on environmental ethics.

It demonstrates that the conservation of the river can be done independently by the people in upper watershed area to resuscitate the public understanding of the condition of the organisms living in the environment as bioindicator. This understanding will enhance intentions of the community and to support sustainable river conservation.

CONCLUSION

Activation on river conservation can be raised by increasing public interest in living organisms to their environment such as macroinvertebrates. With increasing the intentions of the public on the environment will gradually change the perception and behavior of people on the river environment towards better conservation. Understanding of the existing macroinvertebrates bioindicator in the environment will lead to more public attention on their aquatic environment and awaken of the connections between human interrelationships with other living things around them.

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Applicability of Easy-to-use *Escherichia coli* Test Strip for Community Development Program on Drinking Water Safety

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ABSTRACT

In many developing countries, people consume shallow groundwater and river water for drinking and other daily water consumptions in the country side. The monitoring of pathogens in daily water sources is needed for effective problem mitigation. However, it is impossible adequately to tackle the demand of water quality monitoring by standard methods due to high cost for sample analysis. Hence, a strategy that accept wider flexibility was accommodated. Subsequently, an easy-to-use *E. coli* contamination check kit was applied in this research. The aim of this study was to examine the applicability of the easy-to-use *E. coli* testing technique to make tailor-made locally affordable water quality information by non specialist. An exploratory survey was performed taking example in an annual community development program by Universitas Islam Negeri Malang, Indonesia. Dempok hamlet, East Java, Indonesia was the community where the field work was performed. The *E. coli* test strip was easy-to-use, and also fun-to-use. A baseline data for *E. coli* contamination was successfully collected by only nonspecialist without any laboratory facility. The data indicated general moderate level *E. coli* contamination in villagers' daily consuming water ($2 < E. coli$ CFU < 10 /mL). The device of *E. coli* contamination test kit took unique role as a communication support tool between students and villagers in the community development program. The methodological advantage was potential to generate assimilated water quality data for *E. coli* contamination with local ecosystem process, people's feeling for water demands, and local knowledge. Toward the next step, a potential device was designed to implement affordable *E. coli* testing capacity in community development programs.

Keywords

Community development program, *E. coli* contamination, participatory water ecosystem monitoring.

INTRODUCTION

The rapid rate of industrialization as well as urbanization has been improving quality of life and economies, globally. It is also simultaneously causing the tide of pollution ubiquitously increasing contaminants in river and reservoir waters. The associated risk for pathogen contaminated water consumption is one of the great concerns from a health perspective (OECD 2003). Wherefore, fecal contamination has been a general indicator for the abundance of disease-causing organisms (Leclercq et al. 2001; Tallon et al. 2005). Water quality monitoring has been performed for *Escherichia coli* (*E. coli*) contamination, and currently, WHO recommends *E. coli* as the best indicator of fecal contamination (Tallon et al. 2005). According to Moe et al. (1991) in Philippines, good quality (< 1 *E. coli* per 100 ml) and moderately contaminated (2-100 *E. coli* per 100 ml) children's drinking water has little difference for illness rates on children. Therefore, drinking water for children with > 1000 *E. coli* per 100 ml had significantly higher rates of diarrhoeal disease than those less contaminated drinking water. As a risk management indicator, international drinking water standards and guidelines do state no *E. coli* contamination (Tallon et al. 2005). The capacity shows a tolerance of up to 100 *E. coli* per 100 ml (Moe et al. 1991), which is due to the robustness of peoples' immunity to *E. coli* contamination level, though it does not indicate the safety of drinking water.

2. Aim of this research

In Indonesia, the infrastructural capacity for chlorinated pipe water is limited for the country side, where many people consume shallow groundwater and river water for daily life. Subsequently, on demand assessment and mitigation of the pathogen contamination risk gives the rationale to preserve human health. How can *E. coli* contamination with the geologically,

ecologically, and sociologically diverse phenomena be monitored on-demand? Hitherto a critical point can be assumed for the capacity of on-demand tailor made water quality information for pathogenic bacterial contamination in peoples' daily water source. Hereby, if the application of standard method is technically only compulsory for water quality testing, its high cost restricts the information density in time and space. Besides, the analysis can only be conducted by specialists that depend on laboratory facilities, and also capacity of the specialist's job. On the other hand, on demand tailor-made information is enabled with wider flexibility of monitoring methods as if it is purposely accepted (Graveline et al. 2010). In this research, we focused on this strategy, and an easy-to-use *E. coli* contamination check kit was purposely selected that has detection limit for several hundreds *E. coli* contamination per 100 ml water sample. The aim of this study was to examine the applicability of the easy-to-use *E. coli* testing technique to make tailor-made locally affordable water quality information for non specialist. An exploratory survey was performed taking an example from an annual community development program conducted by Universitas Islam Negeri Malang, Indonesia.

MATERIALS AND METHODS

1. *E. coli* test strip

Coliform bacteria test strip SC-N06 (Sankori, Japan) was used (Figure 1). The test strip utilizes two different assays, simultaneously. Coliform bacteria, generally stated as total coliform, is gram-negative bacillus that generate B-galactosidase (B-GAL). Total coliform is tested by B-GAL and 5-bromo 4 chloro 3 indolyl B-D galacto pyranoside (XG) based on colorimetric assay. Existence of a coliform bacteria on a test strip produce B-GAL, and the XG, which does not have color, is broken down by B-GAL into Bromochloro indigo that has blue color (Stevens et al. 2001). Next, amongst coliform

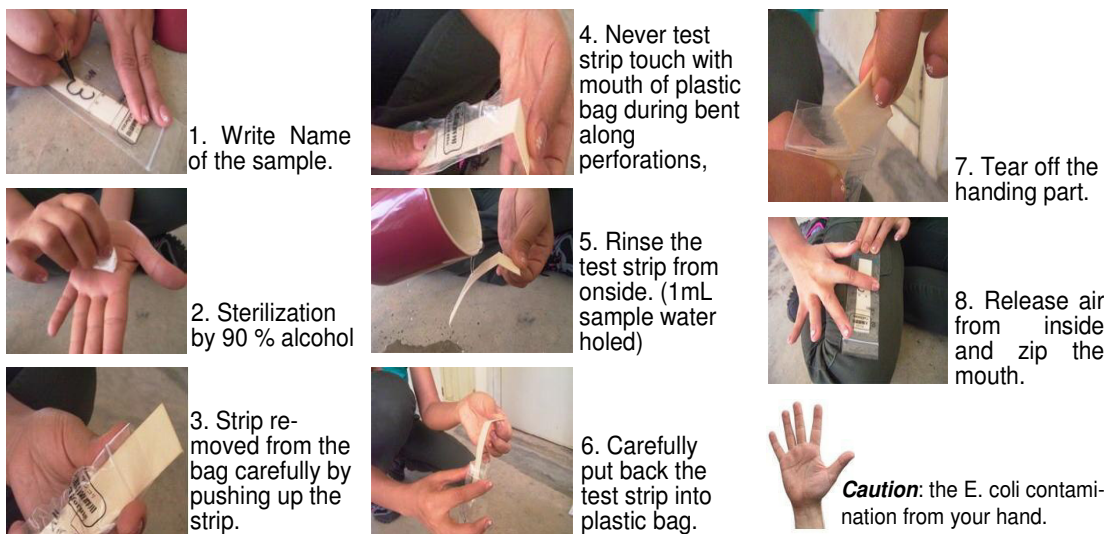
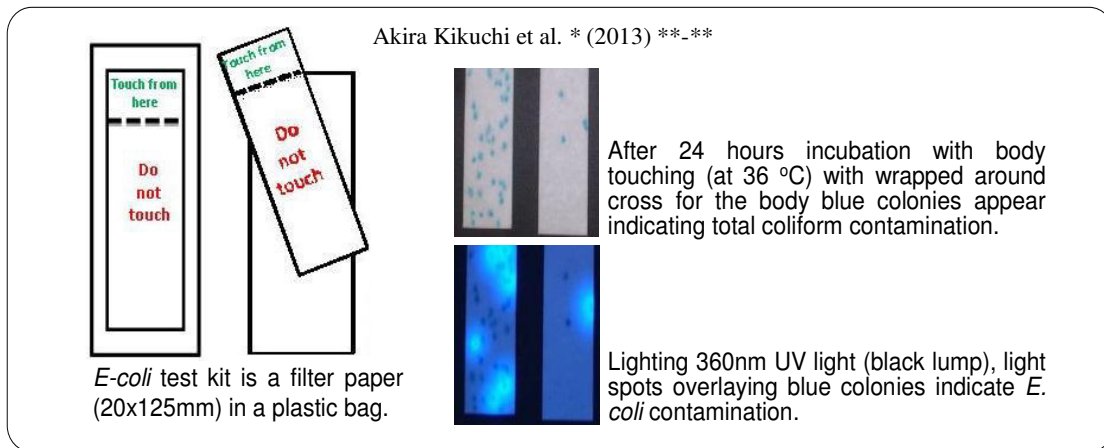


Fig. 1. *E-coli* test strip (SC-No.6, Sankori, Japan) to test both Total coliform and *E. coli* which are B-galactosidase-positive Enterobacteriaceae (Stevens et al. 2001) and B-glucuronidase-positive coliform group bacteria (Huang et al. 1997), respectively.

bacteria, only *E. coli* has B- gluconidase (B-GLU) activation. The existence of *E. coli*, 4-Methylumbelliferyl-B-D(-)-glucuronide broken down by B-GLU into 4-umbelliferone that has bright fluorescence light for 360nm UV light (Huang et al. 1997).

2. *E. coli* as fecal pollution indicator

E. coli is now stated as the best indicator for fecal contamination including WHO (Tallon et al. 2005). Though, it is a fact the applicability of *E. coli* for fecal tracking has exception in the nutrient-rich tropics, as *E. coli* is a member of the natural microflora in such habitat (Rivera et

al. 1988). *E. coli* have been found without known sources of fecal contaminations in tropical area (Jimenez et al. 1989; Lopez Torres et al. 1987). The particular environment for the exception is related with high concentrations of

free amino acids and sugars that property has similarity with the enteric environment of homoiothermal animal (Savageau 1983). Thereby, fecal contamination from feces of homoiothermal animals (e.g. domestic animals,

and birds) are also to be potential back ground noise of *E. coli* contamination in environmental water. The situation is requesting more research for *E. coli* ecology in tropical area (Tallon et al. 2005), regarding with the worse



Fig. 2. Typical water facilities in the village. 1: Pipe water from faucet that water provided through local piping system without chlorination, 2: Water reservoir at a household, 3: Sampling of water from reservoir, 4: relay tank in the local pipe water system, 5: water in reservoir basin for washing use, 6: the source of river stream flow (punden: spring), 7: regiments' daily activity at spring (punden), 8: water sampling from a well, 9: inside of a well.

still, we assume to apply *E. coli* contamination for fecal tracking in research site.

3. Research site

Neighborhood association 15, Dempok Hamlet was research site, where is part of the village Gampingan, Dempok, Pagak district, East Java, Indonesia. In the Gampingan village, diarrhea was the third highest disease after the common cold with the number of cases as many as 136 or 2.3% in 2012 (Gampingan 2012). The number seems small, but it is only a fraction of the total number.

4. Community development program

In this year, 1920 students were participated with Community development program as a compulsory subject at 1st year educational program in University Islam Negeri Malang. The students divided into 16 groups went 14 subdistric in Malang Regency. One group was appointed for this experimental activity. The program started with training in 16th and 23rd of July 2013, and the main body was from June 30 to 30 of July 2013. The community development program was hosted by management of Posdaya Based Mosque in the community. The theme of University's community development program, which can be shared among Islamic Universities, was imitation of the historical development of Islam brought by the Prophet Muhammad, he started the activities of knowledge and development of the community mosque. Whereas, the activity was not only about religious education only instance where the Qur'an (TPQ), moreover the activities include education, economic, entrepreneurship, and environment. By the context, students become agents of community transformation. This activity is expected to be an initial step in the process to increase the public awareness for potential resources of the community which are potentially to be improved or developed.

RESULTS AND DISCUSSIONS

1. *E. coli* contamination

The field work started by a survey on the daily needs for local water consumption. In the village, pipe water supply has been available since 2000, with an increased coverage since April of 2013 by the village development program (activities of Bina Desa). 85% of the population in RT 15 participated in the program with 20 families. 17 pumps and meters were installed at crucial points in the village. The management is assigned to the village up to collection of charge for monthly water consumption. Faucet water is meant at research site was water from natural stream via pipes without any treatment. For the each of house holds, most local people prepare water tank in their

Table 1. Result of total coliform and *E. coli* testing in Dusan Dempol, Gampingan villages, Pagak district, East Java, Indonesia.

Sample name	No.	Total coliform	E. coli
		[number/ ml]	[number/ ml]
Water in reservoir tank	1	26	2
	2	6	4
	3	10	3
	4	6	2
	5	2	2
	6	5	g, 2
	7	2	6
	8	10	5
	9	3	4
Pipe wate from faucet	1	14	2
	2	6	5
	3	11	1
	4	?	9
	5	12	9
Water in relay tank of pipe water supply system	1	5	2
	2	14	2
	3	3	4
	4	13	b-g
	5	17	?
	6	1	1
Sumber	1	13	6
	2	?	2
Well water	1	?	1
	2	?	1
	3	21	5
	4	11	2
	5	?	2
	6	23	5

g: full green color, b-g: full blue-green color. Green color indicates injured E-coli cell contaminated.

kitchen, and then used it to daily life. Even though, such pipe system is developed,

springs (*Suber*) that are locally called “*punden*” still had high demand for daily water consumption. Well water was also used in some households.

In the testing, first, total coliform contamination was checked by counting the blue spots, which were visibly observable on the surface of test strip. The number of total coliform is per 1mL sample water that held in the test strip. The results are shown in Table 1. The result of total coliform contamination showed 100% (24 samples out of 24 samples, with 4 missing value). Thereby, contamination with coliform bacteria for 13 samples were equivalent or higher than 1000 colony forming unit (CFU) per 100 ml level. Then, *E. coli* contamination was checked using 360nm UV light (black lamp for counterfeit bill checking). The result was that the samples were generally contaminated by *E. coli*. 13 samples showed >3 *E. coli* CFU, 2 samples showed > 6 *E. coli* CFU for 1mL sample held in the test strip, respectively. According to Moe et al. (1991), > 10 *E. coli* CFU per 1 ml had significantly higher rates of diarrheal disease for children’s drinking water. Comparing with the criteria, the results were lower than the significant risk level. However, it was deduced that more than half of the samples were moderately contaminated by *E. coli* ($2 < E. coli$ CFU/ mL <10). Moreover, all the water sources, such as, wells, and water distributed through pipe system were not eligible for direct drinking by *E. coli* contamination level regarding any water quality regulations and guidelines (Tallon et al. 2005). The base line data showed the general *E. coli* contamination in daily consumption of water in the research site. Here an important research outcome extrapolated was the fact that the local community is geologically, ecologically, and socially just common state in East Java. It was mostly indicated that there is general *E. coli* contamination, i.e. fecal contamination, in daily consumed environmental water in East Java. After the

fieldwork, the students’ impression for the *E. coli* test strip was easy-to-use and also fun-to-use. Hereby an important research out-come was not only for the baseline data, but also there is the fact that research activity was possible only by non-specialists without any special equipment.

3. *E. coli* test strip as a communication device

In general, the improvement of impacted environmental water resource request interactive framework of ecology, sociology, and economy (Timmerman & Ward 2000; De Jong et al. 1994; Raskin et al. 1997). Problem mapping is one of the general approaches to extract local information and to establish framework of existing problems. Whereas, current technical problem is weakness of sufficient device to generate assimilated real water quality data with the establish framework of existing problems. Moreover, there are demand for unique technique to fuse water quality information, local ecosystem process, people’s demands, and local knowledge. By the aspect, there is sense of significant to reconsider the atmosphere of fieldwork. During a community development program, students immersed themselves in the life of a charged community. According to students participated, when students came into village people's houses for water sampling, they-students narrated the activity was for their research. Responding with it, villagers started to talk about the water sources they have, the use of their daily water, academic work that had been done about the content of lime in the water by a University, including the importance of their drinking and cooking water from their sense. It was one of the most interesting experiences for students to visit peoples’ homes in order to test water quality and to interview them about people’s activities in the daily use of water. The experiences are indicating an important potential to afford access to the backstage culture for multi-dimensional water issues via

participant observation (DeMunck and Sobo 1998). The approach allows for richly detailed description, which they interpret to mean that one's goal of describing "behaviors, intentions, situations, and events as understood by one's own informants", provides opportunities for viewing or participating in unscheduled events. Villagers were open for communication during the field work, and friendly to students who performed community service. Thereby the role of the *E. coli* test strip was to activate interaction between villagers and students. While the effect of the tool was a natural occurrence of an interactive interview by students on villagers' daily water issues.



Fig. 3. A potential device designed. Manual, alcohol in microtube, and *E. coli* test strips are packed in a plastic bag.

According to a case study by this research, applicability of *E. coli* test strip made sense of significance in a University's community development program. Accordingly, a trial device has designed as shown Fig. 3, which was "flyer with *E. coli* test strip" taking analogy with prior study (Akira et al. 2012). The device consists of *E. coli* test strips, a flyer, and 2ml alcohol in microtubes. These were all packed in a plastic bag. The flyer contained explanation about importance the for *E. coli*

testing, manual to use *E. coli* test strip, water quality criteria to interpret the result.

CONCLUSION

The *E. coli* test strip was easy-to-use, and also fun-to-use. A baseline data for *E. coli* contamination was successfully collected by only no specialists without any laboratory facility. The data indicated general moderate level *E. coli* contamination in villagers' daily consumption water ($2 < E. coli \text{ CFU} < 10 / \text{mL}$). The device took role as a communication support tool between students and villagers in a University students' community development program. The methodological advantage was potential to generate assimilated water quality data for *E. coli* contamination with local ecosystem process, people's demand, and local knowledge. Strictly to say, the reliability of this result is required to be tested by comparison with laboratory based standard method. The validation has planned in this year.

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HARMONY INDUCTION OF CASSAVA UNDER AGRO-FORESTRY: CEASE ECOLOGICAL REDUCTION AND REACH ECONOMIC SEDUCTION

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ABSTRACT

As a strong carbon absorber and converter into biomass, cassava is now very popular source of inexpensive carbohydrate to meet various ultimate usages. This biological potential promotes cassava with initially for staple and supplemental food from subsistence stage of the poor community, and then develops into commercialization for fulfilling raw material of industries. Aside as food, the role of cassava is becoming significant to supply as raw material for industrial endeavor from pharmacy, cosmetic, paper, textile, sorbitol, maltose, glucose, high fructose syrup, and edible as well as biodegradable plastic. As part of global community, Indonesia has strong endeavor to realize Millennium Development Goals (MDGs) which put a top priority to reduce hunger and alleviate poverty till 50% at 2015. Unfortunately, the severe of global warming locked a dream into a reality. Expanding of agricultural land by additional of the newly areas from forest faces criticize from national and international parties. Recently a strong commitment to conserve forest is a must. Cassava development into the newly area converted from forest is not able to meet sustainability criteria. Hence, agro-forestry is an appropriate way out to harmonize the dilemma countenance. To gain better accomplishment cassava agro-forestry can be undertaken in line into production forest management neither in the conservation forest.

Keywords: cassava agro-forestry, ecological and economic demand.

INTRODUCTION

Among the root and tuber crops, cassava (*Manihot esculenta* Crantz) is the most essential source of carbohydrate, however it is the third rank important of food after rice and maize. Unlike rice and maize and other cereal crops as well as grain legumes such as soybean, peanut and mung-bean that are strongly worried by

government, cassava and other root crops were less received attention from government. However, cassava is always grown by farmers both for subsistence and commercial needs (CIP, 2001; Widodo, 1995a; 1995b; 1999; 2008, 2009). Conforti and Sarris (2011) as well as Food and Agriculture Organization/FAO (2012) indicated that global food production was under uncertainty and as consequence price

volatility could not be avoided. Therefore, recently government interests to increase the role of cassava in the agricultural development especially to meet the domestic demand as well as international market (Widodo, 2011a; 2011b; 2012a; 2012b). As a strong carbon absorber and converter into biomass, cassava is now very popular source of inexpensive carbohydrate to meet various ultimate usages. This biological potential promotes cassava with initially for staple and supplemental food from subsistence stage of the poor community, and then develops into commercialization for fulfilling raw material of industries (Kementerian Pertanian, 2011; Widodo, 2012b). As source of food cassava can be prepared directly from fresh especially non bitter varieties (HCN<50 ppm) as well as from dried form and starch derivatives products originated from bitter varieties (HCN>50 ppm). There is hundreds recipes of staple and supplemental food generated from cassava fresh, dried form and starch as well. Aside as food, the role of cassava is becoming significant to supply as raw material for industrial endeavor from pharmacy, cosmetic, paper, textile, sorbitol, maltose, glucose, high fructose syrup, and edible as well as biodegradable plastic. Recently, due to shortage of fossil fuel therefore cassava is also used as raw material for bio-ethanol to be mixed with gasoline into gasohol. As consequence production of cassava has to be increased to meet the greater demand of various uses including food, feed, fuel etc. (Widodo, 2012b).

Development of cassava into new areas out side of Java faces many difficulties particularly labor as well as restriction to convert peat land and forest for agricultural area. It means improving productivity is preferred than expansion of new planting area. In Java as densely populated island of

Indonesia, cassava is also grown in hilly mountainous areas. Fortunately, those areas are mostly under the forest domain belong to Perhutani a State Forest Enterprise. Under such system, cassava is planted by small poor farmers under intercropping with cereals and legumes in between forest to improve land equivalent ratio with fully organic farming (IFOAM, 2000; 2011). Moreover, under such system, due to farmer have better attention and care to crop and forest, therefore the positive impact in preventing fire in the forest can be attained (Green Peace, 2012). Contribution of cassava from forest area under Perhutani will be recommended for larger implementation to improve community income, especially those who living around forest area. This work is expected to contribute an endeavor to meet Millennium Development Goals (MDGs) objective number one, where hunger reduction and poverty alleviation as priority (United Nation, 2008; UNDP, 2009; UNFCCC, 2009).

METHODOLOGY

Participatory Research Action with the involvement of farmers around forest to grow food crops including cassava and the other root and tuber crops was supported by Perhutani. This activity was started from 1996 at Karangbendo Blitar then developed to several areas of East Java. In 1998-2001 during the reformation period euphoria of people to cope country asset was strongly knowledgeable included doing illegal logging? As the impact several forest areas of Java was seriously damaged (Widodo, 2011a; 2011b; 2012a; 2012b). Fortunately, to recover the worst condition through the Social Forest Program initiated in 2003, in which farmers and stakeholders around forest were actively participated. In this

program farmers and poor landless people were allowed to grow food crops in between the rejuvenated forest tree. However, the farmers had responsibility to manage and care the forest tree to develop vigorously. Farmers working in the forest build association as so called LMDH (Lembaga Masyarakat Desa Hutan) or Rural Community around forest. Positively under LMDH farmers also have the right to share the profit from timber as well as yield of associate crops.

Field demonstration plot was carried out at several sites under Perhutani at Subang West Java from 2009 to 2011 in order to supply the demand of cassava for bio-ethanol of Korean Factory at Tomo Subang. Five cassava genotypes consisted of local edible not bitter as check (1) to be planted with four bitter genotypes, namely Adira-4 (2), Menyok Putih (3), Caspro (4) and Cccek Ijo (5). Each genotype was planted at the area of around 5 ha. Land preparation was undertaken by tractor entering the space of land between teak trees with 6 m width. Cassava stem cuttings were planted in between teak trees with five rows at the distance of 1 m between rows. It meant the distance of edge rows 50 cm to teak tree rows. Fertilizer application in the form of 500 kg Phonska was applied twice at two weeks after planting 150 kg Phonska and at 2.5-3 months 350 kg Phonska. Fertilizer demonstration plot with five levels of fertilizer application was carried out at the period of 2010/2011 at the same area as genotypes demonstration plot. Bitter variety of Adira-4 was used as planting material, and planted of 0.5 ha for each fertilizer application. So the total of around 2.5 ha Adira-4 was planted to be used for fertilizer demonstration plot. The treatments evaluated were as follow: 1) Indigenous fertility (no fertilizer application); 2). Additional cows manure 10 t/ha; 3).

Additional 300 kg Urea+150 kg SP-36+200 kg KCl/ha; 4). Additional Phonska 500 kg/ha; 5). Additional Phonska 500 kg + 10 t/ha cows manure. Both of demonstration plots have cassava population density around 8,000 plants/ha. To keep the better quality of cassava cutting from stem, therefore harvesting was undertaken at 12 months. So, continuous planting from season to season need only around 15-30 days.

Aside results from demonstration plot with the response of community to grow cassava in various shade intensity at around forest as well as its leverage into economic aspects were observed. Contribution of cassava as food, feed and other uses in national level will be discussed from secondary data collected.

RESULTS AND DISCUSSIONS

Demonstration Plot For Further Implementation

The width of space between the teak rows are adequately to be spent for growing cassava and other food crops under the forest teak. Unfortunately, in some rural remote areas such as the site for demonstration plot, where the labor is not easily available therefore mechanization especially for soil tillage is very important. Indeed there is a worse leverage of soil tillage by tractor into the growth of teak, because several lateral roots of teak are damaged due to deep tillage of plough and ridge maker attached in the tractor (Figure 1). Generally cassava is planted traditionally without better soil tillage by manual (Figure 2). Until five years old of teak, the space between rows with 6 m width very possible to be grown for cassava. Because shade effect of teaks only 40%. Four rows of cassava can be inserted in between rows of

teak. To reduce shade effect of teak to cassava, lateral branches of teak can be cut. During cassava growing period, 6-8 lateral branches of teak were cut, especially during early rainy season. This practice will induce the growth of apical dominant, so teak will vigorously grow higher.

In the forest area of Subang as well as in whole of Java, cassava is widely planted to produce food and cash income. Sweet or not bitter varieties that can be directly consumed, are preferred than did the

bitter one. Because, in around areas there are many food processing especially fermented form (tape/peuyeum). Cassava fermented form is processed by simple way, from peeling ->washing ->steaming ->inoculating with yeast -> incubating 24-36 hours -> ready to consume (tape/peuyeum). Bitter cassava is mostly processed in small till medium starch industries. However, due to during harvest period there was a peak season therefore cassava from this demonstration plots were shipped to Lampung that paid better price.



Figure 1. Mechanization for soil tillage in between teaks row for food crops including cassava, Subang West Java 2009.



Figure 2. Cassava grown in between of forest with minimum soil tillage, Subang West Java 2008.

Among five genotypes evaluated, Caspro gave the highest yield 40.05 t/ha and highest profit Rp 18,785,000 per hectare. Meanwhile the productivity of local genotype non bitter which suitable for direct human consumption was only 21.50 t/ha, so the profit obtained only at amount of Rp 5,800,000 per hectare (Table 1). By introducing the high yielding genotypes for industry it is expected small till medium scale of cassava extraction process in the domain around will grow and develop to provide new as well as additional income for the dwellers. Ginting and Widodo (2013) revealed that starch generate from bitter cassava has a better quality than did from

non bitter cassava. Moreover, they suggested that for direct human consumption or table use non bitter cassava is more recommended. The lethal dose of cyanide for human was reported to be 0.5-3.5 mg HCN/kg body weight, which was about 30-210 mg HCN for a 60 kg adult. the content of HCN at the bitter cassava genotypes is above than 50 mg/kg fresh of root. Cassava very bitter genotypes may consist of HCN above than 100 mg/kg fresh weight of root. During hunger calamity people in rural community consumed cassava without considering the HCN content, as a consequence toxicity of cassava to be serious problem which induces

to passing away. Indeed, the human body is able to detoxify as high as 100 mg of HCN for 24 hours by rapid conversion of cyanide into the much less toxic thiocyanate, which is then excreted in the urine. There was no evidence of acute effect of cyanide exposure rates below 100 mg HCN per 24 hours. However, high levels of cyanide intake may cause severe cyanide intoxication, which particularly may occur in cassava eating population due to consuming insufficient removal of HCN during food preparation.

In remote areas of Java including around forest under Perhutani, bitter cassava is easily processed by peeling and directly sun drying onto soil to be dried cassava as so called Gapek. Indeed by simple processing of cassava root into gapek, the significant removal of HCN content was accomplished. Then from gapek many kinds of food products can be generated by rural people around forest with very safe to be consumed.

Table 1. Yield and profit gain from four genotypes of cassava under agroforestry, Subang West Java 2009/2010.

Cassava genotypes	Root yield (t/ha)	Starch content (%)	Cultivation cost (Rp/ha)	Revenue (Rp/ha)	Profit (Rp/ha)
Local	21.50	22.00	9,250,000	15,050,000	5,800,000
Adira-4	38.25	27.00	9,250,000	26,775,000	17,525,000
White Manioc /Menyok Putih	38.60	26.75	9,250,000	27,020,000	17,770,000
Caspro	40.05	26.50	9,250,000	28,035,000	18,785,000
Cecek Ijo	39.75	26.60	9,250,000	27,825,000	18,575,000

Note: Each genotype planted in area 5 ha. Cassava price during harvest Rp 700/kg (1 USD= Rp 9,000).

The results of fertilizer application demonstration plot indicated that by applying 500 kg Phonska with additional of cow manure 10 t/ha produces the highest yield namely 44.85 t/ha of fresh root, so the highest profit at amount of Rp 28,743,750 can be obtained. Unfortunately most of the farmers in around forest were not apply fertilizer to cassava, so it means that cassava planted by farmers in forest areas is mainly depended on the indigenous soil fertility. Unlike upland agriculture which cassava is grown yearly, the soil fertility especially from organic matter content commonly is low <2%, in forest areas organic matter

content is relatively adequate from contribution of teak litter as well as weed decomposition. Therefore, farmers' practice in order to recover the organic matter content as well as soil fertility by let the land below teak areas are fallow and weedy. Then, during rejuvenation of forest the space in between young tree can be utilized for growing food crops including cassava. Based on this demonstration fertilizer application plot revealed that farmers under forest area were not supported to enjoy fertilizer subsidy; because forest area is considered similar to that estate crop area whereas non subsidy farmers. Subsidy of

fertilizer is mainly focused on food crops grown by farmers at landscape group. Fertilizer subsidy has to be prepared by planning of farmers group with definitely in certain area with fix amount. A strict regulation of subsidy fertilizer resulting group of farmers in forest area to be more

difficult to provide fertilizer for their food crops intercropped under teak. It is the main argument that farmers only spend indigenous soil fertility of forest.

Table 2. Yield and profit gain from four different fertilizer applications of cassava Adira-4 variety under agroforestry, Subang West Java 2010/2011.

Fertilizer application	Root yield (t/ha)	Starch content (%)	Cultivation cost (Rp/ha)	Revenue (Rp/ha)	Profit (Rp/ha)
Indegenous fertility (no fertilizer application)	22.35	27.25	8,250,000	19,556,250	11,306,250
Additional cows manure 10 t/ha	38.25	27.00	9,000,000	33,468,750	24,468,750
Additional 300 kg Urea + 150 kg SP-36 + 200 kg KCl/ha	38.40	26.75	9,500,000	33,600,000	24,100,000
Additional Phonska 500 kg/ha	39.50	26.70	9,750,000	34,562,500	24,812,500
Additional Phonska 500 kg + cow manure 10 t/ha	44.85	26.80	10,500,000	39,243,750	28,743,750

Note: Each treatment planted in area 0.5 ha. Cassava price during harvest Rp 875/kg (1 USD=Rp 9,250)

Exploiting The Potential Of Cassava

To provide more cassava as food, feed and various industrial usages from this activity was observed cassava grown by farmers' under various forest condition related to its space and shade intensity (Table 3). Recently, due to the better of awareness on renewable fuel and green

economic with minimizing pollution, cassava is promising as raw material for bio-ethanol. Furthermore, waste produced from various industrial endeavors in a form of solid as well as liquid can be processed into animal feed and other by products addressed for such as fertilizer and soil conditioner. Biomass other than root, both leaves and stem are also utilized in the food and animal

feed system. Young leaves and shoots can be eaten for human consumption in a form of vegetable with various preparations. Preliminary research indicates that cassava leaves also has potential to be used for silk worm rearing. Under subsistence circumstances, cassava stem is mainly used

for propagation of planting material and the remaining is burnt as source of firewood. In case of cassava incorporating into forestry of teak, firewood is abundant from brushwood of teaks as well as from stem of cassava.

Table 3. Cassava root yield at various conditions and shade intensities, Subang West Java 2010/2011.

Cassava growing condition	Shade intensity (%)	Cassava population density (plants/ha)	Cassava root yield (t/ha)
Open area forest rejuvenated, cassava local variety (not bitter)	2-10	9,500-12,500	33-52
Open area forest rejuvenated, variety Adira 4	2-10	10,000	68
Adira 4 planted in between teak 6x1 m at 3 years old	25-40	8,000	43,5
Adira 4 planted in between teak 3x2 m at 3 years old	35-50	6,500	38
Adira 4 planted in between Mindi tree 3x2 m 2 years old	35-50	6,500	30
Adira 4 planted <i>Melaleuca</i> tree 6x2 m at 6 years old	20-30	8,000	45

Note: fertilizer application in the form of Phonska 500 kg/ha applied twice at plot size of 0.5 ha.

It is the advantages of cassava which suitable to be incorporated into biomass program as suggested by Carbon Initiative World Bank (2009) as well as Winarso (2009) in line with the endeavor to reduce emission of CO₂ at the atmosphere. At commercialization stages, particle board and plywood industries also utilizing part of cassava stem especially the old hardly lignified portion as filler. Utmost utilization of cassava from root till new shoot influencing the whole of crop is removing from the area which very little return back to the soil, except the litter from leaves senescence and fall as organic matter (Table

4). This way of practices ultimately assumed that cassava is soil depleting crop. Fortunately planting cassava under agro-forestry indicates a better synergistic phenomenon for both of crops cassava, cereals and legumes as well as teak or tree of forest. Litter of forest in the form of leaves and branches are the worthy organic matter promotes to organic substance in the soil as well as the admirable material to produce edible mushroom (Jin Tomg Peng, 2010). So, exhaustion of soil organic content of cassava under agro-forestry could be minimized from contribution of leaves

senescence from forest trees (Conforti and Sarris, 2011; Eco Summit, 2007).

Table 4. Utilization of whole cassava plant in around of forest under Perhutani of Java Indonesia, 2011/2012.

Part of crop	Generated from	Processed under
Leaves		
Young leaves & shoot	Lateral buds from < 2 months growth & cassava tips during harvest	Fresh vegetables mixed with shredded young coconut or consumed with chili sauce; side dishes mixed with small shrimp or the other seafood; deep soup with high viscous coconut milk and traditional spices. Etc.
Mature leaves	Topping or reducing canopy of cassava which has high biomass production	Wilting to reduce HCN to be used as animal feed such as goat, cattle and swine. Protein content of cassava leaves able to reach 25% dry basis.
Stem		
Young sprout	<i>Cassavargus</i> the use of young sprout from cassava stem as Asparagus.	Fresh vegetable can be prepared with various recipes
Cutting for planting material	5-10 good quality of cuttings can be obtained from 1 plant.	Select the thick diameter >2cm, and length of cutting 20-25 cm
Skin of stem	Peeled from stem then wilted under sun drying	as animal feed
Old stem and remaining from planting materials	Original mother stems as well as non green fodder stem	firewood as traditional energy
Tuberous root		
Root skin	Peeled from tuberous root then sun dried	as animal feed
Flesh	Direct consumption for HCN <100 mg/kg fresh root	Various recipes of food preparation traditionally
	Prepared as gaplek dried form by removing skin and sun dried the flesh	Further process as flour to be stored longer. Various food preparation is available traditionally
	Starch extraction processes from small, medium to large scale	From tapioca (cassava starch) many derivatives product can be generated.
Pulp	Solid waste from starch industry by fermentation process produces acetic acid etc.	Acetic acid used in pharmaceutical as well as in food processing
	Second generation of bio-ethanol by degradation of cellulose and hemi-cellulose into glucose for further fermentation	Renewable energy without conflicting with food claim.
	Solid waste inoculated by single cell protein for animal feed	Rich protein substance from cassava pulp waste as animal feed

Wisdom From Cassava Farmers

Planting material from its stem cutting constructs cassava is free for propagation, except in the initial establishment of small garden or large plantation. However, due to stem cutting is bulky therefore handling and transportation to be more expensive than other crops propagated by seeds. To attain efficiency of cost, preparation of planting material should be localized. Farmers mostly will select varieties available from its productivity as well as market or factories preference. The high yielding varieties with high root dry matter as well as starch contents are the most likely planted by farmers for long periods. However, the newly high yielding varieties stimulate farmers to try its productivity compared to the old existing varieties. Farmers will back to grow the old existing varieties if the newly is not perform better than the old one, or vice versa. Indeed cassava is not originally from Indonesia. It was introduced by Portuguese from Latin America at around mid 18th century into Maluku, then entering into Java at early 19th century by Dutch addressed for plantation to meet the demand of European feed market. Varietals development of cassava was started during Dutch occupation to improve productivity of plantation. Local varieties distributed in Indonesia were mostly as the results of previous varietals development.

Sustainability For Harmony

Indeed cassava is very efficient to inputs and has a wide range of adaptation to several of soil conditions. In acid soil of Sumatra and Kalimantan cassava is able to grow under soil pH 3.8-4.2; while in Nusatenggara at the soil pH up to 8 with very minimum input cassava able to produce >40 t/ha. Definitely production of cassava in Indonesia 2010 was around 23.1 million

tons harvested from 1.2 million hectares with average productivity approximately 19.2 t/ha. However, due to the demand tends to increase as consequence the import from Thailand in equal to 3 millions ton of fresh cassava could not be avoided. Since the average of national productivity still less than 20 t/ha and considered behind from its biological potential, this provide opportunity to be improved. In fact international market of cassava is still open, especially to China which consumes huge amount of cassava for bio-ethanol industry. Korea and Japan also need dried cassava for bio-ethanol factories and animal feed. Australia and West Europe need dried cassava as feedstuff. Cassava is upland crop that mainly depend on rainfall, so it is mostly planted in the inception of rainy season. Unfortunately, the agricultural commodities as well as forestry required spatial for its development, so there is a plight of competition in utilizing of upland. To anticipate the sustainable of cassava agro-industrial development, therefore production of cassava has to be increased by intensifying the cultural practices to attain higher productivity and expanding into upland in line with the development of other commodities.

Agroforestry: Compromizing Ecology And Economic

As part of global community, Indonesia has strong endeavor to realize Millennium Development Goals (MDGs) which put a top priority to reduce hunger and alleviate poverty till 50% at 2015. Unfortunately, the severe of global warming locked a dream into a reality. So far food pattern of Indonesian is less diversified, because based on rice in lowland as well as upland. Neue (1993) reported that to produce food from rice wetland was not ecologically sound, due to methane emission from rice wetland culture. Nguyen (2008)

indicated that aside in lowland rice had significant contribution as source of methane, in upland reducing forest to be converted into rice field also increase the area of degraded forest. Expanding of agricultural land by additional of the newly areas from forest faces criticize from national and international parties. Recently a strong commitment to conserve forest is a must. Cassava development into the newly area converted from forest is not able to meet sustainability criteria. Therefore, agroforestry is an appropriate way out to harmonize the dilemma countenance (Mac Donald, 1982). Praxis of past and present food crops production in forest area of Java, indicate that Java able to feed not only domestic dweller, but also outer island and even abroad. Unfortunately during food crops production practices there is a detrimental effect to forest, so basic function of forest as carbon sink is disturb. Forest area of Java is 3.3 million ha only around 2.5% from Indonesian forest. Around 73% of Java forest managed by *Perhutani* (State Forest Enterprise), and of 27% remaining maintained by civilian. The area of critical land in Java is around 4.25 million ha. It means that the whole forest area of Java under critical condition. Although formally

Perhutani did not allow cassava to be grown in between trees of forest areas, however farmers still and always grow cassava due to its suitability in upland with low input as well as easier market acceptability. Thus, economically cassava is very profitable due to the increase of price in line with greater demand and supply limitation, especially at the last twelve years and tends to be better in the future. Despite under peak harvest at the period of June till September such as recently cassava farm gate price up to 0.11 USD/kg and it is considered the highest across 15 years. This higher price promote to more people (not only existing farmers) to grow cassava for generating new business with better income. The increase price of cassava is the manifestation of greater demand from domestic and foreign as well. China, Korea and Japan had the huge demand of dried cassava for feed and as raw material for fuel (bio-ethanol). Expanding planting areas of cassava by agroforestry should be in line with the existing supply and demand as reflected by distribution of harvest area, productivity and production in the provinces of Indonesia (Table 5). So, market saturation can be avoided and better price can be maintained.

Table 5. Distribution of cassava by harvest area, productivity and production in Indonesia 2012.

Province	Cassava 2012		
	Harvest Area (Ha)	Productivity (T/Ha)	Production (Ton)
Indonesia	1129688	21.40	24177372
Aceh	2974	12.86	38257
North Sumatra	38749	30.23	1171520
West Sumatra	5502	38.83	213647
Riau	3642	24.32	88577
Jambi	2744	14.21	38978
South Sumatra	8938	16.06	143565
Bengkulu	4571	12.61	57618
Lampung	324749	25.83	8387351
Bangka Belitung	809	16.65	13469
Riau	697	10.99	7666
Jakarta	4	11.75	47
West Java	100159	21.28	2131123
Central Java	176849	21.76	3848462
Yogyakarta	61815	14.02	866357
East Java	189982	22.35	4246028
Banten	5677	14.58	82796
Bali	9346	15.75	147201
West Nusa Tenggara	5979	13.29	79472
East Nusa Tenggara	89282	9.99	892145
West Kalimantan	10217	15.03	153564
Central Kalimantan	3939	11.84	46630
South Kalimantan	5862	15.36	90043
East Kalimantan	4697	17.63	82786
North Sulawesi	4837	13.06	63187
Central Sulawesi	4702	19.92	93642
South Sulawesi	31454	21.71	682995
South-east Sulawesi	9093	19.33	175719
Gorontalo	307	12.30	3776
West Sulawesi	2598	18.58	48265
Maluku	6243	19.15	119545
North Maluku	9407	12.39	116515
West Papua	844	11.55	9747
Papua	3020	12.15	36679

Source: BPS, 2012 on line

Further Action For Better Future

Previous work had successfully to provide the cultural practice components adequately in order to construct the sustainability of cassava under agro-forestry, particularly from soil management and its erosion control till intercropping with various cereal and legumes. Grafting methods as introduced by Mukibat is also an appropriate way to attain high productivity under agro-forestry due to its shade tolerance better than did ordinary cassava. Harmony induction can be accomplished by preventing from ecological reduction and attain economic seduction simultaneously

with implementation of cassava under agro-forestry. By implementing this way, sustainability based on three aspects explicitly ecology, economy and social, are holistically integrated into practical for better accomplishment to community and natural resources as well. By better utilization as well as market and farm gate price of cassava, farmers will interest to grow cassava by applying inputs including fertilizer. Thus, sustainability of soil fertility could be maintained properly and on the other hand high productivity of cassava could be attained. It meant economic seduction indicated by the increase of greater demand could be assembled by poor

landless community to be the farmers in around forest areas, by utilizing space in between forest trees for growing cassava and the other food crops. The most important objective of community around forest to spend the space in between forest trees for growing food crops is not only to create and increase income, but from ecological point of view is also for safeguarding the forest trees from illegal logging (Direktorat Jendral Planologi Kehutanan, 2012; Wikipedia, 2009). In the long run, the merit of community around forest especially they are actively involved in forest prevention from illegal logging should be awarded by profit sharing receive from timber production. In 2008, first author accompanying the commissary president of Perhutani and teams to India for harvesting and sharing the benefit from the agro-forestry system were

including food, feed, and fuel integration. In case of Java as the densely populated island

with land tenure is less than 0.5 ha/household, agroforestry therefore as an alternative to attain economic seduction amid of global need (Mayrowani et al., 2004; Widodo and Prasetyaswati, 2011). Due to forest is the global facility and international community very concern to keep greener, therefore in practicing agroforestry is only executed in production forest. It means that conservation forest should be kept carefully to provide global facility (Table 6).

Table 6. Conservation forest area and percentage to total forest area of Java and Indonesia from 1981 to 2007.

Years	Conservation forest (1.000 ha)		Percentage to total forest area (%)		Total forest area (1.000 ha)	
	Java*	Indonesia	Java	Indonesia	Java	Indonesia
1981	718	30,345	21.71	26.83	3.307	113,077
1984	718	30,316	21.71	26.72	3.307	113,433
1990	718	29,649	21.71	26.53	3.307	111,775
1999**	425	33,520	18.47	29.86	2.300	112,274
2001	425	29,037	18.47	30.60	2.300	94,894
2004	718	31,685	21.71	23.18	3.307	136,710
2007	718	31,604	21.71	23.05	3.307	137,090

Note: * Data forest area of Java is collected from Perhutani. **During early reformation era illegal logging was very serious. Data selected different years from BPS (1981 to 2010).

CONCLUSION

Based on the results of demonstration plot as well as information

and field facts, the conclusions can be presented as below:

1. Among five cassava genotypes demonstrated in between teak, Caspro gave the highest yield yield 40.05 t/ha and highest profit Rp 18,785,000 per hectare. Meanwhile the productivity of local genotype non bitter which suitable for direct human consumption was only 21.50 t/ha, so the profit obtained only at amount of Rp 5,800,000 per hectare.
2. Fertilizer demonstration plots of cassava in between teak indicated that by applying 500 kg Phonska with additional of cow manure 10 t/ha produces the highest yield namely 44.85 t/ha of fresh root, so the highest profit at amount of Rp 28,743,750 can be obtained.
3. By incorporating cassava in between rows of teak, grassy and shrub-bushy weed that stimulate to fire in the forest during dry season can be avoided. The utilization of space in between teak by cassava and the other food crops providing the additional of food from the forest that strengthening the food security and sovereignty.
4. The increase of production of cassava from the forest area with proper agroforestry will able to meet the greater demand of cassava as food, feed, fuel and other industrial purposes as well. Ecological reduction can be attained by implementing agroforestry without converting forest for agricultural land due to area decrease for settlement as well as industrial estate.
5. Sustainability aspect with holistic and comprehensive approaches in the

implementation of agroforestry should be appreciated tangibility to overcome hunger and poverty as the priority of Millennium Development Goals under global climate change.

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EARTHLING IMAGINATION INTO IMPLEMENTED GREEN INNOVATION FOR COMMUNITY WELFARE: PRAXIS OF ROOT CROPS UNDER AGROFORESTRY

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ABSTRACT

Innovation based on science and technology is considered as triggering and accelerating factors to the progress of business as well as industries including agricultural enterprise. Stagnation of adopted innovation from the source to the users is concerned by many parties, especially from the source innovation producing side due mainly to be funded by government. Evaluation tends to asymmetrical pattern that focus more to user side than retrieving with emphatically sense in the source face. The main argument is mainly the need of innovation based on science and technology was not comparable with the ability to provide, as consequence research and development (R&D) activities as well as its funding increase gradually. In order to robust R&D in preparing the appropriate innovation in line with its implementation by the users, therefore an integrated participatory action is urgently required for synergistic and mutuality accomplishment. Relevance with this issue, praxis of root crops R&D for agro-forestry in order to anticipate the need of food, feed and renewable energy under climatic change affected by global warming is feasible to be discussed. Sharing experiences depart from imagination, intuition and inspiration have to be formulated and argued into collective idea by all parties as stakeholders. Furthermore, a collective idea is extracted into planning of R&D by investigation to obtain a proper invention. A proper and better invention is suggested to be published and disseminated to get critical suggestion widely. Further step is to fund rising for larger investment in order to transform invention into economic scale for tailoring reliable innovation. Benefit and impact of the newly innovation needs to be reviewed and evaluated with objective tool under impact assessment, for implementation into actual and dynamic of larger domains. Thus, there are 10 I steps required for earthing the imagination to be transformed into reliable innovation.

Keywords: imagination, innovation, praxis of root crops, agro-forestry.

INTRODUCTION

As part of the global community, Indonesian Agency of Agriculture Research and Development (IAARD) under Ministry of

Agriculture recently aware that international network is strongly required in order to improve its role to provide innovation based on scientific activities especially through research and development endeavors. The growth of

awareness to the current global condition under truly golden era of information which promotes to borderless physical state barriers could be used as the power to force IAARD as the potential stakeholder to contribute a significant role for particularly Indonesian farmers as well as global agricultural communities in a broad sagacity. Therefore, IAARD put the newly tag line **science.innovation.network**; it reflects the crystallization of mandate as the agency supported by better qualified scientists to actively execute research and development for providing innovation (not only proud and stop at invention stage) as well as to build the network between stakeholders regional and international level as well. As consequence the entities under IAARD especially research and extension for development have to be recognized and understood what is required by end-user or client principally care-to share fairly (Widodo, 1995; 1999; 2007).

Under endeavor for realizing globalization, community welfare is still and going to the most important aspiration for all nations presently as well as in the future civilization. Welfare in macro level is the function of accessibility to food, health, education as well as infrastructure (Eco summit, 2007; World Economic Forum Water Initiative, 2011; UNCSD, 2012). As described by Widodo *et al* (2013) the most essential criteria to measure and feel community welfare by the adequacy of each household to cover the basic human need related to Food, Feed, Fiber, Fuel, Funny shelter, with Friendly ecologically and Fix Greener Environmentally (F7GE). Those kinds of work should be organized and fitted under Community Based Natural Resource Management (CBNRM). Natural resource management previously was considered massy handling without holistic and comprehensive long term justification. Therefore as a consequence, an economic result is only attained in a short term, but ecologically welfare in the long term is more difficult to be obtained. As consequence from past development remained poverty and environmental disorder, which more difficult to be recovered into reversible circumstances. Therefore Millennium Development Goals (MDGs) with 8 objectives put the priority to reduce hunger and alleviate poverty up to 50% till the end of 2015. Unfortunately, although the period 1.5 years almost reach 2015, but the objectives stipulated at MDGs still beyond as discourse not as reality.

Continuation to reduce hunger and alleviate poverty will be accomplished by Sustainable Development Goals (SDGs) that is the implementation of Earth Summit in Rio Brazil at the year 1992 then revised and renewed in the name of RIO+20. Therefore the objectives of SDGs are not mainly covering MDGs, but it should also include creating greener environment in order to prevent the severe of global warming for better civilization (Carbon Initiative World Bank, 2009; UNDP, 2007; 2008; UNFCCC, 2009; UNCSD, 2012).

MATERIALS AND METHODS

The long empirical experiences of author from 1982 to recently were delighted as materials to be evaluated by descriptive method. The interaction of author with international agency dealing with root and tuber crops commodities especially with International Institute of Tropical Agriculture (IITA) Ibadan Nigeria in 1984; then continued with Centro Internacional Agricultura Tropical (CIAT) Cali Columbia in 1985; illumination from International Society of Tropical Root and Tuber Crops (ISTRC) 1986 to 1988; involvement in the pre and post inaugural meeting of User Perspective with Agricultural Research and Development (UPWARD) in Banquet State University Baguio as well as University of Philippines Los Banos in 1989-1992 intermitted with several meeting under Asian Farming System Association at Bangkok Thailand; tailoring curriculum of Integrated Crop Management of sweet potato with Centro Internacional de la Papa (CIP) Asia Pacific in 1993-1995; Sweet potato based farming system in highland of Papua with Switzerland project 1995-1997; facilitator of the migrants in Borneo program funded by Japan Agricultural Land Development Agency (JALDA) 1997-1999; the production and utilization of root and tuber crops in Vietnam in 1996; as Advisory Technical Assistance of ATAS Foundation 1996 till 2005 in the agro-forestry program acknowledged by Center of International Forestry Research (CIFOR) and supported by Perhutani (State Forest Enterprise); involvement in the Eco Summit Beijing 2007; active taking part in Global Cassava Partnership funded by Bill & Melinda Gates Foundation in Gent University Belgium 2008; comparative study of development program of renewable energy (bio-

fuel) with Perhutani to India 2008 then exploring the potential of land to produce bio-fuel in Indonesia supported by New Energy Development Organization (NEDO) Japan 2008-2011. Actively involve in the CIAT cassava program of Asia in Thailand, Laos PDR, and China 2008-2011. Resource person in Food and Environment Summit at Wessex Institute of Technology, United Kingdom 2011, cassava development in Malaysia 2012, and Global Water System in University of Bonn 2013. Recently author dealing with CIAT for cassava scoping study at Nusatenggara, East Java and North Sumatra 2013-2014.

RESULTS AND DISCUSSIONS

Leaving The Greedy Entering Green For Great Civilization

At the end of second millennium in line with globalization the need of better greener future was an important agenda to be executed by global community. Entering the third millennium, the awareness of community to the harmony of environment grows a spirit to care each other by willing to share fairly for conserving the only one earth. The previous habit of global economy oriented to development resulting huge gap of income between the have

and the poor as well as over exploitation of natural resources. This type of habit is so called as greedy economy which is not suitable to be sustained into harmonious global community. Although practically from the profit of enterprises belong to the have was distributed at least 2.5% via Corporate Social Responsibility (CSR) especially spend into environmental recovery program, however the way to gather the profit is often controversial with its distribution. It means that greedy economy is not environmentally sound, because cost for repairing environment is more expensive than the profit obtained. Based on this phenomenon, therefore green economy is considered as an ultimate model integrating between economies without sacrificing ecology. It can be stated that green economy is a representation of future economic model that global community want, because it will accommodate social as well as cultural and even spiritual aspects designated to accomplish sustainability development for benefiting many. However to reduce the environmental disorder in the medium term before green economy could be implemented perfectly, to fulfill the demand of F7GE bio-economy or economic based on bio processes has to be developed (Widodo, 2012a; 2012b; Widodo and Radjit, 2013).

Table 1. The area of forest managed by enterprises potential for agro-forestry, 2004-2009.

Provinces in Indonesia	Acreage (Ha)					
	2004	2005	2006	2007	2008	2009
Aceh	796 723	524 644	524 644	524 644	409 644	409 644
North Sumatera	404 600	294 843	437 393	328 803	328 803	328 803
West Sumatera	361 430	268 840	210 376	210 240	210 240	194 290
R i a u	2 390 457	721 102	812 128	812 128	715 128	318 408
J a m b i	821 995	328 349	299 974	133 705	45 825	45 825
South Sumatera	100 000	56 000	56 000	108 170	108 170	108 170
Bengkulu	0	23 000	23 000	23 000	23 000	56 070
West Nusa Tenggara	31 550	31 550	31 550	0	0	0
West Kalimantan	1 125 756	1 128 860	1 163 890	1 257 670	1 214 065	1 195 570

Central Kalimantan	2 825 221	4 323 608	4 569 645	4 473 755	4 120 635	4 086 305
South Kalimantan	280 811	320 531	361 481	359 421	359 361	279 421
East Kalimantan	5 162 452	6 051 172	6 734 227	6 773 357	6 581 712	6 183 873
North Sulawesi	105 500	60 800	60 800	60 800	60 800	60 800
Central Sulawesi	785 270	1 006 095	992 245	992 245	902 245	854 245
South Sulawesi	409 965	293 407	293 407	0	0	0
South-east Sulawesi	296 000	296 000	0	385 590	385 590	385 590
Gorontalo	348 200	185 570	185 570	185 570	185 570	145 000
West Sulawesi	0	0	0	293 407	249 407	214 245
Maluku	818 339	785 210	815 165	778 865	554 695	697 195
North Maluku	306 000	991 000	921 675	983 040	913 040	804 820
West Papua	0	4 512 350	4 350 470	4 091 590	3 560 590	3 885 970
Papua	4 042 050	5 512 253	5 581 243	5 495 043	5 241 293	5 516 643
Indonesia	21 412 319	27 715 184	28 424 883	28 271 043	26 169 813	25 770 887

Source: Forest managed by Enterprises , BPS 1982-2012.

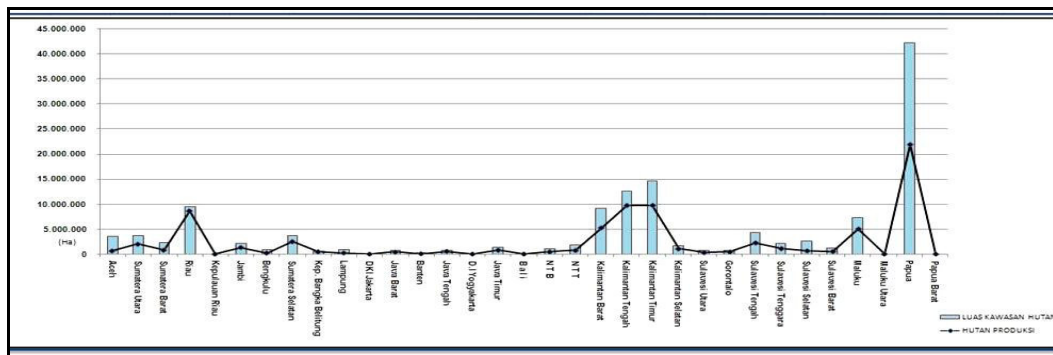


Figure 1: Proportion of natural conservation forest and production forest in Indonesia (Direktorat Planologi Kehutanan, 2012).

Development of agricultural lands in the form of lowland as well as dry upland is mostly designed for increasing food security based on cereals and grains in order to meet the increasing demand due to population increased. If this way out is always undertaken, the natural forest will remain a story, therefore Widodo (2011) strongly recommended stipulating agro-forestry by utilizing current forest production enterprises as well as at critical agricultural lands (Table 1 & Figure 1). According to Widodo (2012a) green economy was perfectly accomplished by

indigenous communities including Javanese. Undeniably from the old traditional teaching, the overall objective of Javanese that so called *Memayu Hayuning Bawono* with the intention of means is to generate, trigger and stimulate (*Memayu*) the beautifulness (*Hayuning*) of the world (*Bawono*) nature (including biodiversity as well as a-biotic/physical elements) under harmony. This old traditional wisdom was performed in the various ritual ceremonies and daily livelihood from prehistoric era (animism) till the last 1970, entering third millennium this

teaching and its practice was really ignored by most of community, except the local community such as Badui in Banten, Suku Naga in West Java, Samin in Central and East Java, Tengger and Osing in East Java as well as rural remote community that still hold old tradition related to sustainable forest management. To respect the soul of forest (big trees habitat), from old traditional philosophy teaches community to provide various food regularly as so called *caos dahar / sesaji*. The foods with special prepared are left and then eaten by animals or the other living things in the forest. This kind of ceremony is still practiced regularly by Balinese till today. From the history of Majapahit kingdom in East Java (1290-1400), definitely Balinese were came from Javanese who disallow new religion (Islam/Moslem from Arab) and still hold Animism and Hinduism. This uniqueness of local wisdom is very important to be recognized by modern community in order to generate the greener world future. As Javanese, in order to generate the harmony of the world should understand *Sastra Jendra Rahayuningrat Pangruwating Diyu*, means for attaining glorious human being (*Rahayuningrat*), wisdom has to be achieved by learning knowledge and science (*Sastra*) under the name of God (*Endra*) and keep a distance far away (*Pangruwating*) from the greediness (*Diyu*). This indigenous knowledge needs revitalization and implementation urgently into modern life style that tends to consumptive and careless. Holistic approaches in sustainable forest management have to be undertaken in line with community development. Some endeavors related to livelihood for fulfilling the basic daily needs especially food and fuel for the community live around forest have to be installed its security and safety. Government policy, gas tank 3 kg must be available in every household till remote rural areas, but due to poor quality and often accident of tank trouble, therefore firewood is still preferred to be used for preparing daily meals. Fuel (energy) for daily needs to prepare foods mostly using the small branches or senescence stems, also requires improvement in order to go along in line with sustainable forest management. Fortunately in agro-forestry, to provide firewood is not allowed by logging tree, but only cut the branches. By reducing canopy of tree light interception is more plenty, so the area under tree could be used for growing sun loving food crops such as grain and cereals, or major root-crops such as cassava and sweet potato. In

the agro-forestry with spacing of tree (forest) is 6x1 m, the branches is regularly cut for both purposes, avoid or reduce shading effect as well as providing firewood. The serious damage of forest in Java and Indonesia during the period of 1998 till 2005 is irreversible, so to get better such condition needs more time and fortunately endeavor to immediately recover was started by implementing agro-forestry. Cereals and grain legumes, cassava and sweet potato as sun loving plants can be associated for agro-forestry with young trees, when shading still less 30%. Fortunately, when shading effect increase till 80% root-crops from family of *Araceae* especially cocoyam (*Xanthosoma* sp), elephant foot yam (*Amorphophallus* sp) are still able to sequester CO₂ to produce carbohydrate, protein, vitamin and mineral as well (Flach and Rumawas, 1996; Jansen and Premchand, 1996; Jansen, et al., 1996). From the progress of root-crops under agro-forestry with fully participatory managed by the community around of forest area, the global issues could be attained simultaneously. Adequate or even abundant of food can be harvested from root-crops without disturbing the role of forest to avoid the alteration of climate (Figure 2). By assuming 50% of dry matter is carbon, in the periods of 6-12 months root-crops under agro-forestry able to sequester carbon 3-7 t/ha. To anticipate future setback, shade tolerant root-crops with more nutritious, high productivity as well as with special trait have to be available easily for the community in order to supply the increase of demand of food and energy under greener circumstances. A mode to memorize that local wisdom or old tradition to respect trees had similar mission with modern movement, so from cognitive aspect must be implemented further into affective and psychomotor for world future greener.

Root Crops Agroforestry: Simultaneous Adaptation And Mitigation

Discovery of the new continent as so called now as America by Columbus at 1492 was induced by the serious hunger at Great Britain and Europe due to failed of potato harvest. Therefore, after that period the development of cereals and grains as source of staple food became stronger. Unfortunately, potato and other root crops remained disregard. Indeed the potential of root and tuber crops is still very huge to be developed. Rhoades and Horton (1990)

revealed that cereals and grains mentality neglecting the opportunity of root and tuber crops to be considered as food alternative to feed the significant increase of world population. Cereal and grain based agriculture oriented to open field by removing forest resulting environment problem especially water scarcity as well as depletion of soil fertility that promotes to the use of artificial fertilizer in the green revolution which ultimately induces global warming (Figure 3).

From European experiences indicating that agriculture is a significant water user, in particular for irrigation. Irrigated areas increased from 1990 to 2000, with grain maize as the most important irrigated crop. In parallel, the water demand rate decreased slightly between 1990 and 2000 (from 6578 to 5500 m³/ha/year). The evolution from 2000 of the regional distribution of the irrigated area and the associated water

demand will need further assessment. The aggregated dated related to industry revealed a decrease in water uses in the 80s and 90s. Further investigation will be needed in the future in order to assess whether increases in water use can be expected in next decades, taking into account continuing growth in economic activity and output (more than 20% in thirty years in most countries) as well as increased water use efficiency with technological changes and IPPC implementation. Cooling water for electricity production mainly concern Western Central and Eastern countries. It will be interesting to further investigate whether decreases in water abstraction can be expected from the possible replacement of older power stations by newer plants in the next thirty years. The activity can have impacts at the points of abstraction even if a large part then flows back farther into the environment (DG Environment Euro Commission, 2007).

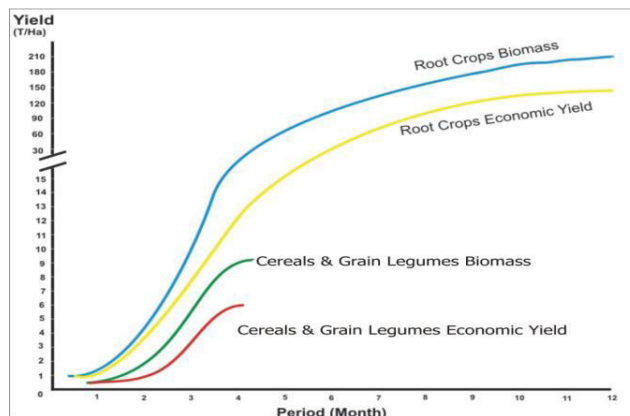


Figure 2: Biological potential of root crops beyond capacity of cereals and grains (Widodo et al., 2013)

Inappropriately, global climate change as direct detrimentally consequence from global warming predicted is going to be a serious obstacle to accomplish the food demand. Indeed global warming was recognized >100 years ago, but the attention of public to that problem begun in <25 years after global community experiencing the alteration of climate which handicapping food production. Forest is considered as a main buffer to prevent from the severe of climate alteration. MacDonald (1982) based on a long series experiments and

experiences in Africa, stipulated by agro-forestry the desert widening phenomenon as well as hunger could be reduced. In line with food security for anticipating global warming, Indonesia as rice based food pattern has to prepare the implementation of food diversification program. Indeed from previous facts, during longer drought calamity when the reserve of rice and cereals at stockroom was empty, community in rural remote areas around forest went to penetrate the forest gathering edible plant especially vegetative portion of

underground crops (root-crops) to be used as source of carbohydrate to combat again the hunger. Drought severity and high temperature coincided with global climate change are going to be the rigorous problems for securing world rice production (Stanford University, 2007; WWF, 2007; IRRI, 2009). Furthermore Nguyen (2008) also pointed out that from alternative slash and burn for cultivating upland rice inducing or even triggering to deforestation and incrementing the global warming due to the decrease of sink capacity to absorb CO₂. Moreover, Neue (1993) based on a series experiments additionally revealed from wetland rice culture, methane was released to biosphere and this also had a major detrimental effect to global warming. Methane proportion is around 15%, while CO₂ is around 55% into the total of green house gases, but due to methane can absorb heat latently, so methane emission is more dangerous, and consequently it should be reduced. It is predicted that 20% of methane in the biosphere is contribution from wetland plus swamp rice culture. Therefore, in order to feed

the human population increase, rice cultivation needs an improvement in the newly scheme of green revolution which more ecological orientation. On the other hand, exploring food other than rice as well as paradigm 'grain cereal mentality' and underground crops with 'dirty image' (Rhoades and Horton, 1990) have to be re-determined, in attempts to nourish current and next generation under the world greener future. To realize the community welfare to fulfill the nourish for needy as recommended by MDGs global community have to be aware that food, energy as well as water scarcity related to climate nexus. This of a connecting link needs international collaboration to overcome (GWSP, 2013; World Economic Forum Water Initiative, 2011). Therefore, in fulfilling F7GE and accomplishing better future civilization under world greener, the innovative natural resource management have to be able to provide the nexus of nutrients cycle naturally for generating renewable feedstock for industry, produce ecosystem benefits and increase food security (UNDP, 2007).

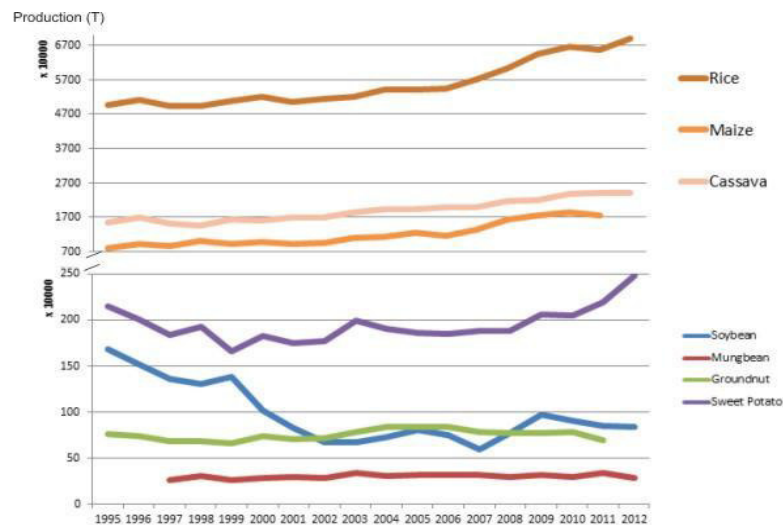


Figure 3. Production trends of various food crops in Indonesia 1995-2012 (BPS, 2013).

The advantage of root crops are not only less require water and shade tolerant that suitable to be grown under agro-forestry, but also from nutrition value is comparable with rice as main staple. Fact representing the critical condition of Java forest is due mainly to over exploitation. From coastal region, mangrove forest which very useful to provide suitable environment for fish and shrimp nurseries is still conflicted with daily need as source of firewood and construction

work as well. Luckily in Tuban, Lamongan, Gresik till Pasuruan community in coastal area the communities are aware about the function of mangrove. Recently in several coastal areas of East Java particularly reforestation with emphasis on mangrove was done by community around supported by companies as well as profit institution through Corporate Social Responsibility (CSR). Reforestation and forestations were done mainly at the early of

rainy season through CSR or by own community due to the increase of awareness. In high altitude, around source of water, arboretums are intensively recovered by local communities. Facts indicate in steep slope are utilized for growing vegetables such as potato, cabbage etc (in higher altitude) or food crops like maize, upland rice or cassava (in lower altitude), yet in the near future such situation will change by growing more trees in the area. Current trends revealed the price of fast growing tree species *Albizia* sp. *Acacia* sp. was economically better, but the increase of awareness to ecology will reject logging of tree, thus tree species producing fruit such as breadfruit, jackfruit etc will more preferred than other species. Moreover in the land area outside of forest, especially nearby city or industrial estate, due to wage of agricultural

labor tends to expensive, growing tree imitates to mini forest is an alternative source of income mainly for timber. Food sovereignty produces and consumes locally is also important perspective to attain economic feasibility as well as ecological benefit. Retrieval of food production which ecologically friendly under the shade of forest requires verification and validation before recommended into larger areas across the region for wider community. While root-crops, edible mushroom (saprophytic plants) are important sources of carbohydrate, protein, fat, vitamins plus minerals that can be cultivated under the entirely shade of forest without disturbing the forest, sadly food pattern most of dweller in Java is still based on rice and cereals (Widodo, 2012a).

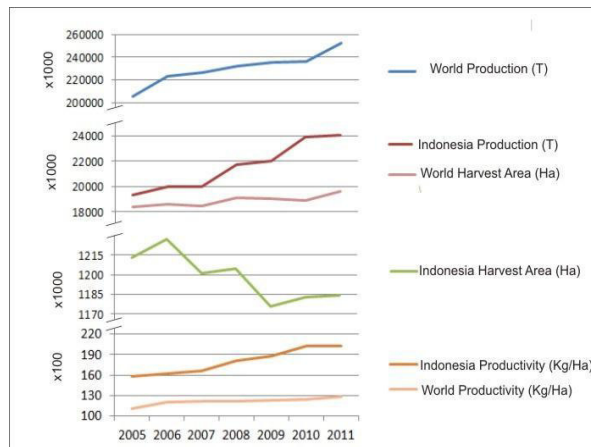


Figure 4. Trends of cassava harvest area, productivity and production in Indonesia and world 2005-2011.

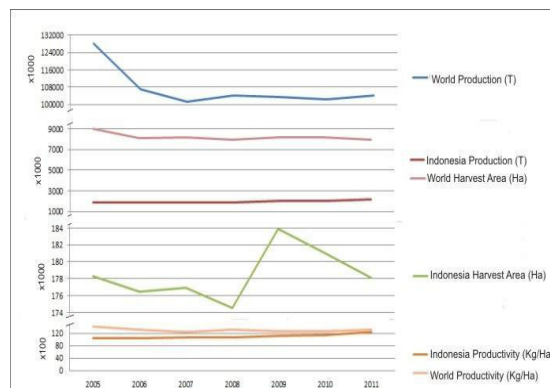


Figure 5. Sweet potato harvest area, productivity and production trends in Indonesia and world 2005-2011.

Praxis Of Research And Development Into Recommendation

Indonesia previously was under centralized management, the whole of government activities

including agricultural enterprises was designed at Jakarta as the center. However, since reformation era decentralization is strongly demanded. In line with the demand to serve current need by bio economy and in the long term that community

want to realize green economy, therefore the newly appropriate approaches to gather imagination into implemented innovation must be designed. Wikipedia (2013) explained the meaning of imagination, inspiration, intuition, invention, investment, impact assessment, implementation of innovation. The explanation of those words is presented below. Imagination is the ability to form new images and sensations that are not perceived through sight, hearing, or other senses. Imagination helps make knowledge applicable in solving problems and is fundamental to integrating experience and the [learning process](#). A basic training for imagination is listening to [storytelling \(narrative\)](#), in which the exactness of the chosen words is the fundamental factor to "evoke worlds". It is a whole cycle of image formation or any sensation which may be described as "hidden" as it takes place without anyone else's knowledge. A person may imagine according to his mood, it may be good or bad depending on the situation. Some people imagine in a state of tension or gloominess in order to calm themselves. It is accepted as the innate ability and [process](#) of inventing partial or complete personal realms within the mind from elements derived from sense perceptions of the shared world. The term is technically used in [psychology](#) for the process of reviving in the [mind](#), [percepts](#) of objects formerly given in sense perception. Since this use of the term conflicts with that of ordinary [language](#), some psychologists have preferred to describe this process as "[imaging](#)" or "[imagery](#)" or to speak of it as "reproductive" as opposed to "productive" or "constructive" imagination. Imagined images are seen with the "[mind's eye](#)". Imagination can also be expressed through stories such as [fairy tales](#) or [fantasies](#). Children often use [narratives](#) or pretend play in order to exercise their imagination. When children develop [fantasy](#) they play at two levels: first, they use [role playing](#) to act out what they have developed with their imagination, and at the second level they play again with their make-believe situation by acting as if what they have developed is an actual reality that already exists in narrative [myth](#). [Artistic inspiration](#) is sudden creativity in artistic production. [Biblical inspiration](#) is the doctrine in Judeo-Christian theology concerned with the divine origin of the Bible. Creative inspiration means impulsive creativity when a new [invention](#) is created. Intuition is the ability to acquire knowledge without [inference](#) and/or the use of [reason](#). The

word *intuition* comes from Latin verb *intueri* which is usually translated as *to look inside* or *to contemplate*. Intuition is thus often conceived as a kind of inner [perception](#), sometimes regarded as real [lucidity](#) or [understanding](#). Cases of intuition are of a great diversity; however processes by which they happen typically remain mostly unknown to the thinker, as opposed to our view of [rational thinking](#). Intuition provides us with views, understandings, judgments, or [beliefs](#) that we cannot in every case [empirically](#) verify or [rationally](#) justify. For this reason, it has been not only a subject of study in [psychology](#), but also a topic of interest in various [religions](#) and [esoteric](#) domains, as well as a common subject of [New Age](#) writings. The [right brain](#) is popularly associated with intuitive processes such as aesthetic or generally creative abilities. Some scientists have contended that intuition is associated with innovation in scientific discovery. [Inventor](#)" and "[Invented](#)" redirect here. An **invention** is a unique or [novel device](#), method, composition or process. It may be an improvement upon a machine or product, or a new process for creating an object or a result. An invention that achieves a completely unique function or result may be a radical breakthrough. Such works are novel and [not obvious](#) to [others skilled in the same field](#). Some inventions can be patented. A [patent](#) legally protects the intellectual property rights of the inventor and legally recognizes that a claimed invention is actually an invention. The rules and requirements for patenting an invention vary from country to country, and the process of obtaining a patent is often expensive. Another meaning of invention is [cultural invention](#), which is an [innovative](#) set of useful [social behaviours](#) adopted by people and passed on to others.^[1] The [Institute for Social Inventions](#) collected many such ideas in magazines and books.^[2] Invention is also an important component of artistic and design [creativity](#). Inventions often extend the boundaries of human knowledge, experience or capability. **Implementation** is the realization of an application, or execution of a [plan](#), idea, [model](#), [design](#), [specification](#), [standard](#), [algorithm](#), or [policy](#). In [political science](#), implementation refers to the carrying out of [public policy](#). [Legislatures](#) pass laws that are then carried out by [public servants](#) working in [bureaucratic agencies](#). This process consists of rule-making, rule-administration and rule-adjudication. Factors impacting implementation include the legislative intent, the administrative capacity of

the implementing bureaucracy, interest group activity and opposition, and presidential or executive support. In water and natural resources, implementation refers to the actualization of best management practices with the ultimate goals of conserving natural resources and improving the quality of water bodies. Innovation is the application of better solutions that meet new requirements, inarticulate needs, or existing market needs. This is accomplished through more effective [products](#), [processes](#), [services](#), [technologies](#), or [ideas](#) that are readily available to [markets](#), [governments](#) and [society](#). The term innovation can be defined as something original and, as consequence, new that "breaks in to" the market or into society. One usually associates to new phenomena that are important in some way. A definition of the term, in line with these aspects, would be the following: "An innovation is something original, new, and important - in whatever field - that breaks in to (or obtains a foothold in) a market or society. While something novel is often described as an innovation, in economics, management science and other fields of practice and analysis it is generally considered a *process* that brings together various novel ideas in a way that they have an impact on society. Innovation differs from [invention](#) in that innovation refers to the use of a better and, as a result, novel idea or method, whereas invention refers more directly to the creation of the idea or method itself. Innovation differs from [improvement](#) in that innovation refers to the notion of doing something different rather than doing the same thing better. An impact assessment (IA) is "a process aimed at structuring and supporting the development of policies. It identifies and assesses the problem at stake and the objectives pursued. It identifies the main options for achieving the objective and analyses their likely impacts in the economic, environmental and social fields. It outlines advantages and disadvantages of each option and examines possible synergies and trade-offs". Two key related areas of work within this field include [Social impact assessment](#) and [Environmental impact assessment](#). Investment has different

meanings in [finance](#) and [economics](#). In economics, investment is the accumulation of newly produced physical entities, such as factories, machinery, houses, and goods inventories. In finance, investment is putting [money](#) into an asset with the expectation of capital [appreciation](#), [dividends](#), and/or [interest earnings](#). This may or may not be backed by research and analysis. Most or all forms of investment involve some form of risk, such as investment in equities, property, and even fixed interest securities which are subject, among other things, to [inflation risk](#).

In short relevance with this issue, praxis of root crops R&D for agro-forestry in order to anticipate the need of food, feed and renewable energy under climatic change affected by global warming is feasible to be discussed. Sharing experiences depart from imagination, intuition and inspiration have to be formulated and argued into collective idea by all parties as stakeholders. Furthermore, a collective idea is extracted into planning of R&D by investigation to obtain a proper invention. A proper and better invention is suggested to be published and disseminated to get critical suggestion widely. Further step is to fund rising for larger investment in order to transform invention into economic scale for tailoring reliable innovation. Benefit and impact of the newly innovation needs to be reviewed and evaluated with objective tool under impact assessment, for implementation into actual and dynamic of larger domains. Thus, there are 10 I steps required for earthling the imagination to be transformed into reliable innovation (Figure 6). Widodo (2012a) simplifying the way to transfer technology from lab to land with 3D, namely discovery stage then continued by development technology participatory and ultimately dissemination technology innovation into larger domain. Under discovery stage imagination, inspiration, and intuition are collected into collective idea to be investigated to produce invention which ultimately resulting implemented recommendable innovation to generate better community welfare (Figure 6).

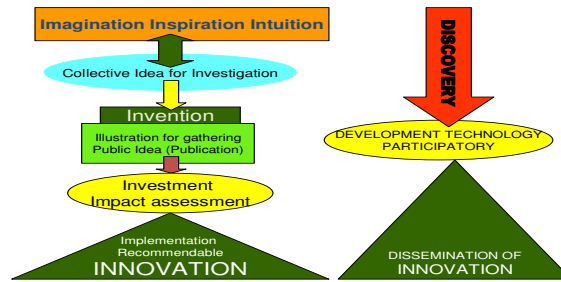


Figure 6: Diagram indicating the transformation of imagination into innovation

CONCLUSION

To summarize the fore above mentioned elaboration into connecting link between imaginations to be implemented into recommendable innovation, the ultimate conclusion can be presented below:

1. Greedy economy vestiges huge gap of income in the social strata and exploiting the natural resources to gain profit without considering the environmental disorders must be reinvented with green economy. Social equitability, ecologically friendly environment, culturally indigenous wisdom as well as spiritually prerequisite should be recognized and have to be incorporated into green economy.
2. The root and tuber crops under agro-forestry provide more biomass for fulfilling F7GE to compliment the potential of cereals and grains as dominant source of food. Under such of condition, production of biomass to meet the demand will go in line with adaptation and mitigation to prevent the severe of climate change and global warming as well.
3. The implementation of green innovation is an urgent agenda in order to avoid the massy handling of an erroneous-track action into on the precise roadmap oriented to sustainable development for better future civilization.

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EFFECT OF N-INORGANIC AND ORGANIC FERTILIZATION ON GROWTH AND YIELD OF SWEET CORN

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ABSTRACT

Sweet corn as food Attracted many people, so the need for growing sweet corn. Many farmers to plant early maturing sweet corn Because of his age around 65-75 days. This study aims to know the effects of N and organic fertilization on growth and yield of sweet corn. The experiment was conducted in rainfed lowland in KP. Kirkcaldy, Kirkcaldy Subdistrict, Mojokerto District on Dray Season (DS II) 2012. Designed a randomized trial with 10 treatments were repeated 3 times in plots measuring 5 m x 3 m at a spacing of 75 cm x 20 cm. Treatment includes: (1) Urea 0 kg/ha, (2) Organic fertilizer 2 t/ha, (3) Urea 100 kg/ha, (4) Urea 100 kg/ha + Organic fertilizer 2 t/ha, (5) urea 200 kg/ha, (6) urea 200 kg/ha + Organic fertilizer 2 t/ha, (7) Urea 300 kg/ha, (8) Urea 300 kg/ha + Organic fertilizer 2 t/ha, (9) Urea 400 kg/ha, and (10) Urea 400 kg/ha + Organic fertilizer 2 t/ha. The results showed that planting sweet corn with fertilizer urea 300 kg/ha obtained a high yield of cob corn and significantly different from urea fertilizer with lower dose.

Keywords: sweet corn, fertilizer, growth, yield cob

INTRODUCTION

Corn is the main commodity crops have an important role in achieving food security and national contribution corn production in East Java reached 31.86% (Diperta Prov. Jawa Timur, 2009). In addition to meeting the needs of food and animal feed, recently began a much-loved type of corn is the sweet corn are harvested young. Land management by rational fertilization is an effort to increase efficiency and optimize production costs increased production of sweet corn. Efficient use of fertilizer is basically giving a good fertilizer macro nutrients and micro nutrients in the number, types and shapes to suit the needs of plants, and by granting the right time according to the needs and growth of sweet corn crop. Excess fertilizer in addition to a waste of funds, also disrupt the balance of nutrients in the soil and environmental pollution (Adiningsih *et al.*, 1989; Moersidi *et al.*, 1991; Rochayati *et al.*, 1991), while the fertilizer is too bit can not provide the optimal level of production.

The use of N inorganic fertilizer is continuously being offset by organic fertilizers can damage soil physical properties such as water holding capacity (Nugroho *et al.* 2000). According to Tisdale *et al.* (1985) and Karama (2000), nutrient needs are only met by the addition of inorganic fertilizer without organic fertilizer leads to the depletion of nutrients in the soil quickly. Such conditions lead to a decline in soil fertility. Decline in soil fertility is related to the low soil organic matter content. The content of organic matter in lowland in East Java, 99% is low at less than 2% (Suyamto, 2003), thus causing the deterioration of land quality. Therefore we need the addition of organic fertilizer to improve soil productivity. Sugito *et al.* (1995) added, the higher the organic matter into the soil will be given higher water binding and the ability to provide more groundwater. Therefore we need

chemical fertilizers and organic fertilizers on crop specific sweet corn.

This study aimed to determine the effect of N inorganic and organic fertilizer on growth and yield of sweet corn

MATERIALS AND METHODS

Fertilization studies at the age of sweet corn harvest 75 days held in lowland Mojokerto village, Mojokerto Subdistrict, Mojokerto District, in DS II 2012. Designed studies using randomized treatment groups with 10 repeated 3 times, consist of : (1) Urea 0 kg/ha, (2) Organic fertilizer 2 t/ha, (3) Urea 100 kg/ha, (4) Urea 100 kg/ha + Organic fertilizer 2 t/ha, (5) urea 200 kg/ha, (6) urea 200 kg/ha + Organic fertilizer 2 t/ha, (7) Urea 300 kg/ha, (8) Urea 300 kg/ha + Organic fertilizer 2 t/ha, (9) Urea 400 kg/ha, and (10) Urea 400 kg/ha + Organic fertilizer 2 t/ha. Experimental plots measuring 5 m x 3 m with spacing of 75 cm x 20 cm, 1 seed per hole. Organic fertilizer used is cow manure.

Dose and timing of fertilizer application, the first fertilizer immediately after planting maize, namely : $\frac{1}{2}$ dose of Urea/ha + 100 kg SP-36/ha + 50 kg KCl/ha + 2 t organic fertilizer/ha, while the second fertilization 21 Day After Planting (DAP), namely: $\frac{1}{2}$ dose of Urea/ha + 50 kg KCl/ha. Data analysis using ANOVA followed Duncan Significant Difference Test (DMRT 5%) (Gomez and Gomez, 1993).

RESULTS

Agroecology of Research Sites

The experiment was conducted in rainfed lowland in the Mojokerto Village, Mojokerto Subdistrict, Mojokerto District, in DS II 2012 (Table 1).

Table 1. Results of soil nutrient analysis before trial in rainfed lowland in Mojosari village, Mojosari Subdistrict, Mojokerto District.

Analysis	Content	Category
Texture :		
Sand %	60	-
Dust %	21	-
Loam %	19	-
Class	-	Sandy loam
pH : H ₂ O	6,0	Rather acid
C-organic (%)	1,05	Low
N-total (%)	0,12	Low
C/N ratio	8,75	Low
P-Olsen (ppm)	69	Low
K (cmol(+) kg ⁻¹)	0,26	Low
Na (cmol(+) kg ⁻¹)	0,49	Medium
Ca (cmol(+) kg ⁻¹)	9,19	Medium
Mg (cmol(+) kg ⁻¹)	3,91	High
KTK (cmol(+) kg ⁻¹)	18,97	Medium

* The results of soil laboratory analysis of BPTP East Java 2012

Plant Growth

Plant height, cob height and number of leaves of sweet corn affected by N inorganic and organic fertilizer (Table 2).

Table 2. Effect of N and organic fertilizer to sweet corn on plant height, cob height and number of leaves, DS II 2012, Mojokerto District

No.	Fertilization	Plant height (cm)	Cob height (cm)	Number of leaves
1.	Urea 0 kg/ha	80,20h	38,60f	8,47e
2.	Organik fertilizer 2 t/ha	92,33g	38,93f	8,67e
3.	Urea 100 kg/ha	125,33e	60,07e	10,07bc
4.	Urea 100 kg/ha + Organik fertilizer 2 t/ha	114,33f	63,60d	9,47d
5.	Urea 200 kg/ha	124,67e	65,07d	9,60d
6.	Urea 200 kg/ha + Organik fertilizer 2 t/ha	134,67d	69,60bc	9,33d
7.	Urea 300 kg/ha	148,67a	75,73a	10,33b
8.	Urea 300 kg/ha + Organik fertilizer 2 t/ha	140,00bc	75,87a	10,20b
9.	Urea 400 kg/ha	142,87b	70,00b	10,93a
10.	Urea 400 kg/ha + Organik fertilizer 2 t/ha	138,33c	67,53c	10,20bc
	C V (%)	10,19	5,86	5,27

The numbers followed the same letter in the same column are not significantly different by DMRT at 5% level

Results and Components of Plant

N-inorganic fertilization (urea) on sweet corn showed an increase in cob length, cob diameter and stover fresh weight (Table 3).

Table 3. Effect of N and organic fertilizer to sweet corn on cob length, cob diameter and stover fresh weight, DS II 2012, Mojokerto District

No.	Fertilization	Cob length (cm)	Cob diameter (cm)	Stover fresh weight (t/ha)
1.	Urea 0 kg/ha	8,75f	3,00e	4.90f
2.	Organic fertilizer 2 t/ha	10,50e	3,03e	5.40f
3.	Urea 100 kg/ha	12,52c	3,76d	7.50e
4.	Urea 100 kg/ha + Organic fertilizer 2 t/ha	11,23d	3,69d	7.52e
5.	Urea 200 kg/ha	12,36c	3,74d	14.93bc
6.	Urea 200 kg/ha + Organic fertilizer 2 t/ha	13,56b	3,84d	12.40d
7.	Urea 300 kg/ha	15,70a	4,20a	16.37b
8.	Urea 300 kg/ha + Organic fertilizer 2 t/ha	13,97b	3,69d	17.93a
9.	Urea 400 kg/ha	15,35a	4,08ab	15.77b
10.	Urea 400 kg/ha + Organic fertilizer 2 t/ha	15,94a	3,98bc	13.87c
	C V (%)	5,87	6,71	6,90

The numbers followed the same letter in the same column are not significantly different by DMRT at 5% level

Further increased dose of fertilizer N (urea) obtained an increase in cob cornhusk weight or cob weight (Table 4)

Table 4. Effect of N and organic fertilizer to sweet corn on cob cornhusk weight and cob weight, DS II 2012, Mojokerto District

No	Fertilization	Cob corn husk weight (t/ha)	Cob weight (t/ha)
1.	Urea 0 kg/ha	4.94f	4.18f
2.	Organic fertilizer 2 t/ha	6.86e	5.72e
3.	Urea 100 kg/ha	9.68d	7.85d
4.	Urea 100 kg/ha + Organic fertilizer 2 t/ha	8.13e	6.67e
5.	Urea 200 kg/ha	10.36d	8.21d
6.	Urea 200 kg/ha + Organic fertilizer 2 t/ha	12.11c	9.46c
7.	Urea 300 kg/ha	16.87a	12.98a
8.	Urea 300 kg/ha + Organic fertilizer 2 t/ha	12.83c	10.27c
9.	Urea 400 kg/ha	16.41a	12.62ab
10.	Urea 400 kg/ha + Organic fertilizer 2 t/ha	15.03b	12.03b
	C V (%)	6,90	6,23

The numbers followed the same letter in the same column are not significantly different by DMRT at 5% level

DISCUSSION

Soil fertility is low with C-organic, N-total, P₂O₅ and K content is low and has the soil texture of sandy loam.

Effect of N fertilization highly significant increase in plant height, cob height and number of leaves of sweet corn. Fertilization application of 300 kg Urea/ha obtained significantly increased plant height compared to other fertilization treatments. Similarly, significantly higher cob highs found in fertilization 300 kg Urea/ha with or without 2 t organic fertilizer/ha in sweet corn. Highest number of leaves of sweet corn significantly encountered in the treatment of fertilization 400 kg Urea/ha.

Fertilization 300 kg Urea/ha in sweet corn obtained cob length and cob diameter more higher, while the highest stover fresh

weight is achieved when the sweet corn crop fertilized 300 kg Urea/ha plus 2 t organic fertilizer/ha. Stover fresh plant sweet corn from a young age are potentially used as animal feed. This is in accordance with the opinion Arifin *et al.* (2003), maize straw of the young age plants have a high crude protein content with a low crude fiber so it very well when used directly for animal feed.

The use of organic fertilizer is less noticeable on growth and yield of sweet corn due to very low nutrient availability following the slow process of decomposition.

Fertilization 300 kg Urea/ha on sweet corn gained increasing of cob cornhusk weight and cob weights significantly more higher, each 16,87 t/ha and 12,98 t/ha. Appropriate soil analysis results (Table 1) which indicates, N-total content in the soil is low so it is the response when added N-inorganic fertilizer into the soil.

CONCLUSION

1. The increased dose of fertilizer N (urea) obtained an increase in cob cornhusk weight and cob weight.
2. Fertilization application of 300 kg Urea/ha plus 100 kg SP-36/ha and 100 kg KCl/ha gave the best cob weight of 12,98 t/ha.

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STUDY OF N FERTILIZATION ON THE GROWTH AND RESULTS OF CUCUMBER LOCAL SUMENEP OF LARGE FRUIT AND CUCUMBER LOCAL SUMENEP OF SMALL FRUIT

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ABSTRACT

Cucumber (*Cucumis sativus* L.) locally with greenish-white fruit color green Sumenep found in many districts, especially in Rubaru Subdistrict. Sumenep local cucumber types, there are two type of cucumber with large fruit and small fruit. This study aimed to determine of different N fertilization on the growth and yield of cucumber. The experiment was conducted on upland Bunbarat Village, Subdistrict Rubaru, Sumenep District on DS II 2012. The randomized factorial experimental design, with three replicates the experimental plots measuring 2 m x 2 m and spacing of 60 cm x 40 cm. The first factor is type of cucumber local Sumenep: (1) Large fruit, and (2) Small fruit, while the second factor was fertilizer N: (1) Urea 10 g/plant, (2) Urea 20 g/plant, (3) Urea 30 g/plant, (4) Urea 40 g/plant, and (5) Urea 50 g/plant. The entire experimental plots fertilized SP-36 20 g/plant + KCl 20 g/plant + organic fertilizer 100 g/plant. Observations of plants include: height of plant, number of leaves, number of fruits, fruit length, fruit diameter and fruit weight. The results showed small cucumber fruit plants with fertilizer urea 50 g/plant most fruitful as 114.333 fruit/ha, but instead that local cucumber of large fruit with fertilizer urea 20 g/plant obtained significantly higher fruit weight (20,94 t/ha) than type of cucumber with other urea fertilizer.

Keywords: Cucumber local Sumenep, large fruit, small fruit, fertilizer N

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one type of vegetable from pumpkin family (*Cucurbitaceae*) (Rukmana, 1994) which is derived from the Indian region. In Indonesia, the prospects are very good crop of cucumbers because

cucumbers much-loved by the community and in the consumption of fresh processed products like pickles, pickles, salads and vegetable (Sumpeno, 2008).

Cucumbers are grown in dry land with low fertility rates required the

addition of adequate fertilizers to boost production. Rational fertilization is an effort to increase efficiency and optimize production costs increased production of cucumbers. Efficient use of fertilizer is basically giving a good fertilizer macro nutrients and micro nutrients in the number, types and shapes to suit the needs of plants, and by granting the right time according to the needs and growth rate of cucumber plants. Excess fertilizer in addition to a waste of funds, also disrupt the balance of nutrients in the soil and environmental pollution (Adiningsih *et al.*, 1989; Moersidi *et al.*, 1991; Rochayati *et al.*, 1991), whereas too little fertilizer is not can provide the optimal level of cucumber production. Tisdale et al. (1985) and Bastari (1996) adds that the application of fertilizer in excess of crop needs done to increase production if done continuously and without any effort to refund the elements absorbed by plants will cause harm and damage the fertility of the soil physical and chemical properties of the soil.

Nitrogen is the nutrient that is needed to increase the protein content of

plants and the development of microorganisms in the soil, as well as serves as a key supplier for vegetative and generative growth and the formation of enzymes and growth hormones (Wijaya, 2008). Therefore, nitrogen fertilization with the right dose can increase the production and efficiency of cucumber production costs.

This study aimed to determine of different N fertilization on growth and yield of cucumber.

MATERIAL AND METHODS

The experiment was conducted in upland DS II 2012 at the dry climates Bunbarat Village, Rubaru Subdistrict, Sumenep District. Research designed using factorial randomized to combination 10 treatment was repeated 3 times. The first factor is the type of cucumber local Sumenep, consist of (a) large cucumber, and (b) small cucumber, while the second factor is fertilizer N, namely: (a) 10 g Urea/clump, (b) 20 g Urea/clump, (c) 30 g Urea/clump, (d) 40 g Urea/clump, and (e) 50 g Urea/clump. Experimental plots measuring 2 m x 3 m at a spacing of 60 cm x 40 cm. Mound size, consist of

width 1 m, length 3 m, height 40 cm and 40 cm spacing between ridges.

Dose and timing of fertilizer application, consist of the first fertilizer when the plants are cucumbers age 5 days after planting (DAP), consist of ½ dose of Urea/clump + 20 g SP-36/clump + 10 g KCl/clump + 100 g organic fertilizer/clump, while the second fertilization at age 20 DAP, consist of ½ dose of Urea/clump + 10 g KCl/clump. Data analysis using ANOVA followed Duncan Significant Difference Test (DMRT 5%) (Gomez and Gomez, 1993).

Observations of plants include : analysis of soil nutrient status before the experiment, plant of height and number of leaves, fruit length, fruit diameter, number of fruits per hectare per harvest

and fruit weight per hectare per harvest. Cucumber harvest interval 3-4 days.

RESULTS AND DISCUSSION

Agroecology Research Sites

Research sites in the Bunbarat Village, Rubaru Subdistrict, Sumenep District have climate type E4 (Oldeman) ie : 1 wet month and 8 dry month (**Fig. 1**), so the cucumber crop in the dry season irrigation pumps require supply vicinity of the river when the water shortage. Conditions relatively less fertile land with sandy soil texture and have an C-organic, N-total, and K content is relatively very low, whereas P₂O₅ were moderate (**Table 1**).

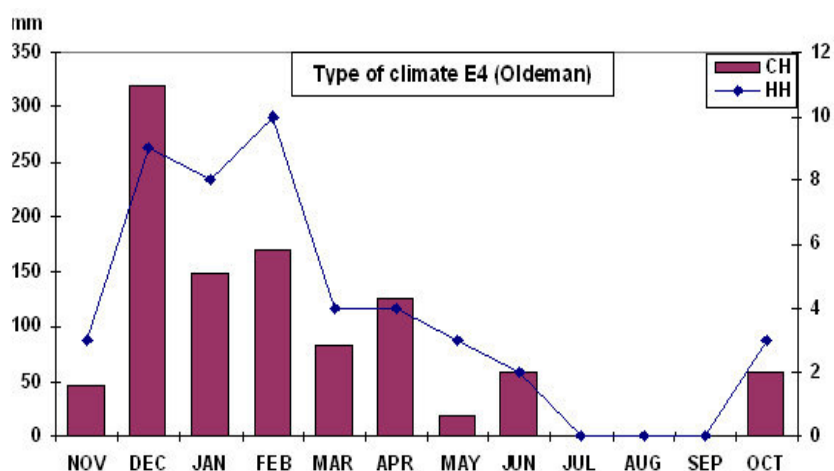


Figure 1. Distribution of rainfall in the study area Rubaru Subdistricts, Sumenep District

Table 1. Results of soil nutrient analysis before the experiment conducted in Bunbarat Village, Rubaru Subdistrict, Sumenep District

Analysis	Content	Category
Texture :		
Sand %	88	-
Dust %	5	-
Loam %	7	-
Class	-	Sandy
pH : H ₂ O	5,9	Rather acid
C-organic (%)	0,51	Very low
N-total (%)	0,05	Very low
C/N ratio	10,2	-
P-Olsen (ppm)	119	Medium
K (cmol(+) kg ⁻¹)	0,10	Low
Na (cmol(+) kg ⁻¹)	td	Very low
Ca (cmol(+) kg ⁻¹)	2,70	Low
Mg (cmol(+) kg ⁻¹)	0,28	Veru low
KTK (cmol(+) kg ⁻¹)	5,66	Low

* The results of soil laboratory analysis of BPTP East Java, 2012

Plant Growth

Cucumber plant growth is influenced by genetic factors and environmental plant growth (**Fig. 2**). N

fertilization with different doses on large cucumber and small cucumber affect plant height, leaf number, fruit length and fruit diameter (**Table 2**).



	
Cucumber local Sumenep of large fruit	Cucumber local Sumenep of small fruit
<ul style="list-style-type: none"> • Plant height ± 207 cm • Fruit length ± 20 cm • Fruit diameter ± 8 cm • Color of white slightly greenish • Colors of fruit base are bright green 	<ul style="list-style-type: none"> • Plant height ± 204 cm • Fruit length ± 16 cm • Fruit diameter ± 7 cm • Color of white slightly yellowish green • Color of fruit base are soft green

Figure 2. Local characteristics of cucumber local Sumenep of large fruit and cucumber local Sumenep of small cucumber

Table 2. Effect of N fertilization and the cucumber type of local Sumenep on plant height, leaf number, fruits number, fruit length and fruit diameter, DS II 2012, Sumenep District

Cucumber type and N fertilization	Plant height (cm)	Leaf number	Fruit length (cm)	Fruit diameter (cm)
Large cucumber + Urea 10 g/clump	203,56 ab	19,56 a	19,67 a	7,52 b
Large cucumber + Urea 20 g/clump	224,11 a	21,22 a	19,67 a	7,26 b
Large cucumber + Urea 30 g/clump	198,67 ab	20,33 a	20,89 a	8,16 a
Large cucumber + Urea 40 g/clump	195,33 ab	19,56 a	19,94 a	7,92 a
Large cucumber + Urea 50 g/clump	212,56 ab	21,11 a	18,78 a	7,51 b
Small cucumber + Urea 10 g/clump	188,44 b	18,56 a	13,78 c	5,97 d
Small cucumber + Urea 20 g/clump	206,11 ab	19,89 a	14,78 bc	6,93 c
Small cucumber + Urea 30 g/clump	207,00 ab	21,67 a	15,78 bc	7,88 a
Small cucumber + Urea 40 g/clump	211,56 ab	21,00 a	15,83 bc	6,18 d
Small cucumber + Urea 50 g/clump	206,78 ab	20,78 a	16,33 b	6,74 c
C V (%)	7,21	7,79	6,49	4,41

The numbers followed the same letter in the same column are not significantly different by DMRT at 5% level

Large cucumber fed urea 20 g/clump obtained the increase plant height, but not significantly different from other treatments, except for a small cucumber fertilizer 10 g/clump. The number of leaves of cucumber planting large and small cucumbers with some urea dosing showed no significant differences.

Fruit length and fruit diameter of large cucumber have larger fruit, which

is 19,79 cm (fruit length) and 7,67 cm (fruit diameter), while small cucumber has 15,30 cm fruit length and 6,74 cm fruit diameter. Large cucumber obtained the more higher fruit length significantly than small cucumber with Urea fertilizer treatment, while the more higher fruit diameter is found in a large cucumber with fertilization of 30-40 g Urea/clump and a small cucumber with fertilization of 30 g Urea/clump.

Result of Plant

The harvesting of fruit cucumbers of local Sumenep as much as four times at intervals of 3-4 days. At

the fourth harvest, the fruit harvest fruit shape is rather small with fruit shape slightly bent (**Table 3** and **Table 4**).

Table 3. Effect of N fertilization and the cucumber type of local Sumenep on number of cucumber fruit per hectare for four harvests DS II 2012, Sumenep District

Cucumber type and N fertilization	Number of cucumber fruit per hectare				
	Harvest 1 st	Harvest 2 nd	Harvest 3 rd	Harvest 4 th	Total harvest
Large cucumber + Urea 10 g/clump	16.333 f	16.333 f	16.333 g	21.000 c	70.000 h
Large cucumber + Urea 20 g/clump	28.000 c	33.833 a	18.667 ef	23.333 b	103.833 bc
Large cucumber + Urea 30 g/clump	25.667 d	29.167 d	19.833 de	17.500 d	92.167 g
Large cucumber + Urea 40 g/clump	29.167 c	30.333 cd	23.333 c	17.500 d	100.333 cde
Large cucumber + Urea 50 g/clump	22.167 e	33.833 a	16.333 g	24.500 b	96.833 ef
Small cucumber + Urea 10 g/clump	23.333 e	32.667 ab	26.833 b	23.333 b	106.167 b
Small cucumber + Urea 20 g/clump	31.500 b	23.333 e	30.333 a	17.500 d	102.667 bcd
Small cucumber + Urea 30 g/clump	35.000 a	22.167 e	17.500 fg	21.000 c	95.667 fg
Small cucumber + Urea 40 g/clump	35.000 a	22.167 e	21.000 d	21.000 c	99.167 def
Small cucumber + Urea 50 g/clump	35.000 a	31.500 bc	17.500 f	30.333 a	114.333 a
C V (%)	5,13	5,46	4,05	5,68	4,29

The numbers followed the same letter in the same column are not significantly different by DMRT at 5% level

The average of number cucumber on total harvest is more small cucumber 95.667-114.33 fruits/ha (an average of 103.600 fruits/ha) compared large cucumber as many as 70.000-103.833 fruit/ha (an average of 92.633 fruits/ha). The first harvest of small cucumber with fertilizer 30-50 g

Urea/clump obtained the more higher number fruit is significant, but harvesting the second, third and fourth more higher number of fruit each found in small cucumber with fertilizer 20 or 50 g Urea/clump. In total harvest, the amount of fruit most significantly is small cucumbers that fertilizer 50 g

Urea/clump, as many as 114.333 fruit/ha.

The amount of fruit that much on a small cucumber does not always result

in higher fruit weight. Although a lower number of fruit on a large cucumber, but obtained the more higher fruit weight than small cucumber (**Table 4**).

Table 4. Effect of N fertilization and the cucumber type of local Sumenep on cucumber fruit standart weight per hectare for four harvests DS II 2012, Sumenep District

Cucumber type and N fertilization	Cucumber fruit weight (t/ha)				
	Harvest 1 st	Harvest 2 nd	Harvest 3 rd	Harvest 4 th	Total harvest
Large cucumber + Urea 10 g/clump	3,27 de	2,39 f	4,78 c	3,27 c	13,71 g
Large cucumber + Urea 20 g/clump	4,67 b	6,30 a	3,79 f	6,18 a	20,94 a
Large cucumber + Urea 30 g/clump	4,26 c	5,48 b	3,79 f	3,85 c	17,38 ef
Large cucumber + Urea 40 g/clump	5,43 a	6,07 a	5,25 b	3,68 c	20,42 ab
Large cucumber + Urea 50 g/clump	3,62 d	5,48 b	4,49 d	5,54 b	19,13 cd
Small cucumber + Urea 10 g/clump	2,92 e	4,84 c	3,97 ef	5,48 b	17,21 ef
Small cucumber + Urea 20 g/clump	4,90 b	4,55 cd	6,88 a	3,44 c	19,78 bc
Small cucumber + Urea 30 g/clump	4,61 c	2,68 f	4,08 e	5,60 b	16,98 ef
Small cucumber + Urea 40 g/clump	4,73 b	3,68 e	4,81 c	3,68 c	16,89 f
Small cucumber + Urea 50 g/clump	4,20 c	4,20 de	4,03 ef	5,72 ab	18,14 de
C V (%)	4,81	6,40	5,31	6,84	5,43

The numbers followed the same letter in the same column are not significantly different by DMRT at 5% level

Fruit cucumber weight significantly more higher first and second harvest found in a large cucumber which fertilizer 40 g Urea/clump, then the third harvest

obtained the more higher fruit weight on a small cucumber with fertilizer 20 g Urea/clump, while the fourth harvest in the more higher fruit weight on a large cucumber with fertilizer 20 g

Urea/clump. Based on the results obtained whole harvest fruit weight significantly more higher at large cucumber crops (20,94 t/ha) are fertilized 20 g Urea/clump.

CONCLUSION

1. Small cucumbers of local Sumenep produce more number of fruit per hectare, but otherwise his weight is lower than large cucumber of local Sumenep
2. Small cucumber plants are fertilized 50 g Urea/clump produces the most fruit reaches 114.333 fruit/ha.
3. Large cucumber plants are fertilized 20 g Urea/clump produces fruit weight of 20,94 t/ha

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The Potential of Lauraceae Family as CO₂ Absorber and Carbon Storage in Purwodadi Botanic Gardens

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ABSTRACT

Increased emissions of greenhouse gases in the earth it caused by deforestation, forest degradation, and the use of various country. It is characterized by rising global temperatures and melting of polar ice. However, these events can be mitigated by planting trees. One group of trees that have the potential as a CO₂ absorbent and store carbon reserves came from the Lauraceae. Species diversity kind of tribe found in many Lauraceae Purwodadi Botanic Garden. Method used is the census (selection) in the collection belonging to the tree (>5meter) and analysis of biomass the model of allometric. Result there are 9 genus and 22 species as an object of research. Average mean annual increment of the Lauraceae is 1.2 cm/year, whilst absorption 578.979 kg CO₂/year, carbon stocks 222.131 kg/year. Kind of out of the Lauraceae have potential to absorb CO₂ and store carbon largest reserves the *Litsea monopetala* (2772.102 kg CO₂/year, 3815.057 kg C/year), *Beilschmiediaroxburghiana* (1584.287 kg CO₂/year, 2180.348 kg C/year), and *Cinnamomum camphora* (1468.909 kg CO₂/year, 2021.561 kg C/year).

Keywords: Lauraceae family, carbon dioxide, carbon storage

INTRODUCTION

Global warming is a large scale climate change caused by increasing emissions of greenhouse gases in the atmosphere. Estimated to be about one-fifth of total greenhouse gas emissions caused by deforestation and forest degradation (Manuri *et al.*, 2011). Emissions gas can be derived from patterns of human life that a lot of fossil fuel technology, automotive, and power plant (R and S, 2008). This has led to global warming with a marked increase in the temperature of the earth (Winarso, 2011).

Independent research shows that Indonesia besides being one of the largest greenhouse gas emitter in the world, is also a country affected by recent changes climate. Therefore, the Indonesian government is strongly committed to reducing CO₂ as much as emissions 26 % until 2020 (Manuri *et al.*,

2011). Efforts in order to tackle global warming by planting tree. Tree can reduce greenhouse gas emissions by the turn CO₂ into other compounds and store the carbon in the long term. Process of absorption and carbon stock in the plant body through the process of photosynthesis (Hairiyah and Rahayu, 2007). So to describe the amount of CO₂ in the atmosphere can be determined via measurement of tree biomass.

Types and diversity of tree has a role different capacities to absorb and accumulate carbon. Effort determine the role of capacity on each tree can be carried out at the Technical Implementation Unit (UPT) Purwodadi Botanic Garden, as one of its tasks is plant. One of the inventory of collection plant in Indonesia is Purwodadi Botanic Garden which has a collection of 1.923 species, 928 genera, and 174 tribes. Part of large collection Purwodadi

Botanic Gardens is form of tree one of the Lauraceae.

Lauraceae is the kind stature of tree. Tribe has a characteristic aroma which have essential on the part of body. Lauraceae has 43 genera and 3000 specimens (Kerrigan and Dixon, 2011). Majority of members of a Lauraceae native plant habitats ranging Indonesia. Distribution of South Sulawesi, North Sulawesi, Maluku, Java, and Sumatra. Lauraceae tall can reach 45 feet in diameter 300 cm. In terms of the potential growth Lauraceae, it can be estimated potential CO₂ sequestration and storing large amounts of carbon. When viewed from directly in terms of the role of the Lauraceae has great potential. Such as reforestation plants *Cinnamomum burmannii* Forestry department. *C. cassia* and *Litsea* sp. as spices and oils at siri. *C. culilawan* as medicine, timber *C. javanicum* as home building materials, *Eusideroxylon zwageri* as the strongest building materials and wood (iron wood) wood insecticide, used his *Persea americana* (Heyne, 1987). The presence of trees in the forest and outside forest has a huge potential to play a role in the global carbon cycle and can reduce global warming.

Diversity of the members of the Lauraceae pretty much in the Purwodadi Botanic Garden Pasuruan. Most are classified as trees and more than ten years old. The purpose of this study was to determine the potential for some kind of Lauraceae as the uptake and storage of carbon in the Purwodadi Botanical Gardens. Research on absorption of carbon reserves and deposits of Lauraceae needs to be done in order to reduce global warming.

MATERIALS AND METHODS

This study was conducted in July 2013 at the Purwodadi Botanic Garden Pasuruan. Purwodadi Botanic Garden be administratively Surabaya-Malang Highway Km. District 65 Purwodadi, Pasuruan in East Java, the area under 85 hectare. Purwodadi Botanic Garden is at a height of 300 meters above sea level, flat to undulating topography. Based on data

Climatology at the Purwodadi Botanic Garden until June 2013, the average minimum temperature is 20.8 °C, the average maximum temperature of 29.5 °C, the highest rainfall in April reached 355 mm, the lowest rainfall in June, 10.3 mm and humidity range 79-82 %RH.

The tools used in this study is a stationery, ruler, measuring tape length of 150 cm, calipers, calculator, camera, tally sheet, scissors plants, and board chest. The material used is a collection of tribe Lauraceae plants and catalogue "An Alphabetical List of Plant Species Cultivated in Purwodadi Botanic Garden". The method used in this study is census. The Method is used to determine the species of Lauraceae in the form of trees (>5 meters). Biomass plant calculated using model allometric.

The procedure begins with the observational studies in the Lauraceae VAK XVIII A and B. However, the study only in the VAK XVIII B as a collection of many types of Lauraceae tree. In addition, some species also grow in VAK IIA, III B, VF, and the small amount. Do IIIH census (selection) large trees and old enough based on books Purwodadi Botanic garden collection, note the date plant. To know the age of the plant can be the difference between the time of planting to the time of measurement.

Amount of each type taken 1 to 5 specimens. Data biomass is obtained by measuring the diameter at breast height (DBH). Measurement average diameter increment (mean annual increment) can be done by dividing the diameter and age. Next was calculated biomass, plant biomass is calculated by allometric equations. Plants that have a density provisions contained in the list of Global Wood Density database contained in Table 2. There are some types that do not have the density that is not written in the table.



Figure 1. XVIII B VAK location in the Purwodadi Botanic Garden

Table 1. Model allometric

Types	Model Allometrik	Reference
Biomass	$0.11 \times \rho \times D^{2.62}$ (Kg)	Ketterings, et al., 2001
Biomass*	$0.118 D^{2.53}$	Hairiyah and Rahayu, 2007
Serapan CO ₂	$C \times 3.67$	Morikawa, 2003; Usmadi, et al., 2011
Karbon tersimpan	$Biomass \times 0.46$	Yuliasmara and Wibawa, 2007

Description:

ρ : density of wood (g/cm³)

D: diameter of plants (cm)

*: Biomass mass of unknown species

Table 2. Mass provision of Plants Used

Species	Massa	Reference
<i>Cinnamomumburmannii</i>	0.46	Seng, 1951 in Soewarsono, 1990
<i>Cinnamomumcamphora</i>	0.49938	Cheng, Yang, and Liu, 1992
<i>Cinnamomuminers</i>	0.564816	Anonymous, 1974
<i>Cinnamomumsintoc</i>	0.38	Seng, 1951 in Soewarsono, 1990
<i>Cinnamomunverum</i>	0.497658	Anonymous, 1974
<i>Litsea firma</i>	0.34	Lemmens et al., 1995
<i>Litsea glutinosa</i>	0.56	Seng, 1951 in Soewarsono, 1990
<i>Litsea monopetala</i>	0.41	Seng, 1951 in Soewarsono, 1990
<i>Litsea noronhae</i>	0.5	Seng, 1951 in Soewarsono, 1990
<i>Persea americana</i>	0.6	Little dan Wadesworth., 1964
<i>Actinodaphne glomerata</i>	0.41	Seng, 1951 in Soewarsono, 1990

RESULT

Species diversity from Lauraceae in the Purwodadi Botanic Garden consists of 13 genera, 33 species and 313 specimens. Highways include: *Actinodaphne*, *Alseodaphne*, *Beilschmiedia*, *Cinnamomum*, *Cryptocarya*, *Dehaasia*, *Endiandra*, *Eusideroxylon*, *Lindera*, *Litsea*, *Neolitsea*, *Nothaphoebe*, and *Persea*. Clan that has a diversity of species and the most that *Cinnamomum* and *Litsea*. *Cinnamomum* consists of 7 species and *Litsea* consists of 11 species.

Based on the selection result obtained 9 genus and 22 species of Lauraceae rate as the object of measurement data obtained research. result diameter increment, biomass, CO₂ uptake, and carbon storage are presented in the take below.

Table 3. Mean annual increment, biomass, CO₂ uptake, and carbon stock of 22 types from Lauraceae

No	Species	Mean Annual Increment (cm/year)	Biomass (kg/year)	CO ₂ Uptake(kg/year)	Carbon Stock (kg/year)
1	<i>Litseamonopetala</i>	1.382	2259.837	3815.057	1039.525
2	<i>Beilschmiediaroxburghiana</i>	0.602	1291.522	2180.348	594.1
3	<i>Cinnamomumcamphora</i>	1.514	1197.466	2021.561	550.834
4	<i>Cinnamomuminers</i>	2.342	1164.323	1965.613	535.589
5	<i>Cinnamomumverum</i>	0.877	1013.896	1711.66	466.392
6	<i>Cinnamomumsintoc</i>	2.097	479.865	810.107	331.107
7	<i>Actinodaphneangustifolia</i>	1.298	533.192	900.134	245.268
8	<i>Linderaagregata</i>	0.707	371.445	627.074	170.865
9	<i>Litseaengleriana</i>	1.637	329.546	556.339	151.591
10	<i>Neolitsea cassia</i>	2.254	329.011	555.437	151.345
11	<i>Cryptocaryabrevipes</i>	1.111	324.067	547.089	149.071
12	<i>Perseaamericana</i>	0.703	214.502	362.122	98.671
13	<i>Cinnamomumlaevigata</i>	1.791	154.407	260.67	71.027
14	<i>Nothaphoebeumbelliflora</i>	0.701	152.349	257.196	70.08
15	<i>Litsea glutinosa</i>	0.646	110.246	186.118	50.713
16	<i>Cinnamomumburmannii</i>	1.006	104.995	177.252	48.298
17	<i>Litsea firma</i>	0.772	96.647	163.159	44.458
18	<i>Linderabibracteata</i>	1.23	85.995	145.177	39.558
19	<i>Actinodaphneglomerata</i>	0.945	70.206	118.523	32.295
20	<i>Litseaodorifera</i>	0.687	59.47	100.394	27.356
21	<i>Cinnamomumcelebicum</i>	0.983	21.786	36.779	10.021
22	<i>Litseanoronhae</i>	1.033	18.954	31.999	8.719
Average		1.2	471.988	796.81	222.131

Diameter increment is the amount of plant growth annually. Species of Lauraceae that has the largest increment is *Cinnamomuminers* with growth reaching 2.342 cm/year. While most small diameter increment is *Beilschmiediaroxburghiana* only 0.602 cm/year. *Cinnamomuminers* stem growth pattern that is by establishing a branch at the base trunk. The meaning the distance grows about 80-110 cm of soil will form a branching stems that big, so it can be expected each year stem growth large. Mean enough kind of average growth rates in the Lauraceae each year was 1.2 cm/year. Growth was classified as slow as less than 2 cm/year.

DISCUSSION

Value describes the amount of biomass carbon stored in trees, especially in trunk. So body, if the value of the biomass can be ascertained CO₂ uptake and carbon storage of the pula. Biomass, CO₂ uptake, and carbon stock that is the largest of the Lauraceae is *Litseamonopetala*, *Beilschmiediaroxburghiana*, and *Cinnamomumcamphora*. Third species has

the greatest value, the main factors that determine the amount of carbon accumulation in plants is an age old tree. More age it can be expected to have biomass, CO₂ uptake, and carbon stock of large age third largest such tree is reached 36-56 years. According to Ramadhan (2011) states that the volume low tree will be directly proportional to the value of tree biomass is low anyway. Comparison of the potential of carbon content on their respective stands directly proportional to the ratio of biomass potential. Slow growth conditions tribe Lauraceae plant was potentially as carbon sinks and high storage. If compared with *Vaticabella* (Dipterocarpaceae) research (Usmadi *et al.*, 2011) potential of absorbing only 385.77 kg CO₂/year 105.21 kg C/year. So Lauraceae can be used as replanting forests, reboisation in areas potential carbon stock, and CO₂ absorber on the highway.

Growth factor is strongly influenced by biotic factors such as physiological photosynthesis. Photosynthesis activity occurs in the leaves, but the biggest photosynthesis proceeds distributed to the constituent

substances trunk. Trunk have wood better than other parts of the tree. Substances making up the timber cavity causing stem cells in many composed by the composition of wood than water, so the weight of stem biomass would be greater (Imiliyana, *et al.*, 2012). Other influential factors of the abiotic environment. Factor that influence the biomass of each tree on the ground (a different site quality). Physical condition of the soil to determine the penetration of roots in the soil, water retention, drainage, aeration and plant nutrients. The physical properties of the soil greatly affect the growth and production of plants (Ramadhan, 2011).

As the development of engine technology increases the amount of carbon emissions that must be offset by tree planting effort. This like needs to be done to reduce the impact of global warming (Sujarwo and Darma, 2011).

CONCLUSION

Amount of species of the Lauraceae in Purwodadi Botanic Garden consists of 13 genres, 33 species and 313 specimens. Many genus is namely *Cinnamomum* sp., and *Litsea* sp. Lauraceae in Purwodadi Botanic Garden show at a mean annual increment of 1.2 cm/year, uptake of carbon dioxide 796.81 kg CO₂/year, while saving carbon stocks per tree reaching 222.131 kg C/year. Deposits highest carbon stocks of the Lauraceae is *Litsea monopetala* (2772.102 kg CO₂/year, 3815.057 kg C/year), *Beilschmiediaroxburghiana* (1584.287 kg CO₂/year, 2180.348 kg C/year), and *Cinnamomum camphora* (1468.909 kg CO₂/year, 2021.561 kg C/year).

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SOYMILK ICE CREAM PROCESSING TECHNOLOGY FROM KABA, ARGOMULYO AND WILIS VARIETIES OF SOYBEAN WITH PURPLE SWEET POTATO SUBSTITUTION

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ABSTRACT

The most certain groups avoid ice cream because it's high in fat. Soymilk ice creams as an alternative to overcome the problems due to the low fat of soymilk. Purple sweet potato contains high anthocyanins and fiber, so soymilk ice cream with purple sweet potato can be a healthy alternative. This research aimed to determine the effect of soybean varieties with different levels of dilution (to make soymilk) as a raw material in ice cream processing towards the physical, chemical and panelist preference level. Observed characteristics are physical properties (melting speed), chemical properties (protein, fat, anthocyanins). Acceptance level panelists using organoleptic test (hedonic) includes colour, aroma, texture, taste, melting speed, ice crystals and general preference level. This research uses a randomized complete block design (3 replications) with two factors, the first factor is Kaba, Argomulyo and Wilis soybean varieties, second is the dilution rate of soybean : water (1:10; 1:13; 1:16). All of treatment are substituted with purple sweet potato. The data obtained were analyzed by ANOVA followed by DMRT at 0.05% level. Agronomic analysis results showed that the highest yields of soybean contained at Kaba varieties (2.4 ton/ha), then Wilis (2.3 ton/ha) and Argomulyo (2.1 ton/ha). Through the analysis of physical, fastest dilution rate found in ice cream with Argomulyo and Wilis soybean varieties with the highest level of dilution (1:16). The chemical analysis of the soymilk ice cream showed that a wide range of soybean varieties and levels of dilution in the soymilk processing, didn't show significant differences on levels of protein, fat and anthocyanin. While on the organoleptic test, showed that a wide range of varieties and levels of dilution in the soymilk processing, showed significant differences for organoleptic parameters. The most preferred ice cream is ice cream with Kaba varieties and dilution level:hot water (1:16).

Keywords :

varieties , ice cream , soymilk , dilution rate

INTRODUCTION

Soybean is one of the major food commodity after rice and maize. This commodity has a variety of uses, primarily as a raw material and a protein-rich foods as a source of vegetable protein, fat, minerals, and vitamins. Soybean consumption continues to increase along with the increase of population, so most have to be imported because domestic production is insufficient (BPS, 2006). To meet the needs of soybean, we need to increase domestic production through the use of high yielding varieties of high yielding potential (Balitkabi, 2008). New varieties of soybeans include Kaba, Argomulyo, and Wilis with an average yield potential of more than 2 t / ha. Soybean is commonly used as a raw material for the manufacture of tofu, tempeh, soy sauce, and soy milk (Nazar et al. 2008). Soy contains essential fatty acids Omega - 3, amino acids, phytoestrogens, protein, minerals and vitamins. One of the soybean processed is soymilk that its amino acid composition similar to cow's milk (Widowati, S. 2007). Soymilk contains no lactose, protein does not cause allergies, low-fat, cholesterol -free, can be further processed into ice cream, yogurt and mayonnaise (Astawan 2004).

Ice cream is a frozen product that very popular, it's just that some people avoid to eat ice cream because ice cream has a high fat content that it interferes with his diet program. Processing soya ice cream can be used as an alternative to overcome this problem. Ice cream soymilk substituted with purple sweet potatoes contain high fiber and anthocyanins but low of fat so that as an alternative healthy food choices appropriate for the diet. Ice cream is one of the frozen food products made by freezing a mixture of dairy products, sugar, stabilizers, emulsifiers and other ingredients that have been pasteurized and homogenized to obtain a

uniform consistency (Arbuckle, 1986 in Syahputra, 2009). With soymilk processing into ice cream then the level of soya consumption will increase because there are some certain people who do not like soymilk caused a distinctive flavor and aroma in soymilk (beany flavor). Beany flavor in soy milk caused by the activity of lipoxygenase enzymes or Lipoksidase contained in soy beans. The enzyme produces ethyl vinyl ketone which causes an unpleasant taste and odor. Immersion in water treatment, the release of the skin, heating at a temperature of 80⁰C for 10-15 minutes, giving the sugar, adding flavor can reduce the unpleasant odor (Astawan , 2004). In individuals with lactose intolerance (allergy to lactose) or for those who do not like dairy cows and for the vegetarians, the soy milk can be used as a substitute for cow's milk selection (Widowati ,S. 2007). So for ice cream consumers but they can't eat it because avoiding cow's milk, the alternative solution is replaced with soya milk as a base for making ice cream. Purple sweet potato (*Ipomea batatas* var. *Ayamurasaki*) is usually called *Ipomea batatas Blackie* because it has skin and tuber flesh purple black colored (dark purple). Purple sweet potato pigment containing higher anthocyanin than other types of sweet potatoes. Total anthocyanin content of purple sweet potato is 519 mg/100 g fresh weight. Purple sweet potato as anticancer because there is an active substance called selenium and iodine and 20 times higher than other types of sweet potatoes. (Kumalaningsih, 2006). Purple sweet potato has a chemical composition of 50-81% water, protein 1-2.4%, fat 1.8 to 6.4%, starch 8-29%, non-starch carbohydrates from 0.5-7.5%, reducing sugar 0.5-7.5%; ash 0.9 to 1.4%; Thiamin 0.1%; Riboflavin 0,06% (Nakashima (1999) in Kumalaningsih (2006) Soymilk ice cream with purple sweet potatoes can be a healthy alternative because it contains high anthocyanin and fiber, but

low in fat content. This research aimed to determine the effect of soybean varieties with different levels of dilution (to make soymilk) as a raw material in the ice cream processing based on the physical, chemical and preference level panelists.

MATERIALS AND METHODS

The research was conducted in the Post Harvest laboratory, East Java Assessment Institute for Agricultural Technology (June-August 2012). The materials used in research related to post-harvest aspects are Kaba, Argomulyo and Wilis soybean varieties, planted from March to May 2012 in the KP East Java AIAT. Observations were done on the agronomic aspects of those varieties that do start growing until harvest. Aspects of post-harvest research to make ice cream from soymilk, using a randomized block design, repeated three times with two factors, the first factor is soybean varieties (Kaba, Argomulyo and Wilis) while the second factor is the dilution rate of soybean : hot water (1:10; 1: 13 and 1:16). The nine treatments were tested (Table 1) then the organoleptic test.

Table 1 . Combination Treatment of Soymilk

Treatment	Interaction
A	Kaba varieties with soy dilution level, soy : hot water (1:10)
B	Kaba varieties with soy dilution level, soy : hot water (1:13)
C	Kaba varieties with soy dilution level, soy : hot water (1:16)
D	Argomulyo varieties with soy dilution level, soy : hot water (1:10)
E	Argomulyo varieties with soy dilution level, soy : hot water (1:13)
F	Argomulyo varieties with soy dilution level, soy : hot water (1:16)
G	Wilis varieties with soy dilution level, soy : hot water (1:10)
H	Wilis varieties with soy dilution level, soy : hot water (1:13)
I	Wilis varieties with soy dilution level, soy : hot water (1:16)

The research was carried out includes three stages: (1) The making of a purple sweet potato

paste, (2) The making of soymilk, (3) The making of soymilk ice cream.

1. Making purple sweet potato pasta by peeling the skin, slicing thin purple sweet potato, washing then boiled with water until the colour get faded (\pm 15 minute). Then separated the purple sweet potato from water. The purple sweet potato then crushing until becomes a smooth paste.
2. Making soymilk shown in Figure 1. Soybean appropriate with the treatment (Kaba, Argomulyo and Wilis varieties) was washing, soaking overnight, boiled with water (soybean : water = 1 : 10). Then soybean crushed with a blender that suitable with a treatment (soybean : hot water = (1:10), (1:13), (1:16). The next step is pressing then will got the filter that called soymilk. Soymilk was used to make ice cream.

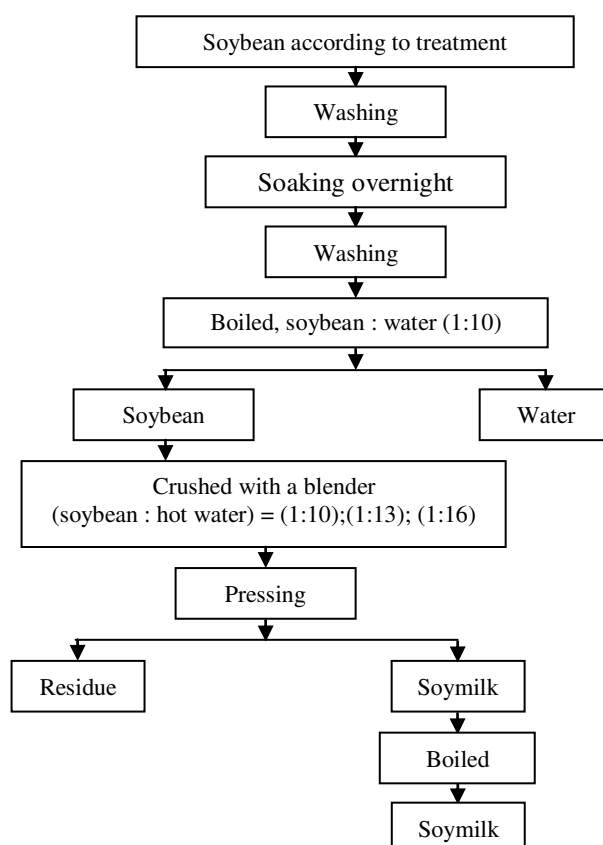


Figure 1. Flow chart of soymilk processing

3. Making a soymilk ice cream as in Figure 2

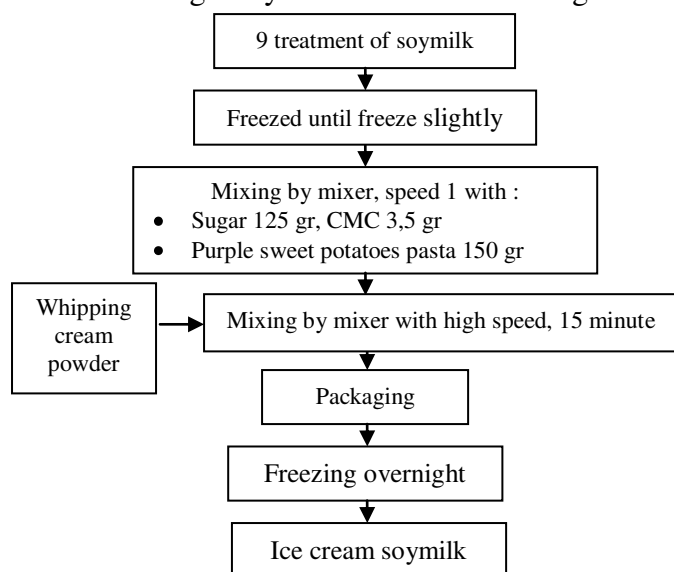


Figure 2. Flow chart of soymilk ice cream processing

Characteristics of soymilk ice cream were observed towards protein levels (semi-micro Kjeldahl method, AOAC 1990), fat content (Soxhlet method, AOAC 1990) and Anthocyanin levels. Physical characteristics were observed include melt velocity (Nelson and Trout, 1965) by measuring the time required for the melting of the ice cream with a weight of 40 g at room temperature. Organoleptic test used hedonic test to determine the most preferred treatment panelists. Organoleptic observations include colour, aroma, taste, texture, melting speed, ice crystals and the general level of preference. The data obtained were analyzed by ANOVA followed by DMRT at 0.05% level

RESULTS AND DISCUSSION

1. Agronomic characteristics of Soybean (Kaba, Argomulyo, and Wilis Varieties)

Characteristics of soybean plants can generally be classified into qualitative and quantitative character. Qualitative characters such as flower color, branching shapes, and

colors beans while quantitative characteristics including plant height, age of the plant, and the yields are easily influenced by the environment (Agustina L., 1994). Therefore, the results observations could be different at different locations due to agroecological variability of place to grow and farming inputs that given. Characteristics of soybean varieties as shown in Table 2.

Table 2. Agronomic characteristics of Soybean Varieties

Criteria	Kaba Varieties	Argomulyo Varieties	Wilis Varieties
Plant height (cm)	58.9	50.2	53.3
Number of leaves	8.2	9.1	12.7
Harvest Time (days)	85	83	89
Weight of 100 seeds(g)	10,5	14.0	10,1
Potential Yield (t /ha) *	3,25	1,5-2	3,0
Yields (t / ha)	2,4	2,1	2,3
Seed color	Yellow	Yellow	Yellow

Information : *) Source (Nazar *et al.* 2008).

Observations showed that the highest plant at Kaba soybean varieties (58.9 cm), at most the number of leaves Wilis soybean varieties (12.7), the harvesting of early maturing soybean varieties on Argomulyo, and the highest yields of soybean at Kaba varieties (2.4 tonnes / ha), then Wilis (2.3 ton / ha) and Argomulyo (2.1 ton / ha).

2. Chemical Characteristic Ice Cream

Fat Content

Fat is an essential key component in the ice cream. Use the appropriate amount is important to note, not only for the balance of the properties of a mixture, but also to meet the minimum requirements established. During the freezing process, the fat particles will be concentrated on the surface of the air cavity, causing fat to be able to provide a soft texture, flavor and a satisfying taste in ice cream (Arbuckle, 1986). Fat content according to SNI No. 01-3713-1995 least 5.0 % w/w. Based on Table 3 fat content of ice cream soymilk ranged

from 2.385 to 2.980 %. While the ice cream is listed in SNI base ingredients are milk cows. So that the ice cream soymilk is low fat content that's very suitable for the diet program. This is because the fat content in dairy cows was higher (3,3%) than fat in soymilk (2%/100 gr) (Astawan, 2004). The chemical composition of soymilk ice cream from various varieties and soymilk dilution rate as shown in Table 3.

Table 3. The chemical composition of soymilk ice cream

Treatment	Protein of Soymilk (%)	Protein of Ice Cream (%)	Fat (%)	Anthocyanin (mg/g)
Variety of soybean				
Kaba	1,02 ^a	2,205 ^a	2,495 ^a	0,897 ^a
Argomulyo	1,06 ^a	2,198 ^a	2,715 ^a	0,813 ^a
Wilis	1,15 ^a	2,402 ^a	2,655 ^a	0,728 ^a
Dilution rate of soymilk				
Soybean : hot water (1:10)	1,18 ^a	2,330 ^a	2,502 ^a	0,745 ^a
Soybean : hot water (1:13)	1,06 ^a	2,218 ^a	2,632 ^a	0,713 ^a
Soybean : hot water (1:16)	0,99 ^a	2,257 ^a	2,732 ^a	0,980 ^a
Interaction				
Kaba variety with dilution rate of soymilk:				
Soybean : hot water (1:10)				
Kaba variety; Soybean : hot water (1:13)	0,90 ^a	2,005 ^a	2,410 ^a	0,810 ^a
Kaba variety; Soybean : hot water (1:16)	0,99 ^a	2,250 ^a	2,645 ^a	1,00 ^a
Argomulyo variety; Soybean : hot water (1:10)	1,10 ^a	2,225 ^a	2,385 ^a	1,00 ^a
Argomulyo variety; Soybean : hot water (1:13)	1,17 ^a	2,310 ^a	2,780 ^a	0,670 ^a
Argomulyo variety; Soybean : hot water (1:16)	0,90 ^a	2,060 ^a	2,980 ^a	0,770 ^a
Wilis variety; Soybean : hot water (1:10)	1,25 ^a	2,405 ^a	2,690 ^a	0,355 ^a
Wilis variety; Soybean : hot water (1:13)	1,12 ^a	2,340 ^a	2,705 ^a	0,660 ^a
Wilis variety; Soybean : hot water (1:16)	1,08 ^a	2,460 ^a	2,570 ^a	1,170 ^a

Note : number in same column with followed by the same letter is not give a really significant differences, according to the DMRT test at 0,05% levels

Protein content

Protein in ice cream act to develop the structure of ice cream including a role in emulsification batter, whipping properties and increase the water-binding capacity. Dough ice cream is an emulsion system where the presence of protein in the system plays a fairly

big. When the protein polymer in contact with the surface of the fat globule layers, protein hydrophobic part will be absorbed in the interface and the hydrophilic parts will remain in solution. The proportion of absorbed and distributed part of this protein affects the stability emulsion (Friberg, 1976). Table 3 shows that treatment (varieties and dilution rate) and its interaction, do not give significant effect on the protein content of ice cream. Soymilk protein levels as materials for ice cream ranged from 0.90 to 1.25 % while the ice cream protein content ranged from 2.005 to 2.460 % . Soymilk protein content after being processed into ice cream has increased since the time of making ice cream is also added sweet potato which containing proteins by 1 to 2.4 % (Nakashima, 1999 in Kumalaningsih, 2006). Soymilk protein give not allergic and also have an essential amino acid composition that most complete more than other types of nuts and similar to cow's milk so it is best used as a substitute for cow's milk, especially for those who are allergic to cow's milk and lactose intolerance sufferers (Astawan, 2004).

Anthocyanin Content

Table 3 shows that the treatment and interactions, does not cause a noticeable difference on anthocyanin content of ice cream. The addition of purple sweet potato in ice cream, can increase the anthocyanin content of up to 1.17 mg/g compared to ice cream without any addition of purple sweet potato.

2 . Analysis of Physical Properties of Ice Cream

The different treatment giving effect to ice cream melts speed, as shown in Table 4.

Table 4 . Average Speed Melt Value

Treatment	Average Speed Melt Value (minute/40 gr)
A. Kaba variety; Soybean : hot water (1:10)	61
B. Kaba variety; Soybean : hot water (1:13)	54
C. Kaba variety; Soybean : hot water (1:16)	47
D. Argomulyo variety; Soybean : hot water (1:10)	60
E. Argomulyo variety; Soybean : hot water (1:13)	48
F. Argomulyo variety; Soybean : hot water (1:16)	35
G. Wilis variety; Soybean : hot water (1:10)	60
H. Wilis variety; Soybean : hot water (1:13)	80
I. Wilis variety; Soybean : hot water (1:16)	35

Table 4 shows that the slowest of ice cream melt contained in the treatment Kaba varieties with dilution rate 1:10, with the value is 61 minute/40gr. While the fastest speed on treatment Wilis and Argomulyo varieties with 1:16 dilution rate. From Table 4 shows that the higher the dilution rate of soymilk, make the rapidity of ice cream melts more faster. With the same amount of stabilizer, but the ice cream with the highest dilution (1:16) make the fluid volume in ice cream will be high anyway so its stabilizer in high dilution rate less than the low dilution rate. As a result of ice cream with the highest dilution, will melt faster. Melting rate of ice cream associated with body and texture and sweetness intensity (Nelson and Trout, 1965 in Pamungkasari, D. 2008). According to Arbuckle (1986) body and texture of ice cream is determined by the total solids contained in the dough that includes sugar, milk solids not fat, protein and hydrocolloids. Weak body is shown with a less sturdy ice cream and always accompanied by the rapid melting. This is due to the low solids and insufficiency stabilizer. According Astawan (2004), total solids of soy milk at 8.5 % which includes 3.6 % protein, 2 % fat and 2.9 % carbohydrate . Therefore, with the higher levels of dilution , the total dissolved solids contained in the ice cream are also lower so that the ice cream resistance decreases so that resulting in rapid melting of ice cream.

3 . Organoleptic assessment

Varieties of soybean and differences on the dilution, affected the consumer acceptance (Table 5).

Colour

Colour is the most determining factor of a food product appealing (Winarno , 1991). Difference in treatment effect on ice cream colors. The most preferred colors are C treatment, with a value of 3.824. According to the panelists in C treatment is more clear in comparison with A treatment (strong white color).

Table 5. Organoleptic value of soymilk ice cream

Parameter	Treatment								
	A	B	C	D	E	F	G	H	I
Colour	3,5 ^{ab}	3,7 ^{ab}	3,8 ^a	3,5 ^{ab}	3,4 ^b	3,7 ^{ab}	3,6 ^{ab}	3,3 ^b	3,5 ^{ab}
Aroma	3,8 ^a	3,7 ^a	3,7 ^a	3,8 ^a	3,9 ^a	3,8 ^a	3,6 ^a	3,6 ^a	3,9 ^a
Aroma preference	3,5 ^{bc}	3,6 ^{ab}	3,7 ^{ab}	3,7 ^{ab}	3,6 ^{ab}	3,6 ^{ab}	3,4 ^{bc}	3,1 ^c	3,9 ^a
Texture	3,1 ^{cd}	3,4 ^{abc}	3,8 ^a	3,3 ^{bc}	3,5 ^{abc}	3,7 ^{ab}	3,5 ^{ab}	2,7 ^d	3,6 ^{ab}
Texture preference	3,2 ^b	3,3 ^{ab}	3,6 ^a	3,3 ^{ab}	3,3 ^{ab}	3,6 ^a	3,4 ^{ab}	2,6 ^c	3,4 ^{ab}
Taste	3,3 ^{bcd}	3,4 ^{abc}	3,7 ^a	3,1 ^{cd}	3,3 ^{bcd}	3,5 ^{ab}	3,1 ^{cd}	3,01 ^d	3,5 ^{abc}
Melting speed	3,2 ^b	3,3 ^b	3,1 ^{bc}	3,1 ^b	3,2 ^b	3,1 ^{bc}	3,0 ^{bc}	3,8 ^a	2,7 ^c
Melting speed preference	3,2 ^{bc}	3,4 ^{abc}	3,5 ^{ab}	3,4 ^{abc}	3,7 ^a	3,6 ^a	3,3 ^{abc}	3,1 ^c	3,4 ^{abc}
Ice crystal	3,2 ^c	3,4 ^{bc}	3,9 ^a	3,6 ^{ab}	3,7 ^a	3,8 ^a	3,6 ^{ab}	2,8 ^d	3,9 ^a
Ice crystal preference	3,1 ^d	3,3 ^{cd}	3,8 ^a	3,6 ^{abc}	3,3 ^{bcd}	3,6 ^{abc}	3,5 ^{abc}	2,6 ^c	3,75 ^{ab}
Preference general levels	3,3 ^b	3,5 ^{ab}	3,8 ^a	3,3 ^b	3,5 ^{ab}	3,6 ^{ab}	3,5 ^{ab}	2,7 ^c	3,8 ^a

Information : number in same column with followed by the same letter is not give a really significant differences, according to the DMRT test at 0,05% levels

Criteria	
Colour,	: 1) Very dislike
Aroma preference,	: 2) Dislike
Texture preference,	: 3) Enough
Taste,	: 4) Like
Melting speed preference	: 5) Very like
Ice crystal preference	
Preference of panelist base on overall parameters	
Aroma	: 1) Very beany flavour
	: 2) Rather beany flavour
	: 3) Enough
	: 4) Beany flavour
	: 5) No beany flavour
Texture	: 1) Very hard
	: 2) Hard
	: 3) Enough
	: 4) Soft
	: 5) Very soft
Melting Speed	: 1) Very faster
	: 2) Faster
	: 3) Enough
	: 4) Slowly
	: 5) Very slowly
Ice Crystall	: 1) Very big
	: 2) Big
	: 3) Enough
	: 4) Small

Aroma

On Table 5 (aroma parameters), the difference in treatment does not give a real difference to the flavor of ice cream. Panelists could hardly distinguish the difference in flavor caused by the treatment. All panelists stated that ice cream flavor is not unpleasant (beany flavor) .

Preference for aroma

Based on Table 5 shows that the difference in treatment provides an influence on the aroma preference. Almost all the panelists expressed like. A highest values is in the I treatment (Wilis variety, with a dilution rate of 1:16) with a value of 3.943 (like)

Texture

In Table 5 shows that the difference in treatment provides a noticeable effect on the texture of ice cream. Ice cream texture values ranged from enough until soft. Highest score is meaning softer, placed in C treatment contained (Kaba varieties, 1:16 dilution level) with a value of 3.830 (soft) .

Preference for texture

Difference in treatment provides significant effect on the texture of ice cream. A value of textures ranging enough to like . A highest values is in C and F treatment with the same value of 3.679 (like) .

Taste

Difference in treatment provides noticeable difference in ice cream taste. According to panelist, a value of ice cream taste is like (Table 5). A highest value is in C treatment (Kaba varieties with 1:16 dilution level) with a value of 3,761 (likes) .

Melt velocity

Difference in treatment gave a significant effect on the speed of melting ice cream. According to the panelists, slowest ice cream melting is ice cream with a Wilis variety treatment with dilution level 1 : 13

Preference of melt velocity

In Table 5 it appears that the melt velocity is the most preferred panelist on E treatment (Argomulyo varieties, the level of dilution 1:13) with a value of 3.736 (like) . According to the panelists, too slow and too fast melt speed is less favored by panelist.

Ice crystals

Difference in treatment provides a noticeable effect on ice crystals (Table 5) . According to the panelists, the ice crystals is enough to small The most small ice crystals present in treatment I (Wilis varieties with 1:16 dilution rate) .

Ice crystals Preference

In Table 5 shows that the difference in treatment provides significant effect on ice crystals preference. Ice crystals are the most preferred in treatment C (kaba varieties , 1:16 dilution level) with a value of 3.824 (like) .

Preference panelist base on overall parameters

According to preference panelist base on overall parameter, the difference in treatment provides noticeable effect on. Based on the overall parameters , ice cream that the most preferred in the treatment of C with consideration for all parameters , treatment C good value.

CONCLUSION

1. Agronomic analysis results showed that the highest yields of soybean at Kaba varieties (2.4 ton / ha), then Wilis (2.3 ton / ha) and Argomulyo (2.1 ton / ha).
2. The most preferred of ice cream is ice cream with Kaba varieties with soy dilution level = soybean : hot water (1:16) . This ice cream has an average value of speed melt 47 minute /40 gr, with the protein content of 2.250 %, 2.645 % fat and anthocyanins 1 mg / g . Organoleptic value in this treatment showed higher for colour preferences 3.8 (

like) , aroma 3.7 (like) ; texture 3.7 (like) ; taste 3.8 (like) and based on the overall parameters, this ice cream has a value of 3.85 (like) .

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PUMPKIN SAUCE PROCESSING TECHNOLOGY

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ABSTRACT

Pumpkin has a high nutritional value mainly antioxidants. When the harvest occurs, the price will be decline. Furthermore, it is necessary to diversify the pumpkin into another product. Sauce is a popular complementary foods in the community. Sauce is one of the flavoring ingredients are often used in various foods by most people. Containing pumpkin as an antioxidants combined with the habits of the people who often use the sauce for seasoning food then their correlation between the two, will be very beneficial in improving public health. Pumpkins sauce can reduce the tomatoes dependent, especially anticipate when the tomatoes got the expensive price. This research was conducted in April to June 2013 aims to determine the concentration of pumpkin pasta to produce sauce that most favored by panelists based on the physical, chemical and organoleptic. Treatment being tested was concentration pumpkin with tomato: A)100:0%, B)80:20%, C)60:40% and D)40:60%. Pumpkin sauce chemical characteristics that observed included beta-carotene, water content, ash, vitamin C, total acid, total dissolved solids and crude fiber. While the physical properties that observed include colour (L,a,b), viscosity and pH. The hedonic organoleptic test to determine the most preferred include colour, aroma, taste, viscosity and general level of preference. The addition of pumpkin pasta influence on physical, chemical and organoleptic properties of sauce. Pumpkin sauce that a panelist received is treatment C (concentration pumpkins: tomato 60:40%) with the beta-carotene (228ug/100 g), water content (77.77%), ash (3.30%), vitamin C (0.26 mg/g); total acid titration (1.4%), total dissolved solids (13.97%), crude fiber (1.34%), viscosity 53.280 cps; L 40.93: a 17.8, b 16.2 and pH 4.31. Based on organoleptic, treatment C occupies most preferred by panelist means the highest value in terms of colour, taste, viscosity, aroma and the general level of preference. In general, the panelists have received this pumpkin sauce well.

Keywords

sauce, pumpkin, tomatoes, organoeptic.

INTRODUCTION

Indonesia is rich in local commodity that has the potential nutritional and bioactive components that are beneficial to humans. One of the local potential that still limited utilization is pumpkin. Pumpkin plants are plants in the family Cucurbitaceae (Sudarto , 1993). Pumpkin is a food that contains calories, carbohydrates, protein, fat, minerals (calcium, phosphorus, iron, sodium, potassium, copper and zinc), β - carotene, thiamine, niacin, fiber and vitamin C, which protects the eyes (of attacks cataracts) and skin, immunity and reproduction. Pumpkin can be used as a food ingredient that can support food security through the diversification process. Besides pumpkin can serve as a vitamin A fortification in processed food products (Anonymous, 2012). The following nutrient content in 100 gr pumpkin material (Table 1).

Table 1. Nutrition of Pumpkin (100 g material)

Nutrients	Content	Unit
Energy	29	30 Kal
Water	91,2	89,7 Gram
Protein	1,1	3,6 Gram
Fat	0,3	0,6 Gram
Carbohydrate	8,79	4,5 Gram
Calsium	45	138 Mg
Phospor	64	99 Mg
Iron	1,4	3,7 Mg
Vit A	180	2750 SI
Vit B	0,08	0,14 Mg
Vit C	52	36 Mg
Edible portion	77	70 %
Sugar	6,6	- gram
	3,43	- Mg

Source: Directorate of Nutrition Department of Health, Jakarta (1996)

It's just that many people do not understand that the pumpkin has many benefits and can be used for a variety product. One of that things is sauce. So far the community has usually to consume sauce with tomato ingredients. Actually pumpkin can also be used as a substitute for tomatoes, especially to anticipate when the price of tomatoes got increased. Pumpkin is a good alternative because pumpkin

also contains a variety of nutritional value that is not less than the tomatoes. Pumpkin meat contains a lot of β carotene or provitamin A is very beneficial for health. In addition, pumpkin also contains nutrients such as protein, carbohydrates, fiber, several minerals such as potassium, calcium, phosphorus, iron and vitamins B and C (Hendrasty , 2007). The sauce is generally defined as a product which come from some commodities considered vegetables, such as tomatoes and chilli (Fardiaz , 1992). The sauce is one of the flavoring ingredients are often used in various foods by most people of Indonesia , which is a time consuming supplementary material meatballs, noodles, pizza, rice, etc. According Astawan and Astawan (1991), seasoning sauce is a food that is like a thick porridge form and generally have orange to red color.

Therefore the presence of antioxidants in pumpkin to prevent cancer combined with the habit of using the sauce for seasoning food then the correlation between the two became very profitable in improving public health. Pumpkin sauce is very promising considering pumpkin readily available, low cost, high durability so that it can be processed into food that is economical and healthy. Sauce can be tick sauce and thin sauce with the main basic taste sour, sweet , spicy , salty , etc. (Susanto and Saneto , 1994). Sauce ingredients that commonly used are tomatoes. The dominant nutrition contain in tomatoes are vitamin A and C are predominant (Tonucci et al (1995) in Dewanti et al (2010), lycopene (Bombardelli, 1999) in Maulida and Zulkarnain, (2010).

MATERIALS AND METHODS

This research was conducted in April to June 2013 in the Post harvest Laboratory of AIAT . This research uses a randomized complete block design (3 replications) with treatment is concentration of tomato and pumpkin. Pumpkins : tomato = A).100:0 %, B) 80:20 % , C) and D 60:40 %) 40:60%. Pumpkin sauce

chemical characteristics were observed included beta-carotene (ug/100 g), water content (oven method), ash (gravimetric method) , vitamin C (mg/g), Total Acid Titration (%), total dissolved solids (%) and crude fiber. While the observed physical properties include color (L , a and b) , viscosity (cps) and pH. Using hedonic organoleptic test to determine the most preferred treatment by panelists include colour, aroma, flavor, consistency and general level of preference. Pumpkin sauce technology by blanching the tomato and pumpkins first. Then by added the seasoning as shown in figure below. Tomatoes are washed, throw away from the seed and then blanched for 3 minutes. The next step is peeling the tomatoes skin. For pumpkin after peeling the skin then slicing and blanching for 10 minutes. Percentage of treatment based on a weight of overall amount of pumpkin and tomatoes that would wanted to make sauce, for example if a sauce with material vegetable weighing 300 g, the other ingredients are added according to the following table 2.

Table 2. Composition of Sauce (per 300 g of raw material)

Raw Materials	Treatment (Pumpkin:tomatoes)%			
	A.100:0	B.80: 20	C. 60:40	D.40:60
Pumpkin (gr)	300	240	180	120
Tomatoes (gr)	0	60	120	180
Garlic (gr)	10	10	10	10
Red chilli (gr)	30	30	30	30
Salt (gr)	5	5	5	5
Sugar (gr)	20	20	20	20
Vinegar(ml)	20	20	20	20
Tapioca (gr)	2,5	2,5	2,5	2,5

Furthermore tomato and pumpkin, seasoning (garlic, red chilli, sugar, salt) then crushing with blender by added 100 ml of water. Cooked pasta and spice until slightly thickened. Further added tapioca starch dissolved in 10 ml of water and vinegar. Mixing continue until the sauce thickens. Long cooking in this same study that for 18 minutes.

Sauce processing as shown in figure 1 below.

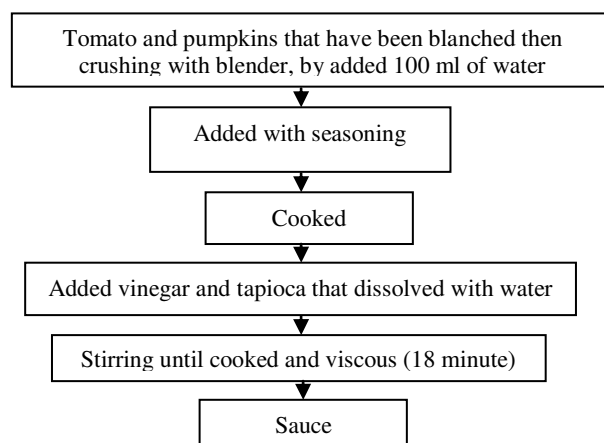


Figure 1. Flow Chart of Sauce Processing

RESULTS AND DISCUSSION

Pumpkin and tomato substitution treatment in the sauce affect the physical, chemical and organoleptic parameter

1. Analysis of the chemical properties

The concentration of pumpkin and tomato in processing of sauce affect the chemical properties as shown in Table 3 below.

Table 3. Value Analysis Chemical Yellow Pumpkin Sauce

Treatment	β caroten (ug/100 g)	Water content (%)	Ash (%)	Vit C (mg/g)	TAT(%)	Crude fiber (%)
A.	189,1 ^d	80,57 ^a	2,27 ^b	0,2967 ^a	1,400 ^a	0,7467 ^b
B	207,1 ^c	73,43 ^a	2,67 ^{ab}	0,3233 ^a	1,400 ^a	1,4630 ^a
C	228,0 ^b	77,77 ^a	3,30 ^a	0,2567 ^a	1,400 ^a	1,3400 ^a
D	236,8 ^a	76,40 ^a	3,30 ^a	0,3400 ^a	1,833 ^a	1,1430 ^a

1.1. β caroten

Concentration of pumpkin and tomatoes influence on the value of beta-carotene as shown in Table 3. Beta-carotene values ranged from 189.1 to 236.8 ug/100gr.

1.2. Water content

According Winarno (1992) the water content in foodstuffs in determining acceptability , freshness, and durability of the material.

Differences in the concentration of pumpkin and tomato paste do not give significant effect on water content sauce (Table 3) . Water content ranged from 73.43 until 80.57 % . Water content values were not significantly different because the water content of tomato (94 g) was nearly the same as pumpkin water content (91.2). Differences in moisture content of the material affects water levels resulting sauce.

1.3. Ash content

Measurement of ash content aims to determine the mineral content contained in the material and are closely related to the purity and cleanliness of the material. According to Sudarmadji et .al., (1989), ash is an inorganic substance which the residual as a result of burning organic material. Differences in the concentration of pumpkin and tomato paste were added, giving a real difference to the ash content (Table 3). Pumpkin sauce ash content ranged from 2.267 to 3.30 %.

1.4 . Vitamin C

Concentration pumpkin and tomato in sauce do not give significant differences in vitamin C parameter. Vitamin C values ranged from 0.2567 to 0.3400 mg/g. Vitamin C sauce content were decreased compared with vitamin C in raw material due to material damage during the manufacturing process. According Winarno (1992) Vitamin C is a vitamin most easily damaged, in addition to water-soluble, vitamin C is easily oxidized and this process is accelerated by heat, light, alkali, enzymes, oxidant, and catalyst by copper and iron.

1.5 . Total Acid Tritation (TAT)

Concentration pumpkin and tomato does not give effect to the value of total acid tritation as shown in Table 3 . The value of total acid tritation ranged from 1.400 to 1.833 % .

1.6. Crude fiber

According Muchtadi (1989), crude fiber is the part of food that can not be hydrolyzed by the chemicals used in determining crude fiber content is H₂SO₄ and NaOH, whereas dietary fiber is the part of food that can not be hydrolyzed by digestive enzymes. Crude fiber consists of cellulose with a few of lignin and pentose (Apriyantono, et al, 1989). Treatment the addition of pumpkin and tomato do not give significant effect on the value of crude fiber dressing. Crude fiber values ranged from 0.7467 to dip 1.4630% (Table 3).

2. Analysis of the physical properties

Concentration pumpkin and tomato give effect to the physical properties of sauce (Table 4).

Table 4. Physical Analysis of Pumpkin Sauce

Treatme nt	Viscosity (cps)	TSS (%)	L	a	b	pH
A	34.000 ^d	13,17 ^a	42,93 ^a	15,27 ^a	20,07 ^a	4,53 ^a
B	80.500 ^a	16,03 ^a	40,97 ^b	18,97 ^a	17,77 ^{ab}	4,34 ^a
C	53.280 ^b	13,97 ^a	40,93 ^b	17,80 ^a	16,20 ^b	4,31 ^a
D	34.500 ^c	14,83 ^a	39,53 ^b	17,03 ^a	14,70 ^b	4,30 ^a

2.1. Viscosity

Viscosity is an important parameter in the sauce because the product viscosity is strongly associated with the appearance of the resulting sauce, ease of packaging and ease of flow when sauce get poured. The sauce that has a very high viscosity (very thick) would complicate consumers when pouring sauce and causing power industry requires a larger pump to drain the sauce into the packaging machine because the more viscous a fluid would require greater force in order to the fluid can flow . The sauce is too thin also less preferred by consumers (Toledo 1991). According to Gaman and Sherrington (1994), if the suspension of starch in water is heated, the water will penetrate the outer layer of granules and these granules begin to swell. This occurs when the temperature increased from 60⁰C to 85⁰C . Granules can be

swell up until the volume become five times its original volume. When the size of the starch granules swell, the mixture becomes thick. According Winarno (1992) increased viscosity is due to the water that to be outside of granule and free to move before the suspension is heated, has now been in starch granule and can't move freely again. According to de Man (1989), starch can be classified based on the properties of the resulting paste. Root and tuber starch (potato , yam and tapioca) form a paste thicker and contains long passages. Pasta is usually clear, on cooling only form a soft gel . In Table 4, substitute of pumpkin in sauce making a real impact on the value of viscosity . Based on organoleptic test, panelists preferred viscosity at C treatment with pumpkin paste concentrate 60% and 40% tomatoes. Viscosity is also influenced by the levels of pectin in pumpkin and tomatoes. This is consistent with Gaman and Sherrington statement (1994) that pectin is a stabilizer that can increase the viscosity, which improves the stability of the emulsion. This fits well by the Sigit research (2007) it's about the sauce technology from chilli, papaya and tomatoes substitution says that with the higher of papaya concentrations then the higher viscosity of sauce (66.76 N.m2.s) as papaya contains pectin which serves as a stabilizer and increase the viscosity of the product. Pectin content in tomatoes quite varied between 0.17 -0.25 % (Anonymous , 2010). While the pumpkin pectin levels ranged from 0.575 to 1.080 % (Yuliani , et.al, 2005). In addition, viscosity is affected by water content. According Mulyanti (2004), a high water content will result an aqueous sauce (low viscosity) and with low water contents will produce dense or thick sauce (table 3and 4)

2.2. Total Soluble Solid

Differences in the concentration of pumpkin and tomato substitution do not give significant effect on the value of Total Soluble Solid sauce (Table 4). Total Soluble Solid sauce values

ranged from 13.17 to 16.03% .

2.3. Colour

2.3.1. L (brightness)

According to Agustin , et al (2003) the value of L is a parameter that states reflected light that produces achromatic colors white , gray and black. Brightness parameter has a value of 0 (black) to 100 (white). Differences in pasta concentration treatments, providing a noticeable effect on the brightness of the sauce. L values ranged from 39.53 to 42.93 (Table 4). L value is highest in treatment A (100 % pumpkin pasta) because at this treatment, brighter color than the others who were added by tomato paste. Tomato pasta sauce contributes a darker color (red inclined) .

2.3.2. 'a'

A value in the color measurement system with Hunter colorimeter system states 'a' is a chromatic colors red and green mix, with a positive value from 0 to +100 for the color red and 'a' negative value from 0 to -100 for the green colour (Agustin, et al 2003). 'a' value on the sauce isn't a give noticeable difference due to the treatment (Table 4) . 'a' value ranging from 15.27 to 18.97. There is a minimum value at the treatment of 100 % pumpkin pasta (the colour is not a really red).

2.3.3. 'b'

'b' values expressed mixed chromatic colors blue and yellow , with a positive 'b' value of 0 to + 70 for yellow and negative 'b' values of 0 to -70 for blue. Difference in treatment provides significant effect on the value of 'b' ranged between 14.70 to 20.07 (Table 4) . B value was highest in treatment A (100 % pumpkin pasta). A treatment of this colour more yellow than others because there is no addition of tomato paste.

2.4. pH

pH measurements to determine the acidity or alkalinity of the food. pH values ranged from 4.3 to 4.5. The pH value decreases with

increasing of tomato paste given (Table 4). This is because tomatoes contain more acid than pumpkin. pH value is highest at 100 % treatment of pumpkin paste (4.533) while the lowest pH value (4.30) found in treatment D (highest tomato paste 60 %) . Pumpkin sauce pH value is in accordance with SII in Mulyanti (2004) which pH ranged of tomato sauce required by SII between 4.0-5.0. According Nurtama (1996) in addition to the pH measurement is one of the SII parameter in determining the quality sauce standards, often used as a parameter to see a durability food product , especially the acid-treated product .

3. Organoleptic analysis of sauce

Concentration of pumpkin and tomatoes give a real effect on a organoleptic analysis as shown on table 5.

Table 5 . The mean value of Organoleptic Test sauce

Treatment	Colour	Taste	Viscosity	Aroma	General of Preference
A	2,704 ^b	2,852 ^b	3,000 ^b	3,111 ^b	3,000 ^c
B	3,630 ^a	3,296 ^b	3,148 ^b	3,519 ^{ab}	3,333 ^{bc}
C	3,815 ^a	3,889 ^a	3,407 ^{ab}	3,630 ^a	3,889 ^a
D	3,741 ^a	3,222 ^b	3,704 ^a	3,593 ^a	3,667 ^{ab}

Note :

Assessment criteria

Colour, Taste, Viscosity, Aroma dan :
General level of Preference

- (1). Very dislike
- (2). Dislike
- (3). Enough
- (4). Like
- (5). Very like

3.1 . Colour

Differences of treatment gives significant effect on colour preference. A value of the sauce colour ranges from 2,704 (enough) to 3,815 (likes). A value of the colour of the sauce is highest in treatment C with a value of 3.815 (like). A value of the sauce colour is the least preferred at treatment A (pumpkin concentration 100 %) as a yellow orange sauce, so the panel is is not used commonly to consume.

3.2 . Taste

Differences in the concentration of pumpkin and tomato pasta gives significant effect on the taste preference. A taste value ranging from 2.852 (enough) to 3,889 (like). A highest taste preference value found in the C treatment with a value of 3,889 (like). The least favored sauce is a sauce with treatment A cause panelists still unfamiliar with the sauce on the A treatment .

3.3 . Viscosity

Differences in the concentration of pumpkin and tomato pasta gives significant effect on the viscosity value. A value of the viscosity of sauces ranging from 3.00 (enough) to 3,704 (like). A value of the viscosity is highest in treatment D with a value of 3.704 as panelists still accustomed to using tomato sauce as the base ingredients. Viscosity of the sauce with tomato paste concentrate most of the panelists preferred than the viscosity of the sauce with the biggest pumpkin pasta concentrations.

3.4 . Aroma

Differences in the concentration of pumpkin and tomato paste gives significant effect on the level of preference panelists in terms of aroma (Table 6) . A value of the aroma of sauces ranging from 3.11 (enough) to 3.63 (like). A value is highest in treatment C (concentration pumpkin pasta : tomato paste = 60:40 %) with a value 3.63 (like). Sauce with biggest pumpkin concentration least most panelists favored because panelists still unfamiliar with the aroma of pumpkin sauce.

3.5 . The general level of preference

A preference based on the overall level of parameters, there is a significant effect of differences as a treatment given. Panelists preference based on the overall level parameters values ranged from 3,000 (enough) to 3,889 (like) . In general, the panelists liked the sauce with treatment C with a value of 3,889 (like). Based on the parameters of colour, taste and aroma, treatment C occupies the

highest preference value. While based on the viscosity parameter, the value of treatment D is highest were not significantly different from treatment C.

CONCLUSION

The addition of pumpkin pasta influence on physical, chemical and organoleptic properties of pumpkin sauce. Pumpkin sauce was a panelist received in treatment C with the chemical composition of beta-carotene (228.0 ug/100 g), water content (77.77%), ash content (3.300%) : vitamin C (0.2567 mg / g); Total Acid Tritation (1.400%), TSS (13.97%) and crude fiber (1.3400%). While based on physical analysis includes viscosity 53.280 cps; L 40.93: a 17.80, b 16.20 and pH 4.310. Based on organoleptic analysis, treatment C occupies most preferred by panelist means the highest value in terms of both colour (value 3.815), taste (3.889), viscosity (3.407), aroma (3.630), and the general level of preference (3,889). In general, the panelists have received this pumpkin sauce well.

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An Alternatif Acoustic Wall from *Gaba-gaba*

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ABSTRACT

Sago is often identified with foodstuffs without knowing that the sago palm is also one of the materials that used in the house. The part that is often used as a building material that is the leaves as roofing, sago leaf midrib as the outer wall and the sliced stalks as floor plates. But such as with the notion of the sago as a food for poor, this material is often left wasted rather than used. Sago leaf midrib usually called *gaba-gaba* own anatomy and fibrous porous like a sponge. From initial observations, these materials can be used as a sound reducing materials. This research was carried out initially by making a 1:10 scale and testing it by using a sound level meter (SLM) to obtain acoustic test value. From testing three models, the results shows the highest difference occurred with interval 24,028 dB depends on how the panels treated. This results strengthen the hypothesis of *gaba-gaba* as one of economically material for acoustic wall and also proves that it can be implemented as architectural element.

Keywords

Gaba-gaba, acoustic wall, sustainable material.

INTRODUCTION

Sago palm (*Metroxylon* spp.) is a native plant from Indonesia. Sago's acreage in Indonesia is the world's largest sago acreage, which is about 1.128 million ha or 51.3% of the 2.201 million ha of world's sago area (Flach, 1997). Papua is one of the region in Indonesia which has a great potential of sago tree. In Indonesia, sago has a wide range of mention among others; rumbia, kirai (Sunda), ambulung or kersulu (Java), and lapia (Ambon). Unfortunately, sago is still often seen as a poor crop.



Figure 1. Sago plam trees
(Source: Yusfan, 2012)

Sago have many benefits. Sago's flour is usually used as a raw material in the manufacture of food or as a food ingredients. The flour is used for ordinary foodstuffs in Papua called *papeda*, also in addition for cakes and raw materials for alcohol manufacture. The leaves are used as the roof of the house, the midrib for the house wall, and the waste can be used as pulp for making paper or animal feed. In some cases of the traditional architecture in Indonesia, especially in the eastern part of Indonesia, its still founded some house that uses the roof from sago leaves and stems of sago as a wall. One of the tribes that still maintain this tradition is Alifuru tribe in Seram.



Figure 2. Traditional houses of Alifuru tribe from sago material in Selumena village (left) and in Binaiya mountain (right)
(Source: Wijayanto, 2013)

Basically, the use of sago leaf material oftenly founded on the type of roofing . Research involving sago leaf / thatch as roofing itself has proven lowering the temperature in the room by 2.75°C (Hanan and Sujarmanto, 2011) . On the walls of traditional houses, sago applied by utilizing the leaf midrib of sago which for most tribes in eastern Indonesia call it the *gaba-gaba*. The sheath has a posture similar to coconut leaf midrib . The difference identified from stem diameter and shape of the circle. When dried, its lighter than the coconut leaf midrib due to the porous material in the middle of the midrib.

Research on gaba-gaba has been carried out by Kongle (2009) and proved that gaba-gaba can be used as an acoustic material. On his research, gaba-gaba treated to identify the value of sound absorption coefficient of panels from gaba-gaba powder, gaba-gaba panel in parallel fibers, and gaba-gaba panel on transverse fibers.

On initial observations of the gaba-gaba, we saw an opportunity to be developed into an acoustic material that can be used to absorb noise. The existence of preliminary research conducted by Kongle (2009), will be strengthened by this research by developed it into architectural form. Therefore, through this study is expected to answer any such hypothesis by implementing the use of gaba-gaba architecturally as a wall on 1:10 scale model of the building and test it using a sound level meter.

In order to make this research more effective, efficient, directional and can be studied more in depth then it is necessary to limit the problem. The main concern of this study is testing the sound absorption panels of gaba-gaba which has been shaped into the house. The research itself strengthen previous research that has been done by Kongle (2009). Object of this research itself is limited to a simple model house type 21 and modeled into 1:10 scale due to its easier to applied on the actual size (for further research). Therefore, based on the background and the identification above, the general formulation of this study are; how does the potency of absorption of gaba-gaba which formed into simple model house type 21 with scale model 1:10?.

Literature Review

2.1 . Overview about acoustics and sound

Acoustics is the study of matters relating to sound, with respect to the sense of hearing as well as the room condition that affects the sound (Gabriel, 2001). Propagation of the sound waves to the object will be reflected, absorbed, or diffused, whose characteristics depend on the characteristics of the object. Propagation of sound waves on the boundary with a gap will experience diffraction (Mediastika, 2005). This is what happens to the sound in the room with the hole. According to Doelle (1985), acoustic materials and sound absorbing construction that usually used as a controller in a noisy spaces can be classified into; porous materials, absorbent panels, resonator cavity, absorbent space, and absorption by the air.

Selection of the proper sound absorbing material required to produce a very satisfactory sound quality. Doelle (1990) explains that one of the sound absorbent material that always used in acoustics design as a wall hanging or as a ceiling is the porous material. The baseline characteristics of all porous materials like these

are changing the sound energy into heat energy comes in the pores and absorbed, while the rest which have less energy reflected by the surface of the material. Weakening of sound that produced by a porous material depends on the flow resistance and the porosity (Attenborough in Kongle, 2009). Meanwhile, Veronina (in Kongle, 2009) identified that the pore diameter is very influential in determining the transmissibility of sound in the material.

2.2. Overview of gaba-gaba as acoustic material

Sago is the world's oldest staple food in the world (Ave in Flach, 1997). Sago palms are often found in peat area. In Indonesia, sago palms are often found in the Riau Islands, Sulawesi, Ambon to Papua. Stanton (in Flach, 1997) mentions some of the advantages of planting corn, such as: 1) economic, 2) sustainable, 3) good for the environment, 4) have a variety of uses, 5) strong, and 6) stabilize agroforestry system.

In addition as foodstuff, some parts of the sago plant can be used as building material. Some tribes in Indonesia still uses material from sago palm such as its leaf and stem.



Figure 3. An example of how gaba-gaba can be used as interior in a school in South Seram (left) and the sago leaves as roof covering (Source: Almascatie, 2007)

Gaba-gaba as raw material for acoustic panels manufacturing have to be considered due to its abundance and until now it still better discarded than used. Results of research conducted by Kongle (2009) have proved that

gaba-gaba can be used as a sound absorber panel material. In his research, gaba-gaba treated as panels from gaba-gaba powder, gaba-gaba panel with parallel fibers and gaba-gaba panel with transverse fiber . These panels are then tested using the impedance tube method consists of PVC Pipe (diameter $D = 4$ inch) with a length of 4 m, 1 microphone, preamplifire , audio function generator (AFG), oscilloscope, and a computer with sound forge 6.0 software to analyze sound signals spectrum that detected by a microphone. Research material consists of: gaba-gaba, sago glue, and pipe glue. From the results, it was concluded among other things:

- Lowest absorption coefficient is 0.83 on transverse fibers panel with thickness of 3 cm and the highest on the panel with sago with thickness of 3 cm and the reflection coefficient from 0.016 to 0.17 and the value of the transmission coefficient between 0.000001 to 0,018631.
- Treatment of gaba-gaba as a panel are highly influential for determining coefficient of acoustic. Absorption coefficient was highest at gaba-gaba panels with pipe glue and the smallest on the panel with parallel fiber sheath.
- Acoustic coefficient depends on the thickness of the panel where the panel thicker will higher the coefficient of acoustic.
- The frequency of the sound of acoustic influence coefficients.

Table 1. Comparison of the absorption coefficient between Gaba-gaba panels with parallel and transverse fibers

Sound Intensity (dB)	Absorption coefficient of gaba-gaba panel	
	Parallel fibers	Transverse fibers
125	0,858793	0,888100
250	0,904848	0,937355
500	0,882274	0,872430
1000	0,945576	0,934931

(source: Kongle, 2009)



Figure 4. Gaba-gaba sliced vertically and horizontally
(Source: Yusfan, 2013)

To complement the research that has been done before, so in this research the treatment of gaba-gaba be simulated in house form so that in addition to strengthening the hypothesis, also will be obtained one method of gaba-gaba composition that most appropriate when applied as an acoustic material in the house form

METHODS

Gaba-gaba which had dried initially will peeled to get the middle part. Then its cut to obtain a similar diameter with a thickness of 1 cm. This thick representing the actual models (10 cm) that can be conditioned as insulation element (not a stand-alone wall). Gaba-gaba edges then arranged vertically so that it becomes a wall .

Mockups was designed using size 52.5 cm x 40 cm as a representation of the type 21 (5.25 x 4 m) and made square without any space in it. In this research, these models will be conditioned into 3 models. Early stage mockups (model 1) was established by using a full wall material of gaba-gaba, roofs of alang-alang as a representation of rumbia roof and the floor and ceiling from plasterboard. This model is then tested using a sound level meter to get the acoustic test model 1 (ATR 1). On model 2 mockups, the treatment differentiated with plasterboard wall on the inside and outside of the model. This model was tested to obtain the acoustic test results of model 2 (ATR 2). On model 3 mockups, similar to the previous

model but with some addition of residential openings such as windows and door. This model was tested to obtain the results of the acoustic test model 3 (ATR 3).

Each test to get ATR follows such a technical methods that is:

1. Measuring the intensity of the sound that occurs during daylight hours on the highway as a representation of most noisy sound intensity. This measurement was conducted on July 24, 2013 at the side of MT Haryono, Malang road at 10:10 am. Furthermore, it was simulated using a handheld mobile as representation of interval highway noise when crowded.
2. Measuring the intensity of sound that occurs in the maket models I, II and III to obtain the average intensity of sounds that occur in the mockups. In each model was performed 50 times test to get an average sound. Results of measurements of each model are recorded and then divided so as to get a valid average noise figure.

The tools used in this study are:

- Mockup models,
- Sound level meter Lutron SL 4010 ,
- Equipment noise source (samsung GT 5510)

RESULTS

This research ran for four months. Description of research activities described consecutively of following activities:

• Testing Model I

Model I is an initial model in this research. This model is made from gaba-gaba which cutted paralely 1 cm as a representation of wall thickness of 10 cm. Its cutted paralely because once done by transverse, it was not strong enough to be arranged into panels. Moreover, it uses more material if the panel arranged transversely. Parallel piece is easier to construct and more able to resist lateral and

vertical loads. All of these pieces then glued using adhesive glue.



Figure 5. Gaba-gaba's slice and wall panel from it

(Source: Yusfan, 2013)

Part of the panel that has been glued formed into square model 50 x 30 cm and 42 x 30 cm each two pieces. These sheets are then put together to get a wall model.



Figure 6. Testing model I

(Source: Yusfan, 2013)

On the top of this model was closed with gaba-gaba panel with a thickness of 1 cm to represent acoustic ceiling, while the bottom of the model was closed by using fiber cement board as a representation of the cement floor.

Model I testing session conducted at the Science Laboratory, Department of Architecture UB with initially stimulating sound condition on the road when crowded. Measurements were carried out at the edge of highway to get real noise conditions that occurs at the peak. From the measurement results, obtained an average noise by 74 dB. The first measurement was conducted on July 24, 2013 using a sound source (highway representation) with 74 dB noise and two Sound Level Meter

(SLM). One speaker of SLM (SLM 1) inserted into the mockups and the other (SLM 2) are outside the model to measure the noise level of the sound source. From 50 times testing of Model I, obtained interval 19.89 dB between the SLM 1 and SLM 2.

• **Testing Model II**

Model II is a development model of model I by adding a fiber cement sheet on some parts of the model I. The addition was done on the outside wall of the model I to represent the real cement wall and the inside to represent ceiling. This attachment glued using PVAc wood glue and mixed with calcium powder.

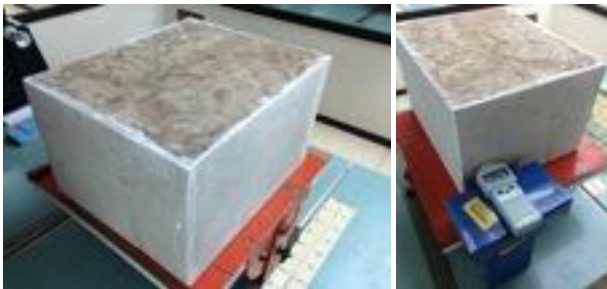


Figure 7. Testing model II
(Source: Yusfan, 2013)

Almost similar to the test performed on the model I, that is by calculating the interval between the SLM 1 and SLM 2. From the test results to the model II, obtained intervals of 26.768 dB.

• **Testing Model III**

This model is a model development of model II with by adding a door and window openings. Moreover, the addition of a thatched roof made to get the model oh house. From the test results to the model III, obtained intervals of 24.026 dB between SLM 1 and SLM 2.



Figure 8. Testing model III
(Source: Yusfan, 2013)

• **Comparison Testing Results Model I, II and III**

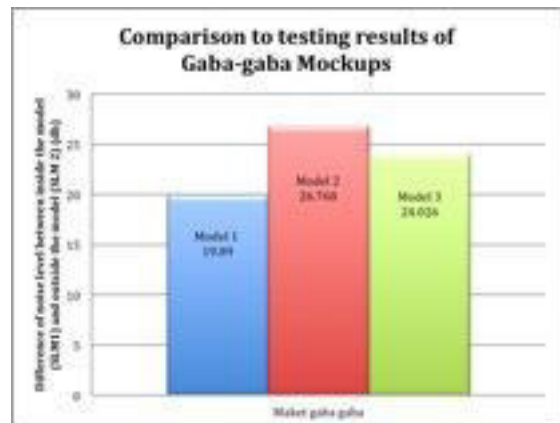


Figure 9. Comparison testing results of model I, II, and III
(Source: Yusfan, 2013)

From the test results of the three models showed that the highest interval obtained on the model II.

CONCLUSION

Conclusions from the results of tests performed on the three models shows that:

1. Test results compared by sound level meter (SLM) to model I (gaba-gaba cube), model II (gaba-gaba cube coated with plasterboard) and model III (model house type 21) shows differentiation in sound intensity. The highest difference occurred in model II with interval 24.028 dB. Treatment to the mockup determines the amount of sound absorption.

2. The panel types which the best to use in this research is made up of gaba-gaba sliced parallelly and then glued. This method is better due to in addition to the intensity of the absorbed sound quite well, also it could strengthen the structure. The recommended way is formed gaba-gaba into hexagonal pieces.

As for suggestions that can be used as basis for developing the research, such as :

1. Need for continuing this research implemented on the model 1:1. It will greatly affect the actual sound absorption.
2. Need for further research on how to preserve gaba-gaba panel so that not easily damaged by bugs and weather.

ACKNOWLEDGMENT

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Urban Green Open Space Conservation of Ijen Sportpark Area in Malang City by Involve User and Ecological Consumption Variables

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ABSTRACT

The sport park (*sportparkbedrijf*) of Ijen area in Malang city was designed socially-ecologically by garden city concept in Dutch era, as a fusion between urban park, Indis (Indonesian-dutch) luxury housing dan kampungs. The Ijen area was covered in the 5th phase of urban masterplan, and then reach the highest successful for Dutch colonial urban design. This successful made Malang city model sent to international urban exhibition in Paris in 1937. The concern of ecological balance of Ijen area appear in using city scenes, urban green open area for social activities, using trees to represent the greeny Indis town. The highness urban-architectural values of Ijen area, make it as the urban conservation area. Futhermore, the sport park of Ijen area change as commercial center is called Malang Olympic Garden (MOG) in 2005. Demolition of urban green open area of the sport park had an effect of ecological. This area usually have flood, increase urban heat island also air pollution from vehicle raising. From theoritical point of view, the understanding of urban green open space have still based on differentiation between mass and space. Meanwhile, the mass of building have potential to increase urban green open space by vertical or horizontal development. In the fact, increasing people of town also influence toward ecological consumption. So, in effort to conserve urban green open area need to involve amount of user (people, vehicles) also ecological consumption (oxygen, site and vegetation quality) as research variables. The purpose of this research is how to conserve urban green open space of Ijen sportpark area toward amount of user and ecological consumption. This research method is descriptive quantitative into two phases. The first is observation of urban green open space theory based on user and ecological consumption. The second phase is calculate green open area requirement.

Key words : conservation, ecological consumption, urban green open space

INTRODUCTION

Ijen sports park area (*sportparkbedrijf*) of Malang city was designed integrated ecologically-socially since the Dutch between city parks, elite settlements and

township residents with the concept of garden city as stipulated in the city development plan (*bouwplan*) phase V. Areas design considered the pinnacle of success designing the city at that time, so the city of Malang became a model urban design of in-

ternational urban exhibition in paris 1937. Attention to the ecological balance appears to involve vista views of the city, the provision of urban green areas for social activities as well as subtle gradations between township-modern settlements, as well as the use of large trees as features Indis verdant city (Handinoto, 1996). Architectural value, knowledge and high culture, make the area designated as a town conservation.

During its development, Ijen sport park changed its function as a shopping center *Malang Olympic Garden* (MOG) in 2005. Disappearance open area became a commercial affects on ecological function, such as frequent flooding, increasing the urban heat island and pollution of vehicles. Ecological damage to Ijen sports park area demonstrated by a study that MOG development has transformed the area of green space by 19%, and decrease the infiltration of water (ground water quantity reduction) of 96327 m³ to 18417 m³. Reducing its power of absorbing water cause rise runoff volume of lower area in kelurahan Bareng subdistrict Klojen from 70907 m³ to 148818 m³ (Maulidi, *et al*, 2013).

The loss of green open space need to be controlled in order to create a comfortable urban space ecologically. Meanwhile, from the theoretical side, the definition of green space still tend to presumption of open space as the rest of the building space or separation between building mass (solid) and outer space (void). If further review, the building also has potential as an addition to green space, either through the development of horizontally or vertically in line with the vast green space is limited due to the increasing population of the city and its activities. Therefore, the determination of green open space also need to involve a variable number of users (visitors and vehicle movements) and variable ecological consumption (consumption of oxygen and quality of site and plants).

1.1. Overview of The Ijen Sports Park Area In Malang City

Ijen sports park area was designed by Thomas Karsten through the development plan of the city in 1924/1925, which is intended to meet the expansion of housing, especially for the European group. Construction of the park including the largest in its time, consisting of the stadium (hereinafter known as the stadium *Gajayana*), field hockey, 2 soccer fields, nine tennis courts along with a club house and swimming pool (*zwembad*). Important matter in the design of the area is the attention of Karsten toward vegetation greening road with mahogany trees to the rhythm increasing visuality and the orientation of the road. Greening the road is an important part in the development of the tropical city of the Dutch East Indies, characterized by a green to obtain the best scenery and reflect the beauty of the city. Application of the concept in the design of the road greening Malang officially reported by Karsten in *Locale Techniek magazine*. (Handinoto, 1996)

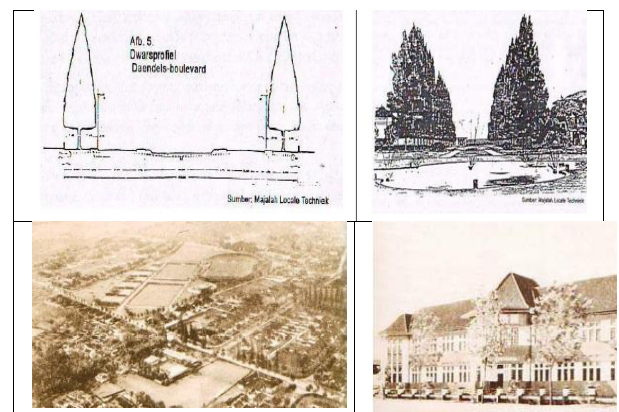


Figure 1. Karsten studies on the use of vegetation in the city of Malang and its application in the sports park with the use of mahogany trees. Source : Handinoto (1996).

In the era of 1980 to 1997 before financial crisis, economic development occurs simultaneously with the physical development on a large scale in the city of Malang included of Ijen area . Downtown area has been dominated by trade sectors and a Central Business District (CBD). This development further influence on the morphology of

cities around the region, both the change of housing functions to the function of trade, as well as the level of residential development that has spread fragmented and penetrated the suburbs. In 2005, the exploitation of the open spaces in the sports park in the form of construction of shopping centers and hotels around the stadium Gajayana known as *Ma Olympic Garden* (MOG).

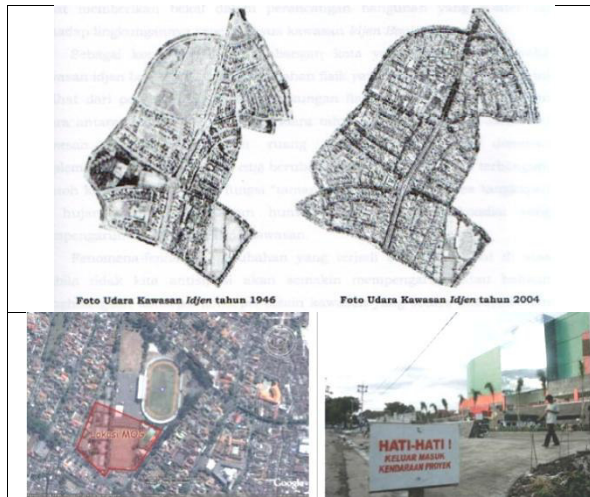


Figure 2. Comparison of morphological changes Ijen Sports Park area of Malang in 1946 and 2004-2005. Source : Wikantiyoso (2005).

1.2. Relationship Between Green Open Space and Oxygen Requirements.

Relating to the use of the term of space still implies a sense of difference and contradiction. Conventionally, green open space measurements are based on a comparison between the land area of the open (non-mass building) toward the plot area. According Madanipour (1996) concept of this kind of thinking is based on the theory of absolute space by Issac Newton who see space as a separation between the mass of the building as a container (solid) and open space, including green space as empty space (void). In the science of urban design, this kind of thinking emerge to the term *Building Coverage* (BC) and *Floor Area Ratio* (FAR).

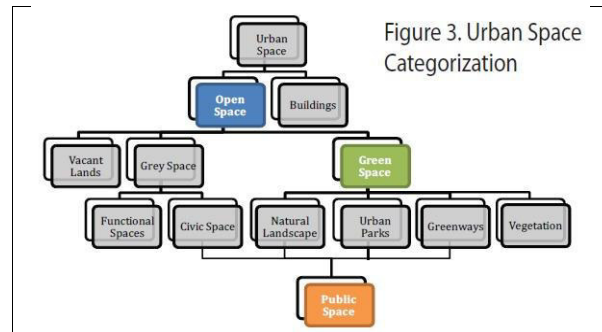


Figure 3. Conventional urban space categorization. Source : Kim, Won Kyung and Wentz, Elizabeth A (2013)

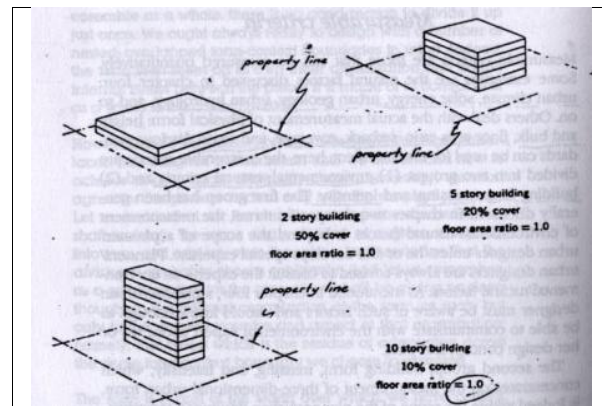


Figure 4 An understanding of mass and outer space as a solid-void. Source : Shirvani (1985)

Meanwhile, the trend of today's world city pay great attention to green space. This concern is not only for the purpose of preservation needs of the urban environment, but also as a way to increase the attractiveness of town. Many cities are now experimenting with innovative ways of bringing urban greenery into the city, and nowhere more so than in emerging ecocities. Innovation in urban green spaces are appeared on vertical, multilevel and roof garden structures and make contemporary urban form, with there are no clear distinct between urban mass and green open space. Thinking in the abstract concept of space is based on the theory as a critique of the notion relationist absolute space. Seeing space as a uniformly extended material that can be modelled in different ways as a main feature of the modernist city is naively realistic. It means dilemma between mass and void, between empirical and conceptual, between real and abstract, which can be understood immediately by the senses, and mental space,

which need to be interpreted intellectually. From this point of view, it sees that the experience of architecture and its spatial effects depends on significant details and argues that the reduction of the effects to space is a misrepresentation of the entire nature of our experience.



Figure 5. Understanding of the mass and outer space as something between solid-void abstract. Source : Husqvarna group (2012).

On the concept of the contemporary space, the determination of the extent of green space should be based on issues important to include the human element as a number of users, both in relation to the vehicle trip and ecological consumption, such as the need for oxygen. Green open space formulas related issue is the oxygen consumption (Direktorat Jenderal Penataan Ruang, 2006) are:

$$L = \frac{a.V + b.W}{20}$$

Description:

L : city green space area (m²)

A : Oxygen demand per person (kg/hour)

b : Average oxygen demand per vehicle (kg/hr).

V : number of residents /visitors

W: number of vehicles of various types

20: constant (kg /hour /ha).

Standard oxygen demand per day for each person is equal in the amount of 0.864 kg / day. At the oxygen demand for motor vehicles amounted to 58.15 kg / day for private cars vehicles, 228 kg / day for buses, 114.40 kg / day for vehicle load, and 2.90 kg / day for a motorcycle.

Malang Olympic Garden (MOG) is a area consisting of shopping centers, swimming pool and stadium. MOG presence would cause a considerable pull movement, through the movement of private vehicles and public transport. Study of the planning of the parking area with a survey method of MOG traffic counting, matching plate, and parking patrol by Rizal, *et al* (2008) showed that the duration of the parking plaza is relatively shorter than the stadium and swimming pool. As for the results of the study show that the model in the parking area requirements in MOG is shown in **table 1.** :

Table 1. The Amount of The Parking Area requirements on MOG

Name of Space	Size of The Area	Proportionality to the Number of Vehicles
Plaza area MOG		
The area of motorcycle parking	5104 m ²	3.403
The area of car parking	10244 m ²	931
Gajayana stadium area		
The area of motorcycle parking	3434 m ²	2.289
The area of car parking	8780 m ²	798
MOG swimming pool area		
The area of motorcycle parking	45 m ²	30
The area of car parking	469 m ²	43
Total number of motorcycles		5.722 units
Total number of cars		1.722 units
Note:		
<ul style="list-style-type: none"> • Assumption 1 module wide bike parking: 0,75 m x 2 m = 1.5 m² • Assumption 1 module wide car park: 2 m x 5.5 m = 11 m² 		

Related to the study of the movement of people in the pull model of MOG by Mendrofa (2007) showed that the results of the number of visitors plaza in MOG pull as much as 3636 people /hour and the number of visitors stadium on MOG pull as many as 2564 people /hour, and the visitors pool is 560 people / hour. Based on these data, the

total number of visitors to the area MOG is 6760 people / hour or **162 240 people / day**.

1.3. Overview of Green Coverage

Calculation of green coverage is intended to measure the greening of an open space. Conventionally, the measurement is based on the green coverage ratio between the area of open land for planting and or water infiltration to the area owned. according Madanipour (1996) thought that sees space as a separation between the mass of the building as container (solid) and open space, including green space as empty space (void) is something naive. Calculation of the contemporary green coverage also requires new definition and approach to understand urban open space by measuring the greenness of urban open spaces. It is based on the premise that the different physical qualities of open spaces (example : humid and arid cities, the kind of vegetation) do not have same affect on spaciality and production of oxygen of cities. In addition there is no standard definition of urban open space that is influenced by different physical qualities, and it is often evaluated with different parameters. In the formula, the calculation of the green coverage is:

$\text{Green Coverage} = \text{vegetation area} \times \text{green index}$
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In general, to find the green index is to use the formula *Leaf Area Index (LAI)*. *Leaf Area Index (LAI)*, is defined as the total one-sided area of photosynthetic tissue per unit ground surface area (m^2/m^2) is an important parameter in climate studies and is a key parameter that affects the surface fluxes of energy, mass, and momentum over vegetated lands. The LAI measurement techniques are not yet standard techniques. Commonly researchers use remote sensing measurements of the radiation levels of vegetation or hemispherical photography spectrum. Although relatively accurate, but this way is quite complicated.

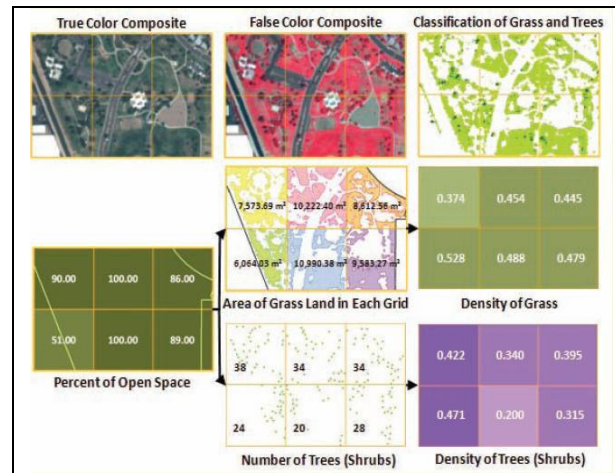


Figure 6. Example of the determination of the green index by using the green Leaf Area Index (LAI) calculation based on the color spectrum of hemispherical photography. Source : Kim, Won Kyung and Wentz, Elizabeth A (2013)

In this study, the determination of estimates of the green index is done by counting green shade area of vegetation (tree / shrub / grass) based on aerial photographs, with attention to the condition of the land to obtain objectivity characteristic of spatial quality. Byrne and Sipe (2010) argue that making the task of classifying various urban green spaces based on issue of scale is virtually impossible and rather pointless. Three criteria stand out as most useful – size, physical condition, and naturalness. These criteria could be used to develop a simple classifying. physical condition, and naturalness. These criteria could be used to develop a simple classifying.

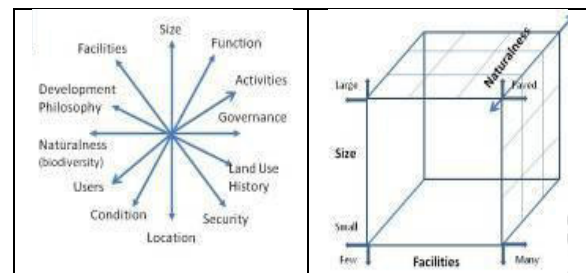


Figure 7. The important aspect of green open space quality. Source : Byrne and Sipe (2010)

According Kurniawaty, et al (2010), the components that affect the quality of green space is shown in **Table 2.** :

Table 2. The Important Aspect of Green Open Space Quality

Aspects Of Quality Green Open Space	Description
Site component	
Intensity of land cover	As an instrument to control the density of buildings and open green spaces. This is the goal for the region compatibility harmonious and ecologically.
Types of Plants	Related to ecological strata plants that can help ease climate amelioration and grow at the desired location.
Density plant canopy	Percentage of sunshine which is retained by the tree canopy. Another benefit is the high density of canopy interception of rain water to prevent landslides.
Distance of trees	Has a range of plants in controlling temperature fluctuations applied to the spacing of the buildings still can create shade without blocking the flow air and follows the security of the building and the plant itself.
Pavement component	
Pavement	The influence of land surface type closure to the formation of local microclimate and water infiltration and surface runoff.

METHOD

Stages of analysis, methods of analysis and data collection methods, including:

1. The first phase, *Analysis of green open space area requirements of MOG*

- a. Methods of analysis:
The area needs the calculation according to the amount of green space in MOG based on Movement People, Vehicles and Oxygen Consumption.
- b. Methods of data collection:
Projection models pull movement of people and vehicles in the MOG area based on previous research by Mendrofa (2007) and Rizal (2008).

2. The second phase, *Analysis Green Coverage in the area MOG*

- a. Methods of analysis:
Calculate Green coverage in the area MOG based weighting site characteristics.
- b. Methods of data collection:
Zoning division toward site of MOG area.

RESULT

ANALYSIS GREEN OPEN SPACE REQUIREMENT IN THE MOG

Based on the calculation formula of green open space which is based issues including the town of oxygen consumption of oxygen released by plants accumulated by Dahlan (1992), the calculation of green open space needs in MOG is:

$$L = \frac{a.V + b.W}{20}$$

$$L = \frac{(0,864 \times 162240) + (58,15 \times 1.722) + (2,90 \times 5.722) \times 24}{20}$$

$$L = 308.280 \text{ m}^2.$$

ANALYSIS OF GREEN COVERAGE IN MOG AREA

Based on the results of site measurement of MOG area showed that the region has an area of 356 977 m², with Building Coverage (BC) area of 237 641 m² (34%) and the open space area is 224 327 m² (66%). To facilitate analysis of the proportion of green coverage, then split zoning of the site based on aerial photographs the area, with the details shown in **Fig. 7**.

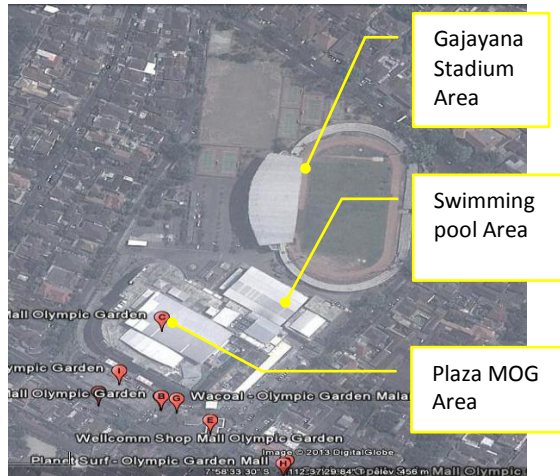


Figure 7. The site based on aerial photographs the area. Source : google image (2013)

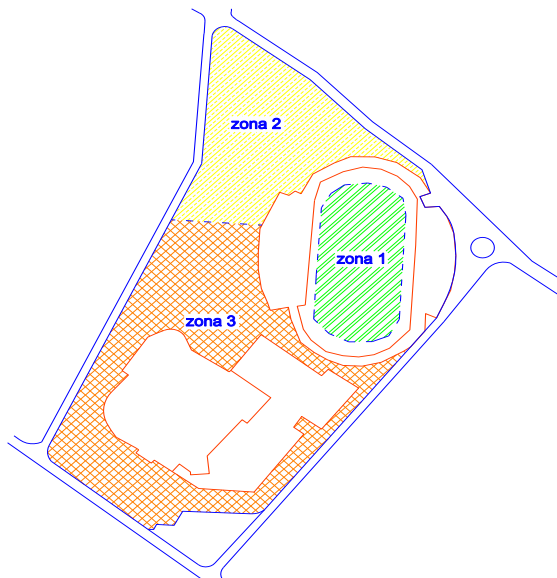


Figure 8. Zoning of the site based on aerial photographs the area.

The operational definition of weight measurement of green coverage (green index) is shown in **table 3**:

Table 3. The Operational Definition of Weight Measurement of Green Index

Green index aspects	Operational Definition		
	Appropriate	Less appropriate	Not Appropriate
Site Components			
Intensity of land cover	□ 66 % site covered by vegetation	66% ≤ x ≤ 33 % site covered by vegetation	□ 33% site covered by vegetation

Types of Plants	Site is dominated by trees, shrubs or grasses cultivated throughout the year	site is dominated by shrubs or grasses cultivated seasonally	Site is dominated by wild vegetation
Density plant canopy	Site is dominated by a dense leafy plants	Site is dominated by loose branchy plant / columnar	Site is dominated by deciduous plant
Distance of tree	Distance between plants / trees do not overlap at optimum height and does not harm the surrounding	Some of distance between plants / trees overlap at optimum height and harm the surrounding	Many of distance between plants / trees overlap at optimum height and harm the surrounding
Pavement component			
Pavement	Predominantly pavement integrated with ground cover	Dominant pavement only allows absorption of water into the soil	Dominant pavement do not allows absorption of water into the soil

The results of measurements green index in the MOG area can be seen in **table 4**:

Table 4. Measurements Green Index in The MOG Area

Green Index Aspects	Green Index Calculation of Land				Amount
	Weight value	Appropriate (N=1)	Less appropriate (N=0.5)	Not Appropriate (N=0)	
Zone 1	Green area= 39694 m ²				

	Coverage open space zone 1 = 39694 m ²				
Intensity of land cover	0,33	1	-	-	0,33
Types of Plants	0,13	1	-	-	0,13
Density plant canopy	0,20	1	-	-	0,20
Distance of tree	0,07	1	-	-	0,07
Pavement	0,27	1	-	-	0,27
Total weight of Green Index zone 1					1,00
Zone 2	Green area = 58006 m ² Coverage open space zone 2 = 70.401 m ²				
Intensity of land cover	0,33	1	-	-	0,33
Types of Plants	0,13	1	-	-	0,13
Density plant canopy	0,20	1	-	-	0,20
Distance of tree	0,07	1	-	-	0,07
Pavement	0,27	-	0,5	-	0,14
Total weight of Green Index zone 2					0,87
Zone 3	Green area =18.876 m ² Coverage open space zone 3 = 114.232 m ²				
Intensity of land cover	0,33	-	-	0	0
Types of Plants	0,13	-	0,5	-	0,07
Density plant canopy	0,20	-	0,5	-	0,10
Distance of tree	0,07	1	-	-	0,07
Pavement	0,27	-	0,5	-	0,14
Total weight of Green Index zone 3					0,38

Based on the calculation of the weight of the quality of green index of sports park site, then the calculation of the real Green Coverage requirement of the existing site is:

Green Coverage(GC) = vegetation area x green index

$$GC = (39694 \times 1) + (58006 \times 0,87) + (18876 \times 0,38)$$

$$GC = 97332 \text{ m}^2$$

With the value of Green Coverage (GC) is 97 332 m², and the calculation is based on the oxygen consumption of green space requirement of 308 280 m², **the area of**

green space should be provided on the site is 210 948 m².

CONCLUSION

Fulfillment in the area of green space in a simple MOG is by replacing lines and parking spaces by paving stone with grass-block. Advantage of grassblock is having space for grass root growth and sufficient capacity to absorb the flow of surface water, as well as able to bear the burden of heavy traffic. area of open space which can be replaced by grassblock amounted area open space zone 3 which is intended for the circulation and parking in the amount of 114 232 m². Lack of green space can be partially replaced by replanting trees greening road that has been lost along the 919 meters. As planting rules refer to the documents the formation of the area within 10 meters between trees. Thus approximately 90 replacement trees can be planted. If we assume that the diameter of the tree crown width of 4 m, then each tree will provide green space area of 12.5 m². This means that replacement trees will create a green space area of 1125m². Lack of green space remaining amount of 95 591 m², must be applied to the building, whether it be roof garden, inner and vertical garden.



An illustration supplying green open space as roof and vertical garden of building.

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PLANNING OF GREEN SPACE AS EFFORT TO CONTROL AIR POLLUTION (A Case Study at Sidoarjo Regency)

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ABSTRACT

The rapid physical development in certain area will influence the ecosystem balance, the development will give positive and negative impacts to the environment, the real impact with the presence of the development is the improvement of social economic of the society, at the other side the change of open space become constructed space cause the higher drainage coefficient that cause the flood. For the matter then it needs development control, such as with the Act No 2007 about Spatial Zoning, where in a city it should contains 30% Green space. The ecological benefits of the green space not only as recreation means and for the comfort feeling, but also as the control for the air pollution, either as natural barrier or as the absorber of CO₂, NO₂, and SO₂. The Sidoarjo regency consist of 18 districts, 322 villages, 31 sub districts, with the private vehicles amount higher than the public transportation. From the calculation results of Green space based on the absorption level of CO₂ emission, it obtains the forest need area to absorb the CO₂ of 0.88 ha, and the grass need of 15.78 ha and for the wet rice field need of 4.33 ha. And the need of RTH to decrease the noise of 65 ha. The suggested green space can be in the form of urban forest in cluser or in path.

Keywords: Barrier, CO₂, Emission, RTH, Noise.

INTRODUCTION

The rapid physical development in certain area will influence the ecosystem balance, the development will give positive and negative impacts to the environment, the real impact with the presence of the development is the improvement of social economic of the society, at the other side the change of open space become constructed space cause the higher drainage coefficient that cause the flood. For the matter then it needs development control, such as with the Act No 2007 about Spatial Zoning.

Suitable with the mandate of Act NO 26 Year 2007 about Spatial Zoning, in a city it should contains 30% Green space. The Green space is aimed as one of instruments to conserve the sustainable urban environment ecologically. The Green space (RTH) all at once as public space that has recreation benefits and comfort feeling because of its aesthetic factors.

The main air pollutants caused by the motorized vehicles that increase rapidly each year. The air pollution contribution that come from the transportation reached 60%, the other from industry of 25% and waste of 5%. The high contribution of the air pollution from the transportation sector produce problems in maintaining the criteria of the air pollution. The imperfect burning process of fuel in the motorized vehicles produced

pollutant such as CO₂, Sox, NO_x, HC, particulate and Pb.

All noise sources basically have potentials to be noise sources. The noise means as unwanted sounds, disturbing, or endanger the health. The noise sources often be met comprised on: traffic noises (passenger car, truck, motorcycle, train, planes, and etc), industrial noises (factory machines), natural noises (explosion of volcano, thunder, and etc) and household noises (tape, radio, mixer, and etc).

The traffic noise today is the dominant noise. Societies are more exposed to the noise of motorized vehicles, either big vehicles, or motorcycles or other sources. Effort to control noise can with control at the noise source, control at the noise propagation, control at the noise receiver, and Barrier.

Based on the ecological considerations and the sustainable spatial use then, at Sidoarjo regency it needs green space that will guarantee its existence as part of physical environment of the city and the people, give positive impact to the city environments.

Efforts that is done to zone the Green space at the Sidoarjo regency in this year it is done the Green space planning. The zoning plan of Green space will allocate protected and defended space for the Green space suitable with the needs that based on the RTH needs based on ecological functions, the population amount, and aesthetic.

The results of the plan in general is directed to anticipate the urban area environment so it reach the functional comfort of the city and also embody the mandate of the Act No 26 Year 2007 about the spatial zoning.

The ecological benefit of the green space not only as recreation means and comfort feeling, but also can be used as the controller of the air pollution, either as the natural barrier or as the CO₂, NO₂, and SO₂ absorber as part of the impact of the city development.

Research Objectives

The objectives of the green space of Sidoarjo regency is to determine the needed green space to control the air pollution especially to muffle the noise and as the CO₂ absorber. So the availability of the harmonious and balanced space between built area and the green space, will guarantee the environmentally friendly and sustainable space use and the secure, comfort, fresh, beautiful, and clean environment.

MATERIAL & METHOD

Sidoarjo regency is regency that wedged in two rivers, Porong river and Surabaya river, so known as Delta city. The administrative area of Sidoarjo regency consist of the land and sea area. The land area of 714.245 Km² and the sea area based on GIS calculation up to 4 miles to the sea of 201.6868 Km².

Administratively, Sidoarjo regency with boundaries as follows:

- North side: Surabaya and Gresik regency
- East side: Madura strait
- South side: Pasuruan regency
- West side: Mojokerto regency

The Sidoarjo regency consist of 18 districts, 322 villages, 31 sub districts.

The spatial pattern of the planning area that is dominated by settlement, the presence of identified existing RTH still in the form of area. For RTH of road corridor and private RTH in existing still not identified in detail. Below is the spatial pattern table at the planning area, look at **table 1**.

Table 1. The Planning Area

No	The Planning Area	Area (Ha)
1	Housing	7.230,96
2	Rice-field	6.333.160
3	Industry	901.270
4	Field	581,01
5	Garden	547.850
6	Fasilitas Umum	248.450
7	RTH	
	- Garden	103,12
	- Field	30.250
	- Grave	27,65
8	Land	332,04
9	Marsh	1.717.240
10	Road	1.213
11	River	1.363
Total Area		20.665

Source : Hasil Survey dan Perhitungan Cad Tahun 2010

Table 2. Total Motorized Which Test in 2008

No	Bulan	Kategori	MPU	Bus	Pick Up	Truck	Truck Tandem	Kendaraan lain	Kereta Gandeng	Kereta Tempelan	Jumlah
1	Januari	Umum	148	330	0	883	49	7	22	1	653
		Tidak Umum	0	35	1.206	561	30	2	29	1	1067.204
2	Februari	Umum	49	349	0	333	68	3	20	11	633
		Tidak Umum	0	39	1.539	206	36	1	11	1	795.539
3	Maret	Umum	208	346	0	222	84	1	26	6	285
		Tidak Umum	0	33	1.461	282	42	7	9	5	679.451
4	April	Umum	208	300	0	338	87	3	17	8	644
		Tidak Umum	0	33	1.644	667	43	2	14	4	964.644
5	Maji	Umum	184	367	0	351	71	2	18	8	601
		Tidak Umum	0	33	1.486	693	57	5	13	7	999.696
6	Juni	Umum	112	253	0	221	66	2	9	2	565
		Tidak Umum	0	36	1.254	684	21	0	11	5	698.254
7	Juli	Umum	132	336	0	274	85	4	20	1	752
		Tidak Umum	0	35	1.629	669	31	7	14	3	984.629
8	Agustus	Umum	113	344	0	352	28	5	12	7	207
		Tidak Umum	0	36	1.583	222	26	7	17	6	695.583
9	September	Umum	129	343	0	318	53	4	9	6	652
		Tidak Umum	5	39	1.576	685	44	5	7	4	990.576
10	Oktober	Umum	83	300	0	347	75	4	21	3	563
		Tidak Umum	0	40	1.466	275	28	2	14	5	625.466
11	November	Umum	176	332	0	349	75	1	22	8	763
		Tidak Umum	0	31	1.368	789	29	5	11	5	631.368
12	Desember	Umum	96	252	0	227	50	2	11	1	539
		Tidak Umum	0	40	1.304	662	22	0	10	2	737.304
Jumlah			1592	4412	17.536	11.801	1291	61	367	110	10314.93

Sumber : BPS Kab. Sidoarjo

Research framework:

Research frameworks of planning of green space as effort to control air pollution shown in Fig. 1 below :

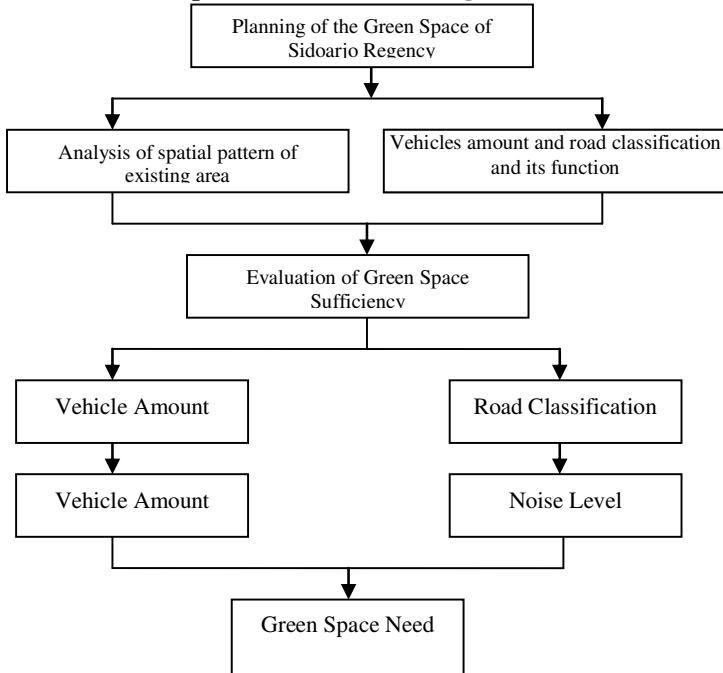


Figure 1. Flow Chart Analisis RTH

RESULTS AND DISCUSSION

The noise level that is experienced at the Sidoarjo regency caused by the daily transportation every day. To know the noise level that produced by the motorized vehicles, then it is made assumptions the produced noise level (dBA). For the motorized vehicles noise, where the sound intensity unit is decibel (dBA), can be seen in the table 3 follow:

Table 3. The sound intensity unit

Type Motorized	The sound intensity Unit Leq (dB)	VLHR (Unit/hari)
Motorcycle	68,5	1.000
car	69,5	1.259
Van (station)	74,9	4.365
Bus	79,1	11.482
Truck	80,4	15.488
Truck twin	84,6	40.738

Source :Subagyo, 1997, Mencari Korelasi Jumlah Kendaraan Yang Lewat Dengan Tingkat Kebisingan Lalu Lintas

From the produced noise level, it can be known the RTH needs in reducing the noise at the calculation below:

The RTH need to reduce noise (K):

$$K = Leq : 7 \text{ dB/ha}$$

K : RTH needs for reducing the noise

Leq : The sound intensity per Unit

7 dB/ha : asumsi 1 hektar RTH and RTH need for reducing. (Embleton, 1963 in Grey dan Deneke 1978)

Calculation RTH need for reducing the noise, can see in the table 4.:

Table 4. Koefisien K for Type Motorized

No	Type Motories	K
1	Motorcycle	K = Leq : 7 dB/ ha = 68,5 dB : 7 dB/ ha = 9,78 ha
2	Car	K = Leq : 7 dB/ ha = 69,5 dB : 7 dB/ ha = 10 ha
3	Van (Station)	K = Leq : 7 dB/ ha = 74,9 dB : 7 dB/ ha = 10,7 ha
4	Bus	K = Leq : 7 dB/ ha = 79,1 dB : 7 dB/ ha = 11,3 ha
5	Truck	K = Leq : 7 dB/ ha = 80,4 dB : 7 dB/ ha = 11,5 ha
6	TwinsTruk	K = Leq : 7 dB/ha = 84,6 dB : 7dB/ha = 12 ha

Source; analisis, 2010.

The RTH needs in reducing the noise at the calculation below:

Table 5. Total Area For RTH

Type Motoraires	Total Area For RTH (ha)
1.000 Motorcycle	9,78
1.259 Car	9,93
4.365 Van (station)	10,7

11.482 Bus	11,3
15.488 Truck	11,48
40.738 TwinTruck	12
Total	65,19

Source; analisis, 2010

The analysis of dioxide carbon absorption (CO₂) is useful to get information about the ability of the green space to absorb the carbon dioxide. The carbon absorption analysis can be done through two ways: that is the coverage analysis and analysis based on direct measurement in field, for direct absorption of CO₂, based on table below.

Table 5. Penetrate Carbon(C) and Carbon Dioksida (CO₂) in vegetasi

Type Vegetasi	Penetrate CO ₂ (ton/ ha/ Year) (RCO ₂)
Forest	58,2576
Grass	3,2976
Shrubs	3,2976
Plantation	52,3952
Rice-field *	12,00

Sumber : Iverson et. al, 1993 dalam Tinambunan, 2006, Analisis Kebutuhan RTH di Kota Pekanbaru

* Prasetyo et.al 2002 dalam Tinambunan 2006, , Analisis Kebutuhan RTH di Kota Pekanbaru

Calculation Penetration CO₂/ ha (R)

Formula :

$$R = E / R CO_2$$

Keterangan :

R : Penetration CO₂ (ha)

E : Total Emisi CO₂ from motorcycle (ton CO₂/year)

R CO₂ : Value penetration CO₂ (ton/ha/year)

Forest:

$$R = E / R CO_2 = 51,59 / 58,2576 = 0,88 \text{ ha}$$

Grass :

$$R = E / R CO_2 = 51,92 / 3,2976 = 15,78 \text{ ha}$$

Rice-field :

$$R = E / R CO_2 = 51,92 / 12 = 4,33 \text{ ha}$$

From calculation green space as effort to peneration emission CO₂, to divide by forest 0,88 ha, grass need 15,78 ha and rice-field 4,33 ha. RTH to decrease the noise of 65 ha.

CONCLUSION

Green space one method to care the ecosystem balance, The ecological benefits of the green space not only as recreation means and for the comfort feeling, but also as the control for the air pollution, either as natural barrier or as the absorber of CO₂, NO₂, and SO₂. From the calculation results of Green space based on the

absorption level of CO₂ emission, it obtains the forest need area to absorb the CO₂ of 0.88 ha, and the grass need of 15.78 ha and for the wet rice field need of 4.33 ha. And the need of RTH to decrease the noise of 65 ha. The suggested green space can be in the form of urban forest in cluser or in path.

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IMPROVEMENT PRIORITY OF SERVICE QUALITY OF PURWOASRI TERMINAL KEDIRI

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ABSTRACT

Problems in decreasing or low service quality of public transport terminal in Indonesia to become problem that is common and cannot be resolved what are the solution. Decline in the service quality was caused partly because of decreased public interest in public transport. Passenger road transport passenger terminal is one of road transport infrastructure has important roles as a node and exchange place of public transport modes with road infrastructure as its trajectory. Purwoasri terminal is one of road transport terminal type B in East Java province, Kediri regency for now virtually this terminal is unused, because the majority of public transport such as bus, MPU, and taxi had no activity over the transit or transport modes in this terminal. This condition has long since established in year 2000 by the local government of Kediri. This study seeks to evaluate services of Purwoasri terminal and set improvement priorities of service quality based on the user importance and satisfaction such as public transport passengers that pass in the terminal. The method used in this study is extracting information needs of user that is called voice of user. The analysis applied is importance-performance analysis (IPA) which is considered a priority to improve services based on user importance and satisfaction. IPA results explained service quality attributes that have highest priority to be repaired and improved according to user importance and satisfaction among others are attributes of good parking area, no extra fees or exactions, and availability of travel information boards.

Keywords: improvement priority, service quality, Purwoasri terminal

INTRODUCTION

Problems in decreasing or low service quality of public transport terminal in Indonesia to become common problem and cannot be resolved the solution. Decline in the service quality was caused partly because of decreased public interest in public transport. Passenger road transport passenger terminal is one of road transport infrastructure has important roles as a node and exchange place of public transport modes with road infrastructure as its trajectory. As we know this condition nowadays, many

people change to private vehicles and leave public transport has significant effect on the terminal performance. Along with the decline in the performance and effectiveness of the terminals, public transport services are also lower because of less demand by the public (Rauf, 2002), and even if they use public transport most of the passengers did not get into the terminal, but they prefer to move down and change public transport modes outside of the terminal (Siddik, 2008). Agung (2013) conducted a study to evaluate the performance

in service improvement of Joyoboyo terminal in Surabaya, East Java Province which is terminal type-B. This study obtained service attributes that can be developed by the terminal management institution. The one of all service attributes is provision good parking area for public transport. Kediri regency is part of East Java province supported by three terminals types-B and two sub-terminals (type-C). The three terminals Type-B are Gumul terminal in Ngasem district, Pare Terminal in Pare district, and Purwoasri terminal in Purwoasri district. While the two sub terminal are Pasar Pamenang sub terminal in Pare district and sub Sambu terminal in Ringinrejo district. In accordance with its function as a terminal type B, Purwoasri terminal has strategic location and position to serve the public transportation to connect Surabaya with the western towns of East Java province such as Madiun, Ngawi, even the cities in Middle Java province and D.I. Yogyakarta province.

At this present, Purwoasri terminal is used for logistic transport terminal because the majority of public transport such as bus, MPU, and the taxi had no activity over the transit or change transport modes in the terminal. This condition has long since established in year 2000 by the local government of Kediri, so that this studies and research is necessary to evaluate Purwoasri terminal services and performance in determining improvement priority of its services based on the perception of user importance and satisfaction. The user is public transport who passenger that pass the terminal. The passenger change public transport modes in Purwoasri terminal. The method used in this study is extracting the information needs of user which is called voice of user. The analysis is importance performance analysis (IPA) which considered a priority to improve services based on user importance and satisfaction. Figure 1 shows the location of Purwoasri terminal in geographical mapping from wikimapia.org (2013), we can see that Purwoasri terminal has good position

in transport connecting. **Fig. 2** show the entry gate of Purwoasri terminal at this time.



Figure 1. The location of Purwoasri terminal in white stripe



Figure 2. The situation of Purwoasri terminal at the entry gate of terminal access

MATERIALS AND METHODS

The conceptual framework of this study refers to Performance Based Design of Buildings (PeBBu), Final Domain Report. CIBdf (Spekkink, 2005). PeBBU provide a concept of service quality of performance-

based infrastructure by considering the balance between technical aspects of terminal to user needs, so there is no discrepancy between technical aspects and functional aspects according to the user. Terminal in Indonesia has been widely researched, studied, and technically planned as queues, modeling vehicles flow, vehicle parking capacity, passenger capacity, both passenger and vehicle circulation, and is still sparse and little consider user satisfaction. This research focuses on determination of service quality of terminal from user importance and satisfaction aspect. This study location is Purwoasri terminal in

Kediri which is terminal type B. Terminal Type B according to Transportation Minister Decree No. 31 of 1995 (KM 31/1995) provide definition of terminal that serving public transport vehicle for transportation between cities in the province, urban, and rural transportation. Data that to be analyzed derive from user perception so the method to identify attributes of terminal services that is survey techniques to user by voice of the user. The method developed in this study is shown in **Fig. 3**.

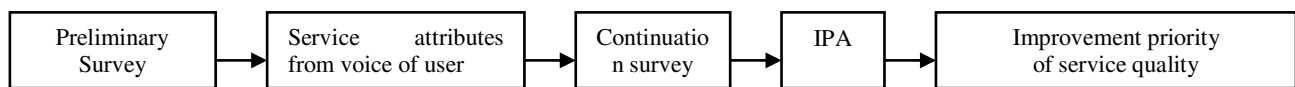


Figure 3. search method development

The first stage in this research is extracting information and determination service quality indicators of terminal by preliminary survey (See Figure 3). Preliminary survey use questionnaire by combining several variables

from previous researches. Table 1 shows the variables of previous research carried out by the method.

Table 1. The previous researches

No	Researcher	Year	Variables	Methods
1	Constantine	1999	Security, information, facilities availability, and aesthetics	Survey and factor analysis
2	Dragu dkk	2001	Security, reliability, frequency, accessibilities, commodities, information, comfort, and aesthetics	Survey and simulation
3	Rauf	2002	Facilities availability and performance, comfort, and safety	Survey, IPA, QFD and Benchmarking
4	Harsanto	2007	Reliability, Responsiveness, Assurance, Empathy, and Tangible	Survey and QFD
5	Rini	2007	Security, facilities availability, services and management, parking services, bus service, ticketing, cleanliness and comfort, pedestrian facilities and access roads to shelter, accessibility, safety, and service operators.	Survey and factor analysis
6	Marliana	2008	Employees ability, comfort, punctuality, speed and accuracy of employees service to passengers, the number of bus routes, shelter facilities, bus density, and disabled facilities	Servqual and QFD
7	Purba	2009	Facilities and management, accessibility, level of service, safety, and environment comfort.	Analytical Hierarchy Process (AHP)
8	Weningtyas	2009	Reliability, physical aspects, and responsiveness	Servqual and survey
9	Pati	2009	Time, flexibility of tickets payment, passengers and goods safety, and the ease of telephone service	Survey and linear regression analysis
10	Saputra	2010	Arrival and departure time, services information systems, brokers and baggage employees regularity, road conditions, and terminal facilities.	Survey, <i>Customer Satisfaction Index</i> (CSI), and IPA
11	Agung Sedayu	2013	Reliability, availability, amenity, durability, Responsiveness, comfort, assurance, frequency, performance, and aesthetics	Survey and IPA

Table 1 shows the variables and methods used in previous researches. It shows that the variables to become concern for terminals user include security, safety, comfort, availability of facilities, reliability of public transport services, management service or terminals management, and other facilities for user amenities. This suggests that the average terminal in Indonesia and in other countries that the variables become very important service attributes for users who are mostly passengers use the terminal facilities. The research method used was a survey and importance performance analysis (IPA). Stages of analysis in this study consist of:

a. Arrangement of voice of user

The initial stage of this study is to explore service attributes of terminal with surveys and field interviews. Surveys and interviews result are shown in Table 2.

b. Validity and Reliability Test of Instruments

Validity test was conducted to determine the validity of questionnaire or the questionnaire that will be distributed to a sample of this study. The test carried out on 30 people (Sugiyono, 2009). Data collection tool used was a questionnaire with a measurement scale as follows:

- Questionnaires of level of user importance with 5 priority scale :
 - 1 = not important
 - 2 = less important
 - 3 = fairly important
 - 4 = important
 - 5 = very important
- Questionnaires of level of user satisfaction consisted of 5 scales :
 - 1 = not satisfactory
 - 2 = less satisfactory
 - 3 = fairly satisfactory
 - 4 = satisfactory
 - 5 = very satisfactory

In this study, an instrument is called strongly correlated if the correlation value above 0.6

(Sugiyono, 2009). For correlation test, then use of the Pearson product moment correlation, i.e. formula that will calculate correlation coefficients of each item with the total score. The equation by Pearson is:

$$r_{xy} = \frac{N \sum XY - (\sum X)(\sum Y)}{\sqrt{[N \sum X^2 - (\sum X)^2][N \sum Y^2 - (\sum Y)^2]}}$$

Where:

- r_{xy} = correlation coefficient of searched items
- X = respondents score for each item
- Y = total score of each respondent for all items
- $\sum X$ = sum of scores in the distribution X
- $\sum Y$ = sum of scores in the distribution Y
- $\sum X^2$ = sum of squares of each score X
- $\sum Y^2$ = sum of squares of each score Y
- N = number of subjects

Reliability test performed after the validity test that aims to determine whether the data collection tool basically shows the level of precision, accuracy, stability, or consistency of the tool in revealing certain symptoms of a group of individuals, even if done at different times. Reliability test conducted on the claims that have been strongly correlated. For Internal Consistency test using consistency coefficient (Cronbach alpha). Cronbach Alpha equations used in the reliability test are:

$$r_1 = \left[\frac{k}{k-1} \right] \left[\frac{1 - \sum \sigma b^2}{\sigma t^2} \right]$$

Where:

- r_1 = consistency of instruments
- K = number of the questions or the number of question
- $\sum \sigma b^2$ = Number of items variance
- σb^2 = total variance

With the rule that if the value of coefficient alpha (Cronbach alpha coefficient) is above 0.60 (Sugiyono, 2009). Validity and reliability test of the questionnaire by using SPSS 15.0.

c. Importance-Performance Analysis (IPA)

IPA is done to get the level of user importance

to service attributes. IPA is conducted on continuation survey data. The level of user importance represented in the Importance Classification Diagram that is divided into four quadrants (Figure 4) with the following explanation,

- 1) Quadrant A is the area that contains attributes which considered important by user but not as expected (user satisfaction level is still very low). In this area, the terminal management institution doing improvements continuously in order to increase performance in this quadrant.
 - 2) Quadrant B is the area that contains the attributes which considered important by the user and the attributes considered in accordance with the user perceived to the level of satisfaction is higher.
 - 3) Quadrant C is the area which contains the attributes that are considered less important by user, and in fact its performance is less special.
- Quadrant D is the area that contains the attributes that are considered less important by user and the perceived excessive.

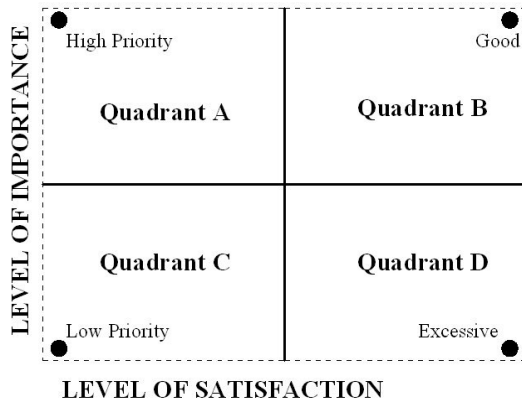


Figure 4. Importance Classification Diagram
Source: Wijaya, 2011

The respondents of this research are user that public transport passengers who use Purwoasri terminal. The reason for using this sampling type, because the population has heterogeneous characterized, and the heterogeneity has a significant meaning to the achievement of the research objectives. Determination of the sample by using Bernoulli equation:

$$N \geq \frac{\left(Z_{\alpha/2}\right)^2 p.q}{e^2}, \text{ become}$$

$$N \geq \frac{(1,96)^2 \cdot 0,95 \cdot 0,05}{(0,05)^2} \rightarrow N \geq 72,99 \approx 73$$

Where,

N = number of minimum samples, Z = the value of normal distribution, e = error rate, p = the proportion of the number of questionnaires that are considered true, and q = the proportion of the number of questionnaires that are considered wrong. Value is considered correct by 95%, and the number of questionnaires that are considered wrong is 5%. We decided by using 75 respondents to avoid fault due to lack of data or not return the questionnaire.

RESULT

a. Preliminary survey result

Stages of preliminary survey get voice of the user which consists of ten primary attributes of Purwoasri terminal. **Table 2** shows voice of user and score ranking of each attributes.

Table 2. Voice of user of Purwoasri terminal

No	Terminal Service Attributes	Total Score	Ranking
1	Assurance in Security, safety, health, and availability of transport modes	127	2
2	Staff responsiveness in provision of care, responsiveness to problems, polite and friendly, and have good skills	110	8
3	Terminal facilities Performance include lighting, air circulation, parking lots, roads, waiting room, small mosque, stalls, kiosks, hall, corridors, toilet, sculpture, and waste management	122	3
4	Facilities aesthetics include waiting rooms, corridors, arrival and departure gate, parks, and landscaping	117	5
5	Amenity and Easy accessibility in location, circulation, tickets, prices, information, facilities, and no additional cost (extortion)	112	7
6	Reliability in arrivals and departures, waiting time, and public transportation ticketing service	143	1
7	Durability of public transport services and facilities	105	9
8	Frequency in passenger queues, overcrowding, and the level of traffic congestion	104	10
9	Convenience and comfort from cigarette smoke, fumes, odors, noise, glare, view, brokers, and gain terminal cleanliness and regularity	114	6
10	Availability of terminal facilities	119	4

b. Validity and Reliability Test Results

Validity and reliability test results of the instrument of 30 people obtained User Importance with correlation values greater than 0.6, whereas alpha coefficient (Cronbach's Alpha) by 0.982 and this value is greater than 0.6, it means all question items in user importance instruments are valid and reliable. For user Satisfaction instruments have correlation values greater than 0.6. While alpha coefficient value (Cronbach's Alpha) with 0.924 (greater than 0.6). Thus, it means question item in user satisfaction instruments are valid and reliable.

a. IPA results

Development of 10 major attributes as in Table 2 was developed into 50 service attributes to be developed on a continuation questionnaire distributed to 75 respondents. Table 3 shows the results of the survey (continuation questionnaire) which gives a mean value for the level of user satisfaction and importance to Purwoasri terminal services. The mean value for the total score obtained for the user importance by 3.83 and 4.04 for levels of satisfaction. The mean value results of each attribute plotted on the importance classification diagram (see **Fig. 5**) by Importance -performance analysis (IPA).

Table 3. The mean values of level of importance and level of satisfaction in Purwoasri Terminal

No	Service Attributes	Notation	Mean Values	
			Satisfaction	Importance
1	Security and safety protection	A-1	3,920	4,056
2	Providing Health help and aid	A-2	4,008	3,776
3	Obtaining necessary transport modes	A-3	3,568	3,984
4	Clarity Assurance in travel destinations selection	A-4	3,820	3,968
5	Employees attention to all customer complaints	A-5	4,072	3,912
6	Employees responsive to all customer problems	A-6	3,936	3,800
7	Employees serving with polite, friendly, and neat	A-7	4,104	4,016
8	Employees have sufficient skills and abilities	A-8	4,080	3,928
9	Functioning of lighting (natural and artificial)	A-9	3,936	3,704
10	Functioning of bathroom facilities	A-10	4,216	3,880
11	Functioning of air circulation	A-11	3,920	3,712
12	Good road performance	A-12	4,072	3,856
13	Good parking performance	A-13	3,960	3,840
14	Waiting room aesthetically	A-14	3,856	4,136
15	Corridor aesthetically	A-15	4,152	3,984
16	Arrival and departure gate aesthetically	A-16	3,912	3,776
17	Garden and landscape aesthetically	A-17	4,168	3,928

18	Amenity and Easy accessibility in location	A-18	3,984	3,760
19	Amenity and Easy in room or space circulation	A-19	4,000	3,752
20	Easy for getting ticket	A-20	4,272	3,880
21	Reaching prices such as ticket, taxes, food, and drinks	A-21	4,088	3,904
22	Ease of getting information	A-22	3,848	3,792
23	Ease of getting facilities	A-23	3,992	3,832
24	No additional charges or payment (extortion)	A-24	4,008	3,928
25	Arrival and departure time	A-25	3,944	3,896
26	No long waiting time	A-26	3,880	3,860
27	Ticketing service on time	A-27	3,904	3,664
28	Durability of facilities services	A-28	4,144	3,976
29	Durability of transportation services	A-29	4,096	4,000
30	Normal Queuing for passenger ticketing	A-30	3,944	3,800
31	Passenger densities inside and outside of terminal	A-31	4,176	3,992
32	No vehicle flow congestion occurs	A-32	3,856	3,696
33	Free from cigarette smoke, vehicles smoke, and odors	A-33	4,136	3,968
34	Free from noise, glare, and unfavorable view	A-34	4,184	3,936
35	Cleanliness interior and exterior	A-35	3,840	3,744
36	No ticket brokers	A-36	3,768	3,600
37	Regularity in roads, parking, circulation, and space organization	A-37	4,024	3,880
38	Availability of adequate parking space	A-38	3,888	3,744
39	Availability of adequate waiting room space	A-39	4,048	3,896
40	Availability of number of kiosk and retail facilities	A-40	4,208	3,864
41	Availability of adequate waste facilities	A-41	4,104	3,944
42	Availability of canteen, restaurant, and food store	A-42	3,992	3,752
43	Availability of travel information board	A-43	3,920	3,968
44	Availability of information and complaint center	A-44	3,928	3,800
45	Availability of adequate tariffs board and list per route	A-45	3,870	3,992
46	Adequate number of bathrooms and space for clean bathroom	A-46	3,940	3,904
47	Clean religious facilities : place for pray	A-47	3,936	3,878
48	There are transportation routes signs	A-48	3,800	3,984
49	Availability of telecommunication facilities (telephone,internet,TV)	A-49	4,264	3,888
50	Availability of health aid centre	A-50	3,712	3,392

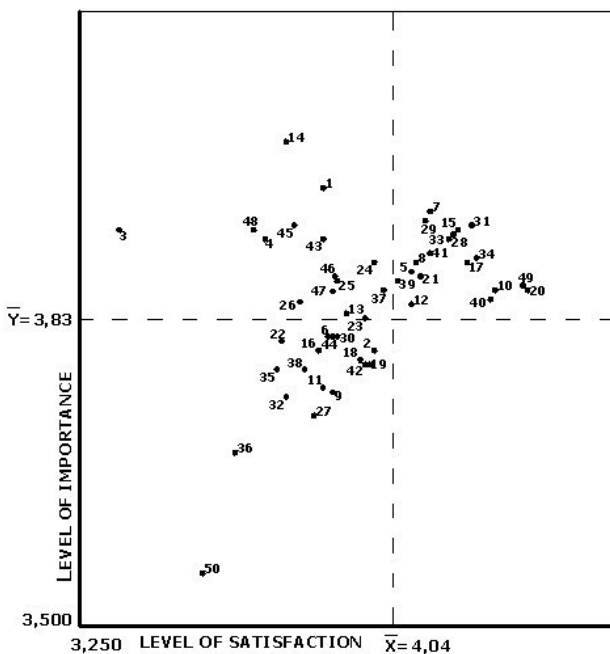


Figure 5.Importance Classification Diagram of Purwoasri terminal

DISCUSSION

The first stages of this research is a preliminary survey obtain 10 primary service attributes into the voice of user. The ten attributes were developed into 50 details service attributes used in the continuation survey. Service attributes with the highest score of the ten primary attributes are reliability in arrival and departure of public transport, waiting time, and ticketing of public transport. Table 3 shows that the mean score for all service attributes for all user satisfaction and importance that is above of score 3. It can be concluded that all service attributes of Purwoasri terminal more than fairly important and fairly satisfactory category. From Importance Classification diagram (see Figure 3) , the total mean score of importance level (\bar{Y} (= 3.83 and satisfaction (\bar{X}) = 4.04. The

total mean value is plotted in the form of straight lines intersecting at the diagram (see Figure 5). From these results it can be seen that 15 service attributes to become the highest priority for improvement by managers get terminal management institution (Purwoasri terminal management) is in quadrant A of the classification diagram (Figure 5) which consists of : attributes of security and safety protection (A-1), obtain the necessary transport modes (A-3), Clarity Assurance in travel destinations selection (A-4), Good parking performance (A-13), Waiting room aesthetically (A- 14), Ease of getting facilities (A- 23), No additional charges or payment (extortion) (A-24), Arrival and departure time (A-25), No long waiting time (A-26), Regularity in roads, parking, circulation, and space organization (A-37), Availability of travel information board (A- 43), Availability of adequate tariffs board and list per route (A-45), Adequate on number of bathrooms and space for clean bathroom (A- 6), Clean religious facilities : place for pray (A- 47), and There are transportation routes signs (A- 48) .

Purwoasri Terminal when studied from the position and location is very strategic in supporting local and regional transport of Kediri regency and East Java province. Purwoasri terminal is terminal transit public transport from the north, east, central, and western regions of East Java. The third attributes is a very important need for services

according to user. While 18 service attributes had good level according to user that is in quadrant B of importance classification diagram (Figure 5) consist of attributes of complaints Employees attention to all customer complaints (A-5), Employees serving with polite, friendly, and neat (A-7), Employees have sufficient skills and abilities (A-8), Functioning of bathroom facilities (A-10), Good road performance (A-12), Corridor aesthetically (A-15), Garden and landscape aesthetically (A-17), Easy for getting ticket (P-20), Reaching prices such as ticket, taxes, food, and drinks (A-21), Durability of facilities services (A-28) , Durability of transportation services (A- 29) , Passenger densities inside and outside of terminal (A-31), Free from cigarette smoke, vehicles smoke, and odors (A-33) , Free from noise, glare, and unfavorable view (A-34), Availability of adequate waiting room space (A- 39), Availability of number of kiosk and retail facilities (A-40), Availability of adequate waste facilities (A- 41), and Availability of telecommunication facilities (telephone, internet, TV) (A- 49). Attributes that are considered good service is maintained and enhanced by the terminal management institution so that the level of terminal service still maintained. The results are plotted in importance classification diagram can be seen in **Fig. 5** and detailed in **Table 4**.

Table 4. Priority classification of service attributes of Purwoasri terminal

Quadrant	Service Attributes (A-x)
A : High priority	1, 3, 4, 13, 14, 23, 24, 25, 26, 37, 43, 45, 46, 47, 48
B : Good	5, 7, 8, 10, 12, 15, 17, 20, 21, 28, 29, 31, 33, 34, 39, 40, 41, 49
C : Low priority	2, 6, 9, 11, 16, 18, 19, 22, 27, 30, 32, 35, 36, 38, 42, 44, 50
D : Excessive	-

Service attributes with low priority (in quadrant C) has a score criterion for user satisfaction are low to the service quality. From Table 4, it can be seen that there are 17 service attributes are included in quadrant C. Three service attributes include attributes Providing Health help and aid

(A-2), Employees responsive to all customer problems (A-6), and Functioning of lighting (natural and artificial) (A-9). For quadrant D that described as excessive service attributes in Purwoasri terminal that were identified no service attributes that fall into this category.

CONCLUSION

Purwoasri Terminal as terminal type B in Kediri regency in position and location that is strategic on supporting public transport transit node in Kediri regency. The methods to determine quality service of Purwoasri terminal through a preliminary survey, continuation surveys, and IPA. Process of preliminary survey and previous reseaches getting 10 services attributes called voice of user, while the continuation survey result 50 detailed service attributes. Analysis results mentioned that service quality of Purwoasri terminal still needs improvement and repair. Overall mean value of service attributes of Purwoasri terminal higher or better than the fairly important and fairly satisfactory category according to the user. IPA results explained that the attributes that get the highest priority is attributes protection of the security and safety protection, Obtaining necessary transport modes, Clarity Assurance in travel destinations selection, Good parking performance, Waiting room aesthetically, Ease of getting facilities, No additional charges or payment (extortion), arrivals and departures on time, no long waiting time, Regularity in roads, parking, circulation, and space organization, Availability of travel information board, Availability of adequate tariffs board and list per route, Adequate on number of bathrooms and space for clean bathroom, Clean religious facilities : place for pray, and There are transportation routes signs While the 18 service attributes of Purwoasri terminal were considered good include employees attention to all customer complaints, employees serving with polite, friendly, and neat, employees have sufficient skills and abilities, functioning of bathroom facilities, corridor aesthetically, garden and landscape aesthetically, easy for getting ticket, reaching prices such as ticket, taxes, food, and drinks, durability of facilities services, durability of transportation services, passenger densities inside and outside of terminal, Free from cigarette smoke, vehicles smoke, and odors,

Free from noise, glare, and unfavorable view, availability of adequate waiting room space, availability of number of kiosk and retail facilities, availability of adequate waste facilities, availability of telecommunication facilities (telephone, internet, TV). As a suggestion for further study and research, methods and steps require further analysis in addition to the IPA in order to obtain a target of terminal services detail to future. Besides, it is necessary that the model can predict the level of service quality of Puwoasri terminal that needs improvement and repair.

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Toxicity, Antioxidant and Antibacterial Activity Test of Methanol Extract of *Chlorella sp.* Microalgae Result Cultivation in Tauge Extract Medium

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ABSTRACT

Chlorella sp. is one of Allah creation that has the potential to be utilized, as state in al Quran surah al An'am verse 99 and surah an Nahl verse 11. *Chlorella sp.* is one of species microalgae Chorophyta that containing kinds of important compound such as flavonoid, tanin, phenolic compound, terpenoid, chlorophyll and carotenoid. The purpose of this research are to know toxicity, antioxidant and antibacterial activity from extract of *Chlorella sp.* microalgae result from extraction with methanol solvent and to know content of active compound group in extract of microalgae *Chlorella sp.* *Chlorella sp.* was cultivated in Tauge Extract Medium (TEM) 4 % and harvesting of at stationary phase (10th day). Microalgae *Chlorella sp.* was extracted by maceration method using methanol solvent. Methanol extracts were tested of its toxicity by BSLT method, antioxidant test by DPPH method, and antibacterial test by diffusion method. Identification of active compound was estimated by reagent tested on qualitative scale include alkaloid, falvonoid, steroid, triterpenoid, and tanin. The result showed that methanol extract of *Chlorella sp.* has strong toxicity with LC₅₀ value 20,516 ppm. Methanol extract of *Chlorella sp.* has strong antioxidant activity with EC₅₀ value 18,610 ppm. Methanol extract of *Chlorella sp.* which has antibacterial activity with inhibition zone 9,9 mm toward *E. coli* and 12,0 mm toward *S. aureus*. The results of the identification of active compound showed that methanol extract of *Chlorella sp.* contains a steroid and tanin compound class.

Keywords

Chlorella sp. Tauge Extract Medium, Methanol extract, Toxicity, Antioxidant, Antibacterial.

INTRODUCTION

Microalgae are photosynthetic microorganism with varying cell morphology, both unicellular and multicellular (formed small colonies) (Becker, 1994). Microalgae has the advantage among others, its life is not depending on the season, doesn't require a large place, and doesn't require a long time to harvest (Borowitzka, 1988). Microalgae can live in fresh water and sea water, don't compete with food production, low water consumption and production costs are not too high (Supriyadi, 2012).

Microalgae produce several important vitamins, such as A, B₁, B₂, B₆, B₁₂, C, E, nicotinamide, biotin, folic acid, dan pantothenic acid. The pigments that resulted include chlorophyll (0,5 to 1 % of dry weight), carotenoids (0,1 to 14 % of dry weight), and fikobiliprotein (Becker, 1994).

One type of microalgae from Chlorophyta groups is *Chlorella sp.* *Chlorella sp.* has potential as a natural food, animal feed, supplements, producing bioactive components of pharmation materials and medicine (Steenblock, 1996). The chemical composition of *Chlorella sp.* contained in **Table 1**.

Table 1. The chemical composition of *Chlorella sp.*

Chemical Compositon	(%)
<i>Chlorella sp.</i>	
Carbohydrate	20,6
Protein	30,9
Lipid	20,1
Others	28,4

Source : Ben-Amotz, *et al.* (1987).

Several studies has reported that *Chlorella sp.* is one of microalgae that has bioactivity as anticancer, antioxidant, and antibacterial. Adams (2009) reported that tumor cells may die completely by injecting *Chlorella sp.* Matsukawa, *et al.* (1998) reported that the

water, ethanol, and methanol extract of *Chlorella sp.* has antioxidant activity of 39,2 %, 52,8 %, and 43,9 %. Sriwardani (2000) reported that chloroform extract of microalgae *Chlorella sp.* able to inhibit 46,2 % the growth of bacteria *E. coli*.

Bioactive components of microalgae *Chlorella sp.* which can produce pharmacological effects obtained from *Chlorella sp.* biomass. *Chlorella sp.* biomass is an accumulation of *Chlorella sp.* cells results from cultivation in a culture medium. To produce a good growth of microalgae, environment with appropriate nutrients is certainly needed. One of culture medium that can used as a cultivation medium of *Chlorella sp.* is Tauge Extract Medium (TEM).

Tauge Extract Medium (TEM) is a natural culture medium that contain components that needed for growth of *Chlorella sp.* microalgae, such as nitrogen and phosphor. The growth of microalgae in Tauge Extract Medium (TEM) more rapidly than other medium such as Sea Water Medium and Guillard Medium (Wulandari, *et al.*, 2010). Prihantini, *et al.* (2007) reported that Tauge Extract Medium 4 % is a medium that suitable for microalgae cultivation.

The purpose of this research are to know toxicity, antioxidant and antibacterial activity from extract of *Chlorella sp.* microalgae which cultivation in Tauge Extract Medium (TEM) and to know content of active compound group in extract of microalgae *Chlorella sp.*

MATERIALS AND METHODS

This research held in Organic and Biotechnology Laboratory, in Chemistry Departement and Ecology Laboratory in Biology Departement, Science and Technology Faculty, UIN Maulana Malik Ibrahim Malang at March-May 2013.

Equipments and Materials

Equipments that used are sets of glass tools, porcelain dish, petri dish, *shaker*, oven, *rotary evaporator vacumm*, cuvet, incubator, aluminium foil, analytical balance, *sentrifuse*, spektronik 20+, and spectrofotometer UV-Vis *Varian Carry*.

Materials that used are isolate of *Chlorella sp.* from Ecology Laboratory, UIN Maulana Malik Ibrahim Malang. Animal test that used is brine shrimp *Artemia salina*, bacteria test that used are *Escherichia coli* and *Staphylococcus aureus*. Other materials that used are taugé of beansprout, methanol *p.a.*, sea water, yeast, KMnO_4 , vitamin C, BHT, solution of DPPH, *Nutrient Agar (NA)*, *Nutrient Broth (NB)*, penicilin dan streptomycin, anhydric acetic acid, chloroform, H_2SO_4 , HCl 2 %, HCl, Dragendroff reagent, Mayer reagent, Wagner reagent, solution of gelatin, FeCl_3 1 %, methanol 50 %, DMSO, Mg metal, dan aquades.

Procedures

Production of Taugé Extract Medium (TEM)

Taugé (100 g) was boiled in 500 mL aquades during 1 hour. Taugé Extract Medium was produced by dissolving Taugé Extract into aquades with each concentration 4 % (v/v) (Prihantini, *et al.*, 2005).

Cultivation of *Chlorella sp.* in Taugé Extract Medium (TEM)

Isolate of *Chlorella sp.* (10 mL) was inoculated into 60 mL of Taugé Extract Medium in Erlenmeyer that located at room temperature 25-30 °C, with lightening used TL 36 lamp (light intensity 1000-4000 lux). Culture of *Chlorella sp.* was incubated until stationary phase with photoperiodicity 14 hours bright and 10 hours dark (Prihantini, *et al.*, 2005).

Harvesting of *Chlorella sp.* Biomass

Harvesting of *Chlorella sp.* biomass was performed at stationary phase at 10th day. Harvesting was performed by culture medium that contain *Chlorella sp.* was sentrifused during 15 minutes with velocity 3000 rpm. *Chlorella sp.* biomass was divorced from its liquid.

Preparation of Sample *Chlorella sp.* Biomass

Sample *Chlorella sp.* biomass from stationary phase which fresh was located in container, then was blowdried at room temperature (25-30 °C) used fan during ± 12 hours. Biomass that blowdried was weighted.

Extraction of *Chlorella sp.* Biomass

Biomass of *Chlorella sp.* that dry was performed maceration used methanol solvent 1:5 (w/v) during 24 hours with *shaker* ± 5 hours, then it was strained. The residue was performed maceration again with methanol until the filtrate that obtained was transparent, then it was strained. The filtrate that obtained was collected and was performed *rotary evaporator vacumm* until obtained thick extract.

Toxicity, Antioxidant, and Antibacterial Activity Test Methanol Extract of Microalgae *Chlorella sp.*

Toxicity Test

Methanol thick extract of *Chlorella sp.* was weighted as many 100 mg and dissolved used its solvent as many 10 mL. Solution that obtained was pipette as many 1000 μL , 500 μL , 200 μL , 150 μL , 100 μL , 50 μL , 25 μL , 10 μL , and 5 μL , then it was included into vial bottle and its solvent was vaporated until dry. Furthermore, included 100 μL DMSO, driblet of yeast, 2 mL sea water, then it was mixed until the extract could soluble in sea water. The solution was moved into measure flask 10 mL, then included 10 brine shrimp *Artemia salina* and added sea water until the volume became

10 mL, so the concentration each solution became 1000, 500, 200, 150, 100, 50, 25, 10, and 5 ppm.

Each solvent as many 1000 μ L was vaporated until dry, then added 100 μ L DMSO, driblet of yeast, 2 mL sea water. The solution was moved into measure flask 10 mL, then included 10 brine shrimp *Artemia salina* and added sea water until the volume became 10 mL (Control 1).

Tauge extract as many 1000 μ L was vaporated until dry, then added 100 μ L DMSO, driblet of yeast, 2 mL sea water. The solution was moved into measure flask 10 mL, then included 10 brine shrimp *Artemia salina* and added sea water until the volume became 10 mL (Control 2). Observation was performed after 24 hours toward death of brine shrimp *Artemia salina*. Analysis data to found the value LC_{50} was performed used probit analysis.

Antioxidant Activity Test

Determination of λ_{maks}

The solution of DPPH as many 5 mL was included into a cuvet. It was measured λ_{maks} using UV-Vis spectrofotometer.

Determination of Stability Time Antioxidant Measurement

Extract solution 30 ppm was pipetted as many 6,75 mL. It was added DPPH solution 0,5 mM as many 2,25 mL, then searched stability time without incubation and after incubation at temperature 37 °C and distance of time is 5-100 minutes with interval 5 minutes. Sample was measured at λ_{maks} that know at phase previously.

Measurement of Antioxidant Potential

Extract was dissolved in methanol solvent with concentration 5, 10, 15, 20, 25, and 30 ppm. The solution of extract was pipetted as many 6,75 mL and added 2,25 mL DPPH 0,5 mM then it was incubated at 37 °C, then was measured absorbance at λ 518,0 nm.

The value percent (%) antioxidant activity was counted by:

$$\text{Aktivitas Antioksidan} = \frac{A_{\text{kontrol}} - A_{\text{sampel}}}{A_{\text{kontrol}}} \times 100 \%$$

Then the value EC_{50} was counted with regression equation used “*Graphpad prism5 software, Regression for analyzing dose-response data*”. Control that used are solution of DPPH 0,5 mM. Comparator BHT and ascorbic acid being regarded as sample.

Antibacterial Activity Test

Production of Agar Media

Nutrient Agar (NA) as many 2,3 g was dissolved in 100 mL aquades and included into erlenmeyer and it covered by cotton. Liquid media (*Nutrient Broth*) was produced by 0,9 gr *Nutrient Broth* (NB) was dissolved in 100 mL aquades, then included into erlenmeyer and it covered by cotton. The suspension of agar media was brought to the boil until boiled, then included into reaction tube. It sterilized by autoclaf during 15 minutes at 121 °C and pressure 15 psi (*per square inchi*) (Volk dan Wheeler, 1993).

Rejuvenation of Bacteria

Pure breeding of *E. coli* and *S. aureus* was scratched with ose needle at agar compact media and it covered by cotton. Then it incubated during 18-24 hours at 37 °C, then located in freezer.

Production of Active Breeding Solution

Bacteria result from rejuvenation as many one ose was fertile in 10 mL liquid media (NB) sterile and it was homogenized.

Antibacterial Activity Test

Solution of active breeding *E. coli* and *S. aureus* bacteria as many 0,1 mL was added into sterile petri dish. Then, 10 mL agar compact media that brought to the boil until molten, it was freezed until temperature 40 °C and poured into petri dish and it was homogenized. Media left until condensed. Paper disc (diameter 5 mm) was panatrated in extract and control

(positive are penicillin and streptomycin, control negative are methanol and TEM). Paper disc was located above the surface of bacteria media used pincers and it was pressed. Bacteria media that gave antibacterial material was incubated at 37 °C during 18-24 hours. Inhibition zone that forming was measured used.

Active Compound Group Test with Reagent Test

Active group compound was performed with reagent test in group of metabolic secunder include alkaloid, flavonoid, triterpenoid, steroi, and tannin.

RESULTS AND DISCUSSION

Cultivation *Chlorella sp.* in Tauge Extract Medium (TEM)

The purpose of cultivation *Chlorella sp.* in Tauge Extract Medium (TEM) are to growth *Chlorella sp.* until the cells of this microalgae increasingly and could produced biomass that increase too. This research used sample of biomass microalgae *Chlorella sp.* at stationary phase (Prihantini, *et al.*, 2005). Stationary phase of *Chlorella sp.* was occurred at 10th day that signed with the highest number of cells *Chlorella sp.* is 4.880.000 cells/mL.

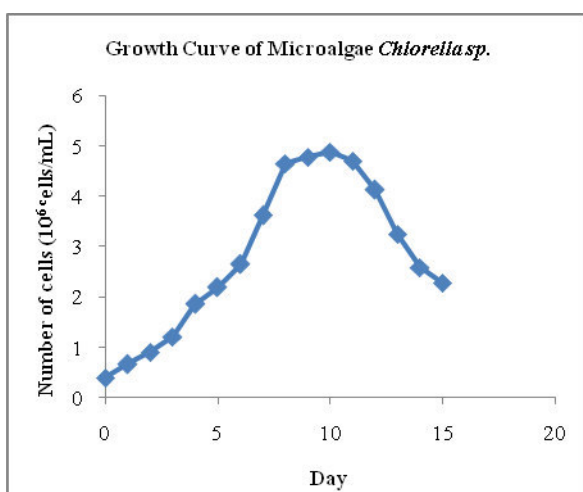


Figure 1. Growth Curve *Chlorella sp.* In TEM

During cultivation process, the culture of *Chlorella sp.* was occurred colour changed. This case showed that cells density of *Chlorella sp.* was changed too. The culture colour changed of *Chlorella sp.* was showed at Figure 2.



Figure 2. Change of *Chlorella sp.* culture colour

Preparation of Sample *Chlorella sp.* Biomass

Preparation of sample microalgae *Chlorella sp.* biomass was performed by blowdried during ±12 hours used a fan. Dried wasn't performed by the oven because doubt with high temperature could destroyed compound that contained *Chlorella sp.* biomass.

Table 2. Change of biomass weight of microlagae *Chlorella sp.* after blowdried

Mikroalgae <i>Chlorella sp.</i> Biomass	Mass (g)
Wet	400
Dry	55

The purpose of sample preparation by blowdried are to decrease water content of *Chlorella sp.* biomass. This case was performed in order to breakage microorganism consequence could minimalized, and to prevent growth of fungi so it could saved in a long time and not damaged chemical composition. Rendemen blowdried of *Chlorella sp.* biomass was 14,096 %, that is from ±400 gram of wet biomass, after blowdried obtained ±55 gram dry biomass that organized as soft powder.

Maceration of *Chlorella sp.* Biomass

Extraction active component of microalgae *Chlorella sp.* was performed by maceration method with methanol solvent. Methanol extract that obtained colored bottle green. The result of rendemen methanol extract of microalgae *Chlorella sp.* showed at **Table 3.**

Table 3. Rendemen of metanol extract microalgae *Chlorella sp.*

Mikroalgae	Rendemen (%)
<i>Chlorella sp.</i>	
Methanol Extract	7,001

Rendemen of methanol extract microalgae *Chlorella sp.* showed that polar compounds in *Chlorella sp.* biomass much too. Methanol was polar solvent that often used because penetration into cells patition more efficient, so it could produced much metabolic secunder. Methanol extract of *Chlorella sp.* biomass has colored bottle green was estimated by chlorophyll that extracted.

Toxicity Test

Table 4. Result toxicity test methanol extract of *Chlorella sp.*

Concentration (ppm)	Number of <i>A. salina</i> that death	% Mortality
0*	0	0
0**	0	0
5	2	0
10	4	0
25	5	0
50	7	0
100	10	0
150	10	30
200	10	80
500	10	100
1000	10	100

Ex: Number of animal test :
 30 brine shrimp *Artemia salina*
 * : solvent control
 ** : media control

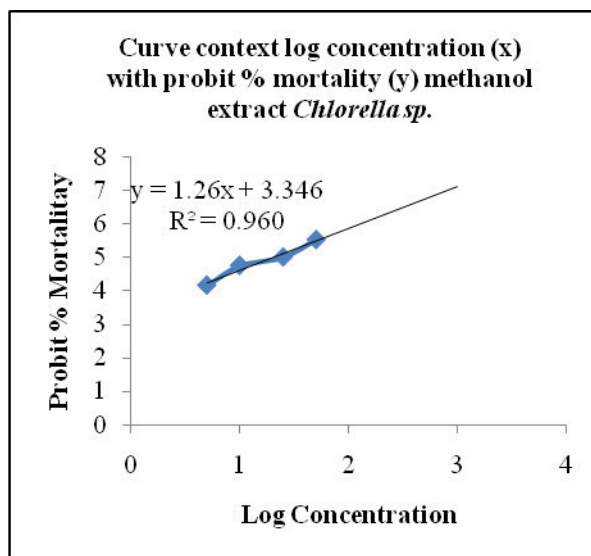


Figure 3. Curve context log concentration (x) with probit % mortality (y) methanol extract *Chlorella sp.*

Building on the result of counting obtained the value LC₅₀ methanol extract was 20,516 ppm. The distance of confidence value (*Upper dan Lower Limit*) 11,873 – 35,452 ppm.

Meyer, *et al.*, (1982) report that extract showed toxicitant activity in BSLT if it could cause death of 50 % animal test at concentration less than 1000 ppm. This case showed that methanol extract of *Chlorella sp.* has toxic toward *Artemia salina* because it has the value LC₅₀ < 1000 ppm.

Antioxidant Activity Test

Table 5. Percent antioxidant activity metanol extract of *Chlorella sp.* and comparator

Concentrat ion (ppm)	% Antioxidant Activity		
	Extract	Vit. C	BHT
5	5,784	67,260	39,460
10	6,041	92,744	66,247
15	9,590	95,056	77,674
20	69,690	96,190	86,498
25	85,383	96,744	89,823
30	90,139	96,795	89,879

Table 6. Value EC₅₀ methanol extract *Chlorella sp.* and comparator

Sample	EC ₅₀ (ppm)
Methanol Extract	18,610
Vitamin C	3,527
BHT	6,552

EC₅₀ was defined as concentration of sample solution that will cause reduction toward 50 % DPPH activity. More little the value EC₅₀ so it antioxidant activity more high (Molyneux, 2004). Methanol extract of *Chlorella sp.* has value EC₅₀ 18,610 ppm, it means that with increasing antioxidant from extract as many 18,610 ppm test solution will catch free radical 50 % from total free radical.

Antibacterial Activity Test

Present of methanol extract at stationary phase could inhibited growth of *E. coli* (negative gram) and *S. aureus* (positive gram) bacteria. Inhibition power methanol extract of *Chlorella sp.* from stationary phase toward *E.coli* appertain medium (5-10 mm) and toward *S. aureus* bacteria appertain strong (10-20 mm).

Table 7. Result antibacterial activity test methanol extract of *Chlorella sp.*

Methanol Extract of <i>Chlorella sp.</i>	Inhibition Zone (mm)	
	<i>E. coli</i>	<i>S. aureus</i>
Stationary Phase	9,9	12,0
Solvent Control	-	-
Media Control	-	-
Penicilin		26,0
Streptomycin	19,3	

Ex : Negative Control : Methanol and MET.
Positive Control : Penicilin dan Streptomycin.

Table 8. Result antibacterial activity test variation concentration

Concentration (% w/v)	Inhibition Zone (mm)	
	<i>E. coli</i>	<i>S. aureus</i>
5	1,0	2,2
10	3,7	4,9
15	6,9	3,0
20	16,5	7,4
25	12,1	13,1
Solvent Control	-	-
Media Control	-	-
Penicilin (5 %)		30,0
Sreptomycin (5 %)	18,7	

Ex : Negative Control: Methanol and MET.
Positive Control : Penicilin dan Streptomycin.

Concentration 20 % produce highest inhibition zone toward *E. coli* bacteria and concentration 25 % produce highest inhibition zone toward *S. aureus* bacteria. Inhibition power methanol extract of *Chlorella sp.* at concentration 20 % and 25 % appertain strong (10-20 mm) toward *E.coli* and *S. aureus* bacteria.

Active Compound Group Test

Active compound group test used reagent showed positive result compound group of steroid and tannin. The result showed at **Table 9.**

Table 9. Result observation active compound group methanol extract of *Chlorella sp.* biomass

Compound Group	Result
Alkaloid	-
Flavonoid	-
Tannin	+
Steroid	+
Triterpenoid	-

Ex: Sign + : contain compound
Sign - : nothing compound

Steroid

Methanol extract of *Chlorella sp.* was contained compound group steroid because it showed colour changed from green yellowness became green deep blue after increasing that reagent. If in the test solution there are water molecule, then anhydric acetic acid will changed became acetic acid before reaction and acethyl derivative do not formed. Triterpenoid was gave forming reaction brownness ring when this compound was dropped sulfuric acid through partition, whereas steroid will produce colour green deep blue (Robinson, 1995).

Steroid disposed non polar. This compound group contained in methanol extract possible because it was bounded as it glycoside. Glycocycle was combination between sugar and alcohol that mutual bonded through glycocycle bond.

Tanin

The result tannin test used FeCl_3 was negative because do not showed colour green blackness or deep blue. Whereas the result tannin test used gelatine solution was positive because formed white stratified in base tube in methanol extract of *Chlorella sp.* biomass. White stratified was formed because hydrogen bond between tannin compound with gelatine.

Gelatine was contained protein, so forming complex tannin-protein compound, because hydrogen bond between tannin and protein in gelatin so it could formed white stratified. This hydrogen bond was formed from H atomic that bounded with 2 atomics that has haigh electronegativity, such as N, O, and F atomic. Hydrogen bond that formed cause H atomic that bounded with 2 atomics O or that bounded with O and N atomic from tannin and gelatin structure.

CONCLUSION

1. Methanol extract of microalgae *Chlorella sp.* has toxicity toward *A. salina* that sowed with the value LC_{50} 20,516 ppm.
2. Methanol extract of microalgae *Chlorella sp.* has antioxidant activity that showed with value of EC_{50} 18,610 ppm.
3. Methanol extract of microalgae *Chlorella sp.* has antibacterial activity toward *E. coli* and *S. aureus* bacteria. Inhibition zone that produced methanol extract of *Chlorella sp.* was 9,9 mm toward *E. coli* and 12,0 mm toward *S. aureus* bacteria.
4. Active compound group methanol extract of *Chlorella sp.* biomass were steroid and tanin.

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SYNTHESIS OF SANSALVAMIDE A, A PEPTIDOMIMETIC AS NOVEL ANTI-TUMOR AGENTS, USING OLEFIN METATHESIS VIA MICROWAVE IRRADIATION

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ABSTRACT

Peptidomimetics has been developed and used to improve the biological and mechanical properties of native proteins to increase bio-availability, bio-stability, bio-efficiency and bio-selectivity. Small protein-like molecules, designed to mimic a peptide, typically arise from structural modification of an existing peptide to enhance its stability and biological activity. Based on the physiological properties of proteins, peptide-based therapeutics have been used and give higher overall success rates. Natural linear peptides are often unstable in vivo, however, because they are susceptible to enzyme-catalysed degradation reactions. This reduces their efficacy in many cases. One means of enhancing their in vivo stability is to cyclise them into cyclic peptides. The use of a temporary dicarba bridge to facilitate the formation of macrocyclic peptides has been developed and used to synthesise cyclic sansalvamide peptide analogues. Linear sansalvamide sequences were synthesised where two of the native leucine residues were replaced with non-proteinaceous allylglycine residues and a terminal leucic acid residue was employed to form the target macrocyclic lactone. Ruthenium-alkylidene catalysed alkene metathesis then led to the formation of a bridging alkene. This temporary tether localises the N- and C- termini of the peptide and facilitates subsequent head-to-tail lactone formation. In this study, after backbone cyclisation, the dicarba-bridge was cleaved via a ring opening metathesis reaction to form the monocyclic peptides. The resulting pair of olefinic residues can then be subjected to further metathesis reactions to generate close structural analogues of the natural products. The use of microwave to synthesised cyclic peptide of sansalvamide has been developed to reduce the organic waste and promote green chemistry.

Keywords: *Peptide, Cyclicpeptide, Sansalvamide, olefin metathesis, microwave irradiation, green chemistry*

INTRODUCTION

Peptides, proteins and other natural polymers, have an important role in living organisms. Every living cell contains peptides or proteins where they serve many roles such as functioning as catalysts in enzyme applications (Bruice, 2007; Schmid, 1996). Peptides, especially antibodies, are attractive therapeutics because of their high specificity and potency and low incidence of toxicity (Carey, 1996). Peptidomimetics is a field of

chemistry that aims to improve the biological and mechanical properties of native proteins to increase bio-availability, bio-stability, bio-efficiency and bio-selectivity (Schmid, 1996). Small protein-like molecules, designed to mimic a peptide, typically arise from structural modification of an existing peptide to enhance its stability and biological activity.³ A recent report by a market and technology research firm, Frost and Sullivan, indicated that more than 40 approved peptide-based drugs are in use today (Bruice, 2007, Carey, 1996).

Furthermore, approximately 800 are being developed to treat allergies and cancer as well as Alzheimer's, Huntington's, and Parkinson's diseases, and more than 400 are in advanced preclinical trials worldwide (Carey, 1996)

Based on the physiological properties of proteins, peptide-based therapeutics have received enormous attention from pharmaceutical companies due to their high potency, bio-reactivity, specificity, safety, and higher overall success rates (Carey, 1996). Peptides can be formulated to provide therapeutic agents which are stable and have effective bio-availability. Peptides typically offer low toxicity and high specificity, and demonstrate fewer toxicology issues than other small-molecule drugs.¹ Protein drugs also have chemical and biological diversity, low drug-drug interaction, low accumulation in organs and tissues and low non-specific binding to non-target receptors (Hu, 2009)

Natural linear peptides are often unstable *in vivo*, however, because they are susceptible to enzyme-catalysed degradation reactions. This reduces their efficacy in many cases. One means of enhancing their *in vivo* stability is to cyclise them into cyclic peptides (Bruce, 2007)

Cyclic Peptides Involving Lactones

Linear peptides can be cyclised via $N \rightarrow C$ backbone cyclisation involving terminal amino and carboxylic groups to give lactone-containing cyclic peptides. In addition, lactones can also be produced from the cyclisation of sidechain residues (Craig, 2006). Reaction of a terminal

carboxylic acid with a hydroxyl group in a hydroxyl-containing amino acid (e.g. serine and threonine) leads to lactone containing cyclic peptides, for example Sansalvamide A.

Cyclic peptides are commercially attractive because they have chemical diversity, can be efficiently synthesized, have defined three dimensional structures, and tend to have greater binding affinity for protein targets than their linear or small molecule counterparts.⁷ This can be attributed to the restricted bond rotation throughout their conformationally constrained ring systems. Cyclic peptides also degrade much more slowly than linear peptides because proteases have difficulty cleaving amide bonds located within the macrocycle. Cyclic peptides are also effective at penetrating cell membranes and can be stable within cells.⁷

Hence, cyclic peptides have become very common as protein based drugs because they exhibit substantial resistance to proteolytic degradation. Some also survive the human digestive tract (Craig, 2006). Cyclic peptides can also be formed through the reaction of sidechain residues and the peptide termini, e.g. condensation of a threonine residue with the *C*-terminus of the peptide. Sansalvamide A, a cytotoxic peptide first isolated by Belofsky from a marine fungus, is a cyclic depsipeptide (Figure 1). This natural product has been structurally modified to enhance bioactivity to provide novel antibiotic, antitumor and anti-inflammatory agents (Otrubova, 2008)

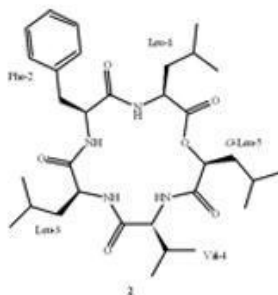


Figure 1. Structure of Native Sansalvamide A

Cyclisation of Peptides Using Olefin Metathesis

Macrocyclic peptides with enhanced stability can be prepared by ring-closing metathesis of olefin-containing peptides *via* the installation of metabolically-inert dicarba bridges below (Illesinghe,2006).

The olefinic residues undergo metathesis when exposed to ruthenium alkylidene catalysts (Scheme 1) (Illesinghe,2006). Several Ru-containing catalysts have been developed and two widely used and commercially available ones are shown

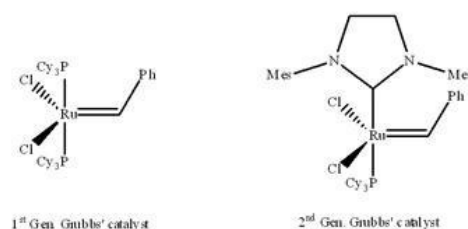


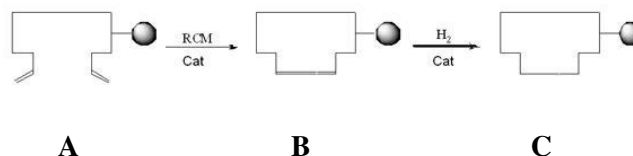
Figure 2. Ruthenium-alkylidene metathesis catalysts³

(Cy = cyclohexyl, Mes = mesitylene)

Catalytic Routes to Enable Replacement of Disulfide Bridges *via* Metathesis and Hydrogenation

The Robinson/Jackson group have developed a metathesis-hydrogenation procedure

for regioselective incorporation of a cystine isostere into a peptide sequence using an on-resin, two step, tandem metal-catalysed sequence (Scheme 1) (Illesinghe,2006).



Scheme 1. Catalytic strategy for dicarba bond formation⁵

Fmoc-protected allylglycine is readily incorporated into linear peptide sequences *via* standard solid phase peptide synthesis (A). Ru-alkylidene catalysed ring closing metathesis (RCM) is then used to metathesise the two alkenes to form the carbocyclic structure (B) *via* microwave irradiation. The initial product of the metathesis possesses an unsaturated dicarba bridge. The newly formed carbon-carbon double bond, which possesses *E*- or *Z*- geometry, is then readily reduced on-resin to give the saturated dicarba analogue (C) (Scheme 1). This two step process has significant advantages over other non-catalytic methods to facilitate a cysteine → dicarba bridge switch (Illesinghe,2006).

Metathesis-Assisted Macrocyclisation

Previous research by the Robinson/Jackson group has also led to the development of methods for achieving peptide macrocyclisation using a metathesis-labile tether (Scheme 2) (Hu, 2009). In

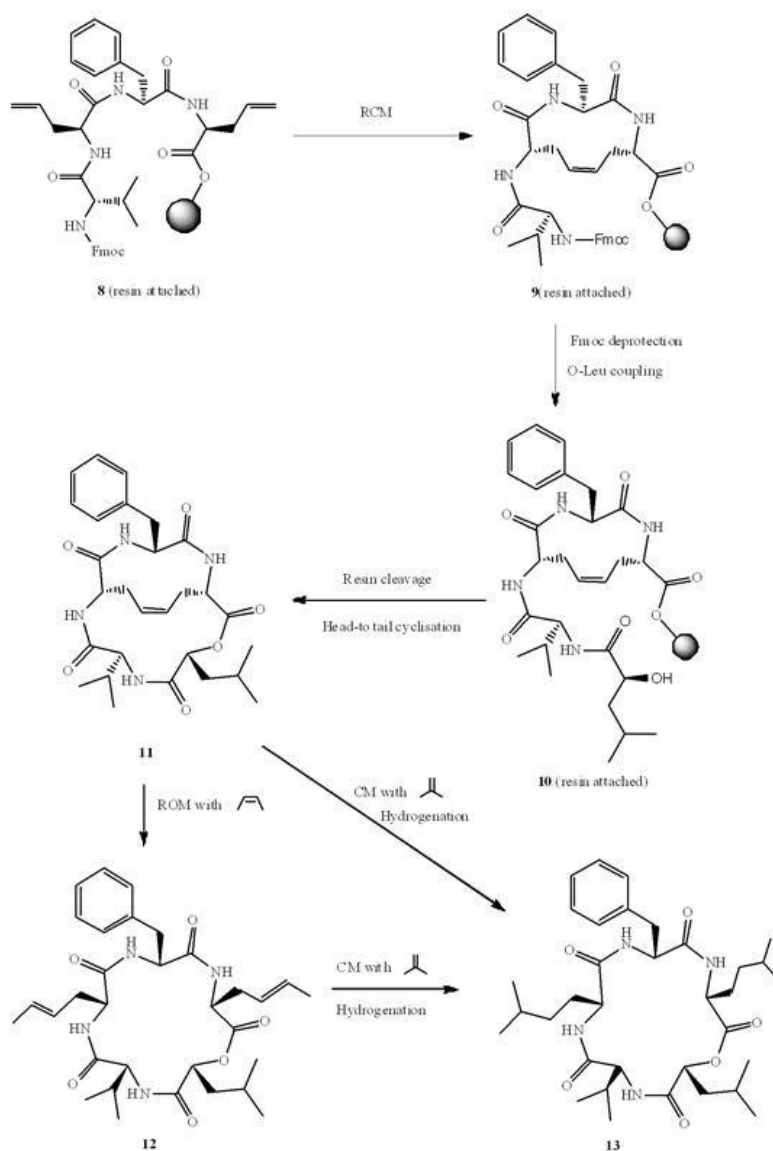
this method, two native residues are replaced with non-proteinaceous olefin-containing residues, such as allylglycine, and these are exposed to ruthenium-alkylidene catalysis. Following olefin metathesis, installation of the dicarba bridge aids localisation of the *N*- and *C*- termini of peptide to facilitate subsequent head to tail lactam formation. After cyclisation, the dehydrosuberic acid bridge can be cleaved open *via* a ring opening metathesis reaction, for example by reaction with 2-butene, to reinstall the olefin sidechains (Scheme 2). These can then be subjected to further reaction to generate native and/or uncoded amino acid sidechains.

The application of an olefinic tether was approach for the formation of the cyclic lactone, Sansalvamide A, by replacing two leucine residues from native Sansalvamide A 2 sequence (Fig. 1) with allylglycine. Post-metathesis of resin-bound

peptide **8**, the newly installed alkene bridge in **9** can be used to promote native lactone formation after installation and cyclisation of the leucic acid residue in **10** onto the C-terminus. Once formed, reaction of bicyclic **11** with isobutylene, followed by hydrogenation, leads to a close structural analogue **13** of native Sansalvamide A **2**. Alternatively, a three step process of ring opening

of **11** by butenolysis with 2-butene, cross metathesis of **12** with isobutylene, and then hydrogenation, could also be used to achieve the same outcome (Scheme 2).

Some of these analogues may retain or possess superior biological activity to their native counterpart.



Scheme 2. Metathesis-assisted lactonisation

DISCUSSION

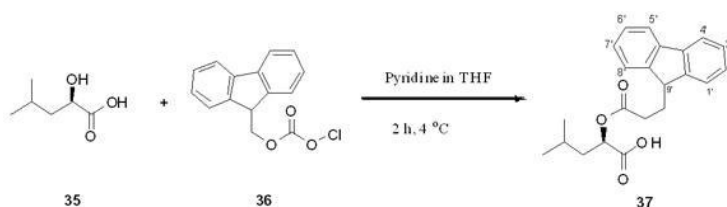
Synthesis of a Sansalvamide A Analogue

Sansalvamide A is a cyclodepsipeptide isolated from a marine fungus (*Fusarium sp*) which exhibits anti-tumor activity.¹² Native Sansalvamide A consists of four amino acids

residues (two leucine, one phenylalanine, one valine) and one hydroxy acid (leucic acid). This chapter investigated the application of the olefinic tether approach to form cyclic lactone derivatives of smaller residues (5 residues) of cyclic peptide. The strategy was to replace two of the native leucine residues with allylglycine to install the

tether and to accomplish lactonisation *via* reaction of the C-terminus with a sequence-installed leucic acid residue. Following macrocyclisation, the tether would be dismantled *via* a ring opening metathesis reaction and converted into homoleucine residues *via* CM with isobutylene and subsequent hydrogenation.

Preparation of Fmoc-protected Leucic acid Single Step method: Preparation of Fmoc-protected α -Hydroxy acid (Synthesis of (*S*)-2-(9'-Fluorenylmethoxycarbonyloxy)-4-methyl-pentanoic acid (Fmoc-*O*-Leu) 37

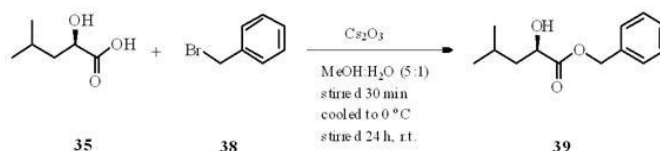


Scheme 3. Synthesis of (*S*)-2-(9'-Fluorenylmethoxycarbonyloxy)-4-methyl-pentanoic acid 37

Reaction of commercially available leucic acid **35** with fluorenylmethoxycarbonyl chloride (Fmoc-Cl, **36**) according to a procedure described by Kuisle *et al.* gave the target Fmoc-protected alcohol **37** albeit in low yield (54%) (Scheme 20).¹² Formation of desired Fmoc-protected leucic acid **37** was supported by ¹H n.m.r. spectroscopy with presence of signals at δ 7.21, 7.30, 7.53, and 7.65, integrating for one proton. These resonances correspond to the aromatic, CH₂O and H9' protons of the attached Fmoc-protecting group respectively. The poor recovery of **37** was

attributed to its high water solubility and poor extraction. Continuous extraction, however, did not improve the recovery. To improve on the yield of **37** an alternative strategy involving initial protection of the carboxylic acid functionality was investigated.

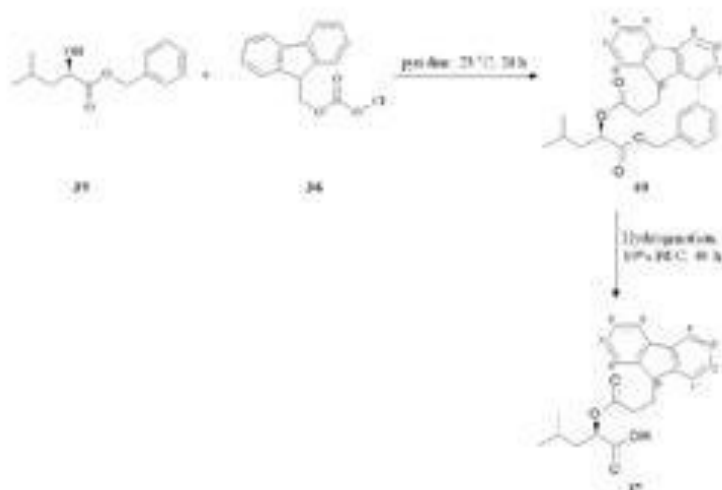
Three step method: Preparation of Fmoc-protected α -Hydroxy acid (Synthesis of (*S*)-2-(9'-Fluorenylmethoxycarbonyloxy)-4-methyl-pentanoic acid (Fmoc-*O*-Leu) 37



Scheme 4. Synthesis of leucic acid benzyl ester 39 (*O*-Leu-OBn)

Benzyl bromide **38** was reacted with leucic acid **35** according to a procedure by Ghosh and Bischoff to prepare (*S*)-leucic acid benzyl ester (*O*-Leu-OBn) **39** in high yield (98%) (Scheme

21).¹² Protection of the leucic acid *via* esterification of **35** was deemed necessary to increase the yield of the following reaction to Fmoc-protect the hydroxyl group.

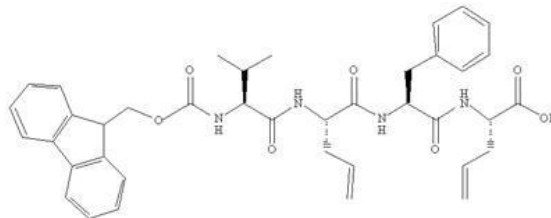


Scheme 5. Synthesis of (S)-2-(9'-Fluorenylmethoxycarbonyloxy)-4-methyl-pentanoic acid 37

The Fmoc-protected α -hydroxy acid (Fmoc-*O*-Leu) **37** was prepared according to a procedure described by Seufert.¹³ The synthesis of **37** was achieved through the reaction of the previously prepared leucic acid benzyl ester **39** with fluorenylmethoxycarbonyl chloride (Fmoc-Cl, **36**) in high yield (90%) (Scheme 5).

Gratifyingly, with the carboxylic acid protected, this transformation proceeded in excellent yield. Subsequent hydrolysis removed the benzyl protecting group and generated the desired product **37** in 74% yield (Scheme 5).

Synthesis of the Linear Peptide 8



8 (resin attached)

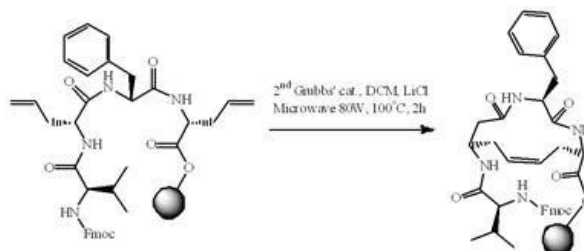
Figure 3. The linear, tetrapeptide sequence of Sansalvamide A analogue 8 (resin attached)

The modified Sansalvamide A sequence was synthesised using Fmoc-protected amino acids and the same SPPS method previously employed to generate Mahafacyclin analogues. Native Sansalvamide A **2** consists of four different amino acid residues, 2 leucines, one valine, one phenylalanine and one hydroxyl acid, leucic acid ((*S*)-2-hydroxy-4-methylpentanoic acid; *O*-Leu).

In this synthesis, two of the leucine residues were replaced by L-allylglycine, to create an olefinic tether for the promotion of lactone formation. Wang resin, a polystyrene-based solid support, was used as the solid phase to synthesise Sansalvamide A analogue sequence. The first amino acid (Fmoc-Agl-OH) was attached to the

resin via esterification reaction. Diisopropylcarbodiimide (DIC) was used as an activator for the amino acid, with dimethylaminopyridine (DMAP) as a base for proton capture. A small sample aliquot of peptidyl-resin was cleaved for mass spectral analysis to confirm attachment of the residue. Mass spectral analysis showed a peak at m/z 360.0 [$M_{21} + Na$]⁺, C₂₀H₁₉NNaO₄ requires 360.1, corresponding to the charged molecular ion of Fmoc-Agl-OH **21**. After attachment of the first amino acid, the resin was reacted with a capping solution (5% acetic anhydride, 94% DMF and 1% NMM) in order to esterify free hydroxyl groups and convert any free amines into amides. Standard coupling procedure was then used to couple the remaining amino acid

residues. After four coupling cycles, a small aliquot of peptidyl resin was removed and cleaved. The crude peptide was subjected to mass spectrometry which showed that synthesis of the target linear tetrameric peptide **8** had been successful (Figure 9); peaks at m/z 681.4 and 703.4 were representative of required $[M8 + H]^+$, $C_{39}H_{45}N_4O_7$ requires 681.3, and $[M8 + Na]^+$, $C_{39}H_{44}N_4NaO_7$ requires 703.3, ions for sequence **8**.



Scheme 5. Ring closing metathesis of Sansalvamide A analogue **8**

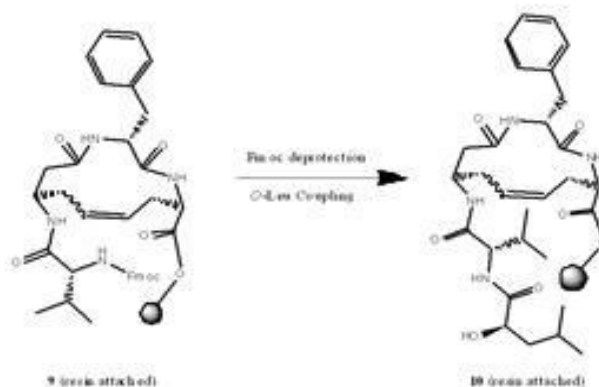
Capping with 5% acetic anhydride, 94% dimethylformamide (DMF) and 1% NMM was performed before RCM reaction to cap any potential free hydroxyl groups on the resin. Ring closing metathesis (RCM) was performed on the resin-attached tetrapeptide **8** to form the target dicarba bridge and cyclic peptide **9**. Once again, microwave irradiation was used to promote the cyclisation reaction.

The reaction mixture containing the resin supported linear peptide **8** and 2nd generation Grubbs catalyst was assembled in a drybox in **O-Leu Coupling**

The mass spectrum was devoid of peaks corresponding to deletion products. An analytical RP-HPLC chromatogram verified that these reaction conditions led to 82% conversion to the desired product **8** relative ($t_R = 14.4$ min) relative to the other minor unknown impurities.

Microwave-Accelerated Ring Closing Metathesis of the Linear Peptide **8**

degassed DCM/DMF and LiCl. The LiCl was used to reduce peptide aggregation during the catalysis and RCM was performed using a microwave radiation at 80 watts and 100 °C for two hours. A small aliquot of peptidyl resin was removed and subjected to the standard Fmoc-deprotection and cleavage conditions. Mass spectral analysis (ESI⁺) on the crude product confirmed the formation of the peptide **9** with the appearance of peaks at m/z 431.1 $[M9 + H]^+$, $C_{22}H_{31}N_4O_5$ requires 431.2. The Fmoc-deprotected starting material **8** peak at m/z 459.2 $[M8 + H]^+$ was negligible.



Scheme 7. O-Leu Coupling

After Fmoc-deprotection of **9**, leucic acid ((*S*)-2-hydroxy-4-methylpentanoic acid; *O*-Leu **35**) was coupled to the resin attached monocyclic peptide **9**. Coupling of leucic acid (*O*-Leu) to the free amine on the resin to form an ester bond required the use of two equivalents of HATU as an activator and six equivalents of NMM (as an activator base). Following the allocated reaction

period, a small portion of the peptidyl-resin was Fmoc-deprotected and cleaved for evaluation. Surprisingly, mass spectral analysis revealed a strong molecular ion peak at m/z 431.2 $[M9 + H]^+$, $C_{22}H_{31}N_4O_5$ requires 431.2, corresponding to the charged molecular ion of the starting material **9** and only a small peak at m/z 545.3 $[10 + H]^+$, $C_{28}H_{41}N_4O_7$ requires 545.2, corresponding to the

charged molecular ion of the product **10**. A second *O*-Leu **35** coupling was performed to improve conversion to the product, yet mass spectral analysis of a small cleaved sample still showed peaks at m/z 431.2 [$M_9 + H$]⁺, corresponding to the starting material **9**, in addition to the required m/z 545.2 [$M_{10} + H$]⁺ for product **10**. Low *O*-Leu coupling was also confirmed by RP-HPLC analysis of the reaction mixture. Considerable time was spent trying to improve this reaction: reaction conditions, and activating agents were all systematically changed without positive change in coupling yield.

An alternative strategy was then investigated to improve access to the target peptide **10**. The protected Fmoc-*O*-Leu **37** prepared above was coupled to the Fmoc-deprotected peptidyl-resin **9** using two equivalents of HATU (as an activator) and six equivalents of NMM (as an activator base). Following an allocated reaction period, a small portion of the peptidyl-resin was Fmoc deprotected and cleaved for evaluation. Mass spectral analysis revealed an ion peak at m/z 431.2 [$M_9 + H$]⁺ which corresponded to the charged molecular ion of the starting material **9**. A second Fmoc-*O*-Leu **37** coupling to the free amine on the resin using two equivalents of DIC (as an activator) and two equivalents of DMAP (as an activator base) was also unsuccessful; mass spectral analysis of a small cleaved sample only showed starting material **9** and minor conversion to **10**. Reasons for this coupling failure were not determined.

It was hypothesised that the cause of the original incomplete *O*-Leu **35** coupling reaction

Head-to-tail Cyclisation

was due to competing side reaction involving the self-condensation of the activated *O*-Leu residue **35**. In order to validate our hypothesis and prevent/minimise this reaction, the HATU and NMM-activated acid **35** was exposed to the peptidyl-resin without delay (i.e. no pre-activation phase of the incoming residue before addition to the peptidyl resin). MS and LC analysis of reaction mixtures showed that though this approach was more successful, the starting peptide **9** was still the major species present in the reaction mixture.

Finally, a different strategy of *O*-Leu coupling was investigated following the procedure described by Spengler *et al.*¹⁴ This approach employed two equivalents of leucic acid (*O*-Leu) **35**, two equivalents of DIC and two equivalents of HOBt. The pre-activation of *O*-Leu **35** with DIC and HOBt was performed for 17 minutes before being exposed to the peptidyl-resin. After 3 h, a small aliquot of the peptidyl-resin was cleaved for evaluation. Mass spectral analysis revealed a strong molecular ion peak at m/z 545.3 [$M_{10} + H$]⁺, $C_{28}H_{41}N_4O_7$ requires 545.2, corresponding to the charged molecular ion of the target product **10**. An analytical RP-HPLC verified that these reaction conditions led to 90% conversion to the desired product **10**.

The crude peptide **10** was purified by preparative, RP-HPLC using a C8 column and *E*- and *Z*- isomers were combined. Mass spectral analysis of the purified peptide showed a strong molecular ion peak at m/z 545.1 [$M_{10} + H$]⁺, $C_{28}H_{41}N_4O_7$ requires 545.2, corresponding to the singly charged molecular ion of the required peptide **10**.



Scheme 8. Head-to-tail cyclisation reaction

The coupled *O*-Leu containing peptide **10** was cleaved from the resin and head-to-tail cyclisation attempted on crude material. A stirred solution of **10** in DMF under an atmosphere of

nitrogen was cooled to 0 °C and treated with FDDP and DIPEA, then warmed to room temperature and stirred for three hours. Mass spectral analysis of the product **11** showed a

molecular ion peak at m/z 527.3 [$M_{11} + H$]⁺, which correspond to the charged molecular ion of the target cyclic peptide **11**, but this ion was also accompanied by a molecular ion (m/z 545.3 [$M_{10} + H$]⁺, $C_{28}H_{41}N_4O_7$ requires 545.2) corresponding to the starting material **10**. Further attempts were made to improve the head-to-tail cyclisation but all failed to give complete conversion to the target bicyclic Sansalvamide A analogue **11**.

Synthesis of the depsipeptide framework of Sansalvamide A *via* the tether approach used for the construction of the Mahafacyclin analogues was not straightforward. Despite considerable effort, a high yielding synthesis of the bicyclic lactone **11** was not achieved. Unfortunately, it is currently unclear whether this arises from detrimental, rather than beneficial, placement of the tether. Acylation of the α -hydroxyl group in the leucic acid residue is the critical step in achieving macrocyclisation and was found to require a more potent reagent to achieve carboxyl group activation. Additionally, once formed, the

ester may have been highly sensitive to hydrolysis. Taking both of these points into consideration, we decided to use the tether strategy to generate the more stable Sansalvamide A amide derivatives. This approach required that the leucic acid residue be replaced by L-leucine.

Synthesis of Sansalvamide A Amide **18** Synthesis of Pentapeptide **14**

Due to the lack of success in making the target lactone analogues of Sansalvamide *via* our tether strategy, we turned our attention to the preparation of lactam analogues of the natural product. We postulated that replacement of the native leucic acid residue with L-leucine would be well tolerated and also potentially provide an easier synthesis of Sansalvamide analogues. Using the strategy previously outlined in Scheme 7 we proposed to install a temporary dicarba tether to facilitate head-to-tail $N \rightarrow C$ coupling. Following macrocyclisation, the tether would then be cleaved and the resulting pair of olefinic sidechain into homoleucine residues.

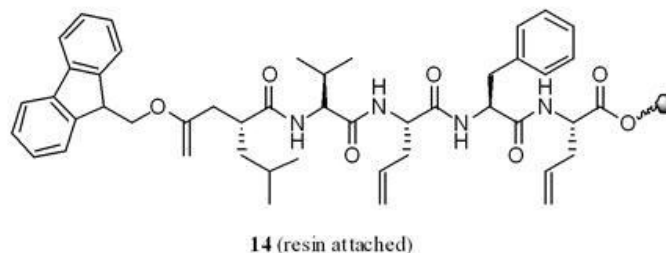


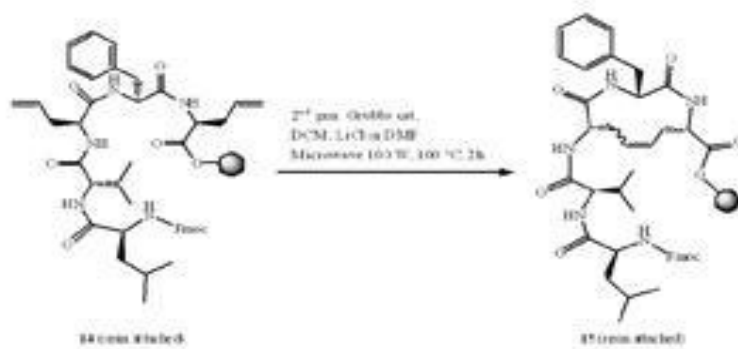
Figure 4. The linear pentapeptide of Sansalvamide A amide analogue 14

The sansalvamide A pentapeptide **14** was synthesised using Fmoc-protected amino acids and solid phase peptide synthesis (Figure 4). This was achieved *via* manual automatic SPPS techniques. In this peptide, two of the native leucine residues were replaced by L-allylglycine and the leucic acid was replaced by L-leucine. Wang resin was used as the solid support.

This first amino acid (Fmoc-Agl-OH) was attached to the resin *via* an esterification reaction. Diisopropylcarbodiimide (DIC) was employed as the activator in the presence of a base, dimethylaminopyridine (DMAP). Following the allocated coupling period, a small sample of the peptidyl-resin was cleaved to confirmed attachment of the first residue. After the first

amino acid was attached, the resin was reacted with a capping (5% acetic anhydride, 94% DMF, and 1% NMM) in order to esterify free hydroxyl groups and convert any free amines into amides. Standard coupling procedures were then used for the remaining amino acid residues. A small aliquot of resin was removed, Fmoc-deprotected, and cleaved. The resultant crude peptide was analysed by mass spectrometry which showed a strong molecular ion peak for desired product at m/z 572.3 [$M_{14} + H$]⁺, $C_{30}H_{46}N_5O_8$ requires 572.3. An analytical RP-HPLC verified that the crude peptide ($t_R = 13.3$ min) was of acceptable purity to progress to the planned catalysis steps.

Microwave-Accelerated Ring Closing Metathesis of the Linear Peptide **14**



Scheme 9. Ring closing metathesis of 14 → 15

Capping with 5% acetic anhydride, 94% DMF and 1% NMM was performed prior to RCM to cap any deprotected amine functionality. Ring closing metathesis (RCM) was performed on the resin-attached pentapeptide **14** to form a dicarba bridge and the target carbocycle **15**. Microwave irradiation was used to increase the rate of reaction, relative to that of conventional heating, and to disrupt aggregation and allow catalyst penetration. The reaction mixture containing linear peptide **14** and 2nd Generation Grubbs catalyst was assembled in a drybox in degassed DCM/DMF and LiCl. The RCM was performed using a microwave radiation at 100 watts and 100 °C for two hours.

At the end of the reaction, a small sample of the petidyl-resin was treated with 20% piperidine in DMF and cleavage solution. The deprotected and cleaved crude peptide was then subjected to mass spectral analysis (ESI+). The analysis confirmed the formation of the dicarba bridge between the two L-allylglycine residues with appearance of new ions of m/z 544.1 [**M15** + H]⁺, C₂₈H₄₂N₅O₆ requires 544.1, corresponding to the charged molecular ions of the *E*- and *Z*-isomers of cyclic peptide **15**.

Head-to-tail Cyclisation



Scheme 10. Head-to-tail cyclisation reaction

The purified monocyclic peptide **15** was cleaved from the resin and head-to-tail cyclisation was attempted according to procedure describe by Sayyadi, Skropeta and Joliffe.¹⁵ A stirred solution of **15** in DMF under an atmosphere of nitrogen was cooled at 0 °C and treated with FDDP and DIPEA, then warmed to room temperature. After three hours, a small portion of the reaction mixture was removed and diluted with water for assessment by analytical RP-HPLC and MS. The LC trace showed only the starting material **15** and mass analysis of this peak showed only the molecular ion peak for **15** (m/z 544.2 [**M15** + H]⁺, C₂₈H₄₂N₅O₆ requires 544.3). Unfortunately, longer

reaction times, up to 5 days, failed to generate the target bicyclic peptide **16**. Due to time constraints, this reaction could not be fully examined. In the future, cyclisation and the *E*- and *Z*-isomers of **15** should be independently investigated to determine whether the stereochemistry of the tether can play a role in the macrocyclisation reaction. A full assessment of activating agents should also be investigated to achieve this end. Additionally, molecular modelling could be used to determined the optimum location of the tether for the promotion of head-to-tail ring closure.

The use of olefin metathesis in peptidomimetics research has emerged as a powerful tool for enhancing bioactivity in naturally occurring peptides. Reasons for its popularity include its high functional group tolerance, high chemical selectivity, and the commercial availability and ease of use of the ruthenium alkylidene metathesis catalysts. Shortcomings, such as the need for high catalyst loading are slowly being rectified with the use of aggregation disrupting strategies, including microwave irradiation and chaotropic salts, as well as higher activity second generation catalyst. Olefin metathesis now provides a highly versatile method for the regioselective ligation of peptides, replacement of metabolically unstable bonds (such as disulphide bridges), the stabilisation of bioactive secondary structure (such as α -helices) and the tethering of small molecules for requirements such as imaging. In this thesis we investigated the use of olefin metathesis to strategically enhance peptide macrocyclisation yield, an inherently difficult process, and expediently generate peptide libraries of native cyclic peptides.

This thesis has demonstrated that olefin metathesis can be used to construct head-to-tail cyclised peptide esters via sequence installation of metathesis-active residues. Cross metathesis of the sequence installed olefinic residues generates a C=C based tether which can be used to promote $O \rightarrow C$ coupling. The peptide sequence and

individual amino acid residues of the peptide can strongly influence the success of this process. Further work is therefore needed to determine the scope and limitation of this approach.

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Aprocessing Heavy Metal In River Water Trough Coagulant – Flocculation Process Using Moringa Seeds (*Moringa oleifera*) or “ biji kelor” as Biokoagulan

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ABSTRACT

The effects of metal ions causes reddish water , whereas manganese oxide causes brown or Blackish water. The effects of manganese occur mainly in the respiratory tract and brain. Magnesium takes the important rules on enzyme systems in the body . that is why, before being used, river water need to be processed first to be able to qualify as clean water. This study uses a coagulation - flocculation reactor- continuous sedimentation stream with coagulant dose variation 1 mg / l and 2 mg / l as well as variations in particle size measure of Moringa seeds powder (*Moringa oleifera*). 100 mesh dan 140 mesh.the sample were taken from one location of river where used coagulants that have high effectiveness and eco - friendly i.e Moringa Seeds (*Moringa oleifera*) .the results showed that the coagulant of Moringa Seeds (*Moringa oleifera*) , is able to reduce 80,7 % of iron and 4,71% of manganese in the river water. The highest degradation percentage occurred in coagulant dose of 2 mg / l and a particle size of 140 mesh of Moringa seeds powder (*Moringa oleifera*) .

Keywords : River Water, Iron , Moringa seeds (*Moringa oleifera*) , coagulation - flocculation - sedimentation , Manganese

INTRODUCTION

Water is a source of vital necessity for the survival of living things, but when the water has been polluted where there are substances or other components put it into the water by humans or natural activity would cause degradation of water quality to the certain level so that water cannot be used well. Government rule No 82 tahun 2001 about water quality management and water pollution control. The obstetrical of iron and manganese in the water are dangerous for health. If those substances are in the water, they would cause discomfort, blemishes, and problems because iron and manganese are chemically similar. they cause the same problems. Iron will cause reddish brown stains on clothes, porcelain, dishes, utensils, and even glassware. Manganese acts in a similar way but causes a brownish black stain. Soaps and detergents cannot eliminate these stains and the using of bleaching fluid only will add the stain. The Accumulation of iron and manganese would be economic problems if the pipes and equipments must be replaced. Energy would also be more wasteful, due to the extra energy is required for pumping through smaller pipes due to deposition of iron or manganese (J Water).

One alternative to physical and chemical processing that is easy to operate and inexpensive is coagulation – flocculation. Coagulation can be defined as the process of destabilization of colloids and particles in the water with the use of a coagulant that causes the formation of clumps of nuclei (precipitates) (Slamet and Masduqi, 2000). in the coagulation process, if the coagulant is added to the water, it will cause colloidal destabilization and formed flocculent particles. Flocculation is the process of merging core of flocks to become larger flocks. (Masduqi and Slamet, 2002). Flocculation process is a continuation of the process of coagulation.

Flocculation process occurs when there is a slow stirring the agitation process in the formation of flocks. Mechanism of coagulation - flocculation process in water electrically charged colloidal similar particles (both negative) repel each other so as not to come closer together and the conditions in which the particles remain in this place called stable condition. Stable conditions do not allow the formation of flocks. If the water is supplied with the positively charged metal ions, the positive charge can reduce power among repel colloids (Repulsion force), so that there will be conditions for destabilization of the particles. Unstable colloidal particles allow flock formation. The Existence of positive charge will form a small flock corps of colloids. In order can be precipitated, small flocks must be always concurrent until become bigger enough to be in precipitated. Sometimes given a positive charge is not able to incorporate small flocks because of destabilization conditions (back to stable) so it is difficult to continue merging into a fairly large flock. This problem can be solved by giving the flocculants. Small flocks will be bounded by flocculants because it has “long arms” as a set of threads. From the description above it is clear that the mechanism of coagulation and flocculation can occur sequentially and can also occur simultaneously making it difficult to distinguish between the two processes. Widely used Sugiharto (1987) coagulant in water treatment is a chemical coagulant such as alum and PAC, however it produces mud / sediment that still have chemical elements that can harm the environment if dumped directly onto the ground or into the waters, therefore, it should be used the coagulants which are not influent sediment and water that is by using Moringa seeds coagulant (*Moringa oleifera*). Moringa seeds (*Moringa oleifera*) contains the active substance rhamnosyloxy - benzyl - isothiocyanate,

which is able to adopt and neutralize the mud particles and metals contained in waste water suspension. with particles of dirt floating in the water . Moringa seeds have a low molecular weight and positively charged . Additionally moringa seeds that have been made to powder can be easily dissolved in water and in solution. Moringa seeds contain 0,086 % iron and 0,008 % manganese. If it is seen from the components . the seeds of Moringa meet the criteria for a substance that can hold a bond in an electrostatic attraction to other particles Leuvinadrie (2005) . Moringa seeds also contain substances that are bactericidal , therefore , the study is conducted to treat river water using more economical coagulant , that is using moringa seeds as shown in the following picture :



Figure 1. Trees and fruits of moringa

Research Objectives

1. How big is the ability of Moringa coagulant (*Moringa oleifera*) in reducing the levels of iron and manganese on river water?
2. How much the dose of coagulant and Optimum Size measure of Moringa (*Moringa oleifera*) in reducing the levels of iron and manganese in the water stream?

MATERIAL & METHOD

Samples Of Water

Water samples which is used in the research is taken from the Metro River in Mergan Bandulan Sukun Malang. Alaerts dan Santika (1984).

Coagulant Materials

Moringa seeds (*Moringa oleifera*) as a coagulant treatment previously as follows:

- a. Moringa seeds (*Moringa oleifera*) selected the ones which are ripe on trees
- b. then it is dried until the seeds of Moringa (*Moringa oleifera*) dry
- c. Moringa seeds (*Moringa oleifera*) are then grinded and sieved with a mesh size of 100 and 140 mesh
- d. Make a solution of Moringa seeds (*Moringa oleifera*) with a dose concentration of 1 mg / l and 2 mg / l
- e. Moringa seed powder is placed in a closed, dry and clean place to avoid contact with air.

Equipments

Series of coagulation-flocculation equipments as a waste treatment reactor consists of the following parts :

- a. Sump container
plastic barrel with a capacity of ± 45 liters of water are used to accommodate the stream to be processed. River water is flown by using a hose, which include discharge valve to regulate the flow.
- b. coagulant container
plastic barrels with a capacity of ± 4 liters of a solution that are served as a coagulant. Coagulant is flown on gravity by using a plastic hose equipped with a valve to regulate the flow.
- c. Coagulation basin
Made of glass with a capacity of around 1 liter which has the following dimensions:

- Length: 10 cm
- Width: 10 cm
- Height: 8 cm

The basin is equipped with a paddle-type stirrer with 2 blades and motor machine which has rotation speed of 200 rpm.

d. flocculation basin

The basin's capacity is 15 liters made of glass equipped with a paddle-type stirrer with 2 blades and motor machine which has rotation speed of 20 rpm. The dimensions are as follows:

- Length: 22 cm
- Width: 22 cm
- Height: 31 cm

e. Sedimentation basin

Sedimentation basin used is made of glass which has a capacity of 25 liters with a mud room with a volume of 3 liters. Placed at the outlet zone valve that is used to take a sample to be analyzed.

Sketching and equipments drawing Dake (1985) can be seen in **Fig. 2**:

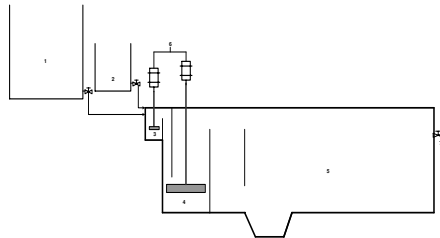


Figure 2. Coagulation-Flocculation Equipments

Research Variables :

1. Iron and Manganese.
These parameters are part of the parameter corresponding by Health ministry rules No. 416/MENKES/PER/IX/1990.
2. Coagulant dose of 1 mg / l and 2mg / l
3. Rapid stirring speed: 200 rpm, with rapid stirring time: 1 min.
4. Slow stirring speed: 20 rpm, with slow

- stirring time: 30 minutes.
- 5. Deposition time: 60 minutes.

Research Stages

Preliminary research

At the beginning of the study conducted a preliminary analysis to determine the initial condition of the river water to be treated. The parameters analyzed were iron and manganese levels.

Continuous Process How it Works:

In this process is done by running a series of tools coagulation-flocculation-sedimentation Reynolds (1982).. Here's how to work for a continuous process:

- a. prepares a water sample that will be processed. Analyze the initial content of iron and manganese levels in the river water.
- b. Prepares coagulant solution with suitable coagulant concentration which are compatible with variation dosage and mesh.
- c. Then put it in the coagulant tank.
- d. flow River water ($Q = 0.5 \text{ ltr / min}$) and coagulant ($Q = 262 \text{ ml / min}$) into the coagulation basin in gravity with a rotation speed of stirring 200 rpm for 1 minute.
- e. Rapid stirring, flows in gravity into a slow stirring basin with stirring rotation speed of 20 rpm for 30 minutes.
- f. slow stirring basin flow in gravity into sedimentation basin and settling for 60 minutes
- g. Measuring the pH of the waste processed once every 60 seconds in each processing basin.
- h. Taking samples from the outlet valve sedimentation.
- i. Analyze the levels of Iron and Manganese after processing.
- j. Repeating steps c to i with dose variation and different mesh

Research framework:

Research frameworks of Iron and Manganese Levels decline in river water using Moringa seed coagulant (*Moringa oleifera*) shown in **Fig. 3** below :

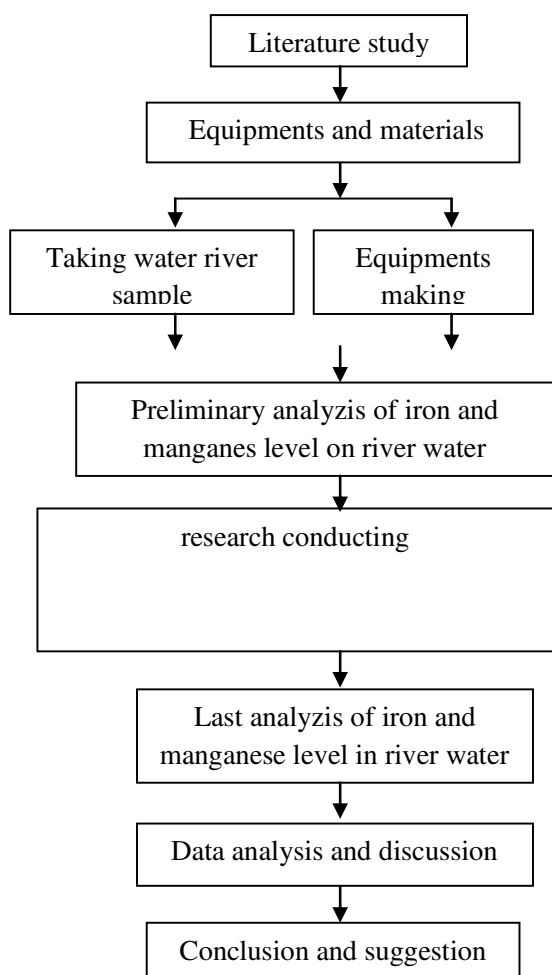


Figure 3. Research framework

RESULTS AND DISCUSSION

Preliminary Research Results

Preliminary research done first to determine levels of Iron and Manganese early on river water is shown in **Table 1** below:

Table 1. Concentration Value Content of Iron and Manganese In Water River Research Introduction

No	Parameter	Concentration (mg/l)
1	Iron	4,25
2	Manganese	0,85

Sources: (Research result)

Results After Treatment

Table 2. Concentration Value Content of Iron and Manganese Water River In Coagulation Flocculation-Sedimentation Process

Coagulant dose (mg/l)	Coagulant measure (Mesh)	Iron concentration (mg/l)	Manganese concentration (mg/l)
1	100	2,66	0,76
	140	1,55	0,61
2	100	1,32	0,58
	140	0,82	0,47

Sources: (Research result)

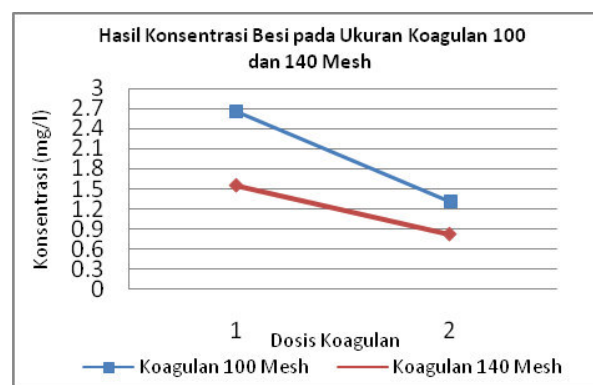


Figure 4. Iron concentration in the coagulant of 100 and 140 Mesh

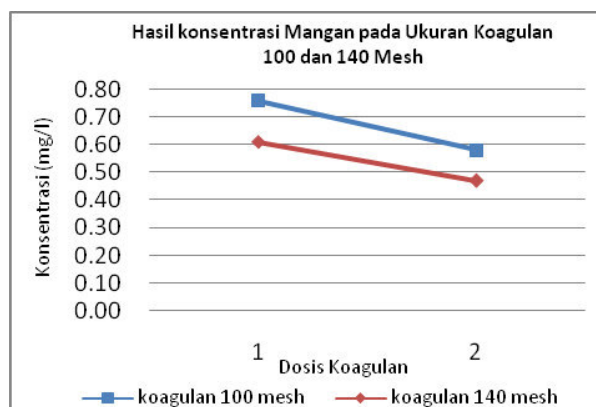


Figure 5. Manganese concentration in the coagulant of 100 and 140 Mesh

Discussion On Preliminary River Water Research

Based on **Table 1** note that the concentrations of iron and manganese exceed Quality Standards of the Health Ministry Regulation of Indonesia Number 416/MENKES/PER/IX/1990 where exposure limits Iron 1 mg / l and manganese 0.5 mg / l

The value concentration discussion of Iron and Manganese In Coagulation-Flocculation Process River Water

Iron and manganese reduction percentage based on Table 2, the smallest value between coagulant dose of 1 mg / l and 2 mg / l was at the addition of coagulant dose of 2 mg / l. Addition of coagulant dose variation of moringa seeds have the great influence to the degradation percentage of Iron and Manganese in river water. while the percentage of Iron and Manganese best drop upon the coagulant addition of 140 mesh. Particle size affects the exclusion levels iron and manganese allowance because the smaller the particle size, the width of area between coagulant and metal will be even greater.

Heavy metal Iron is one of the chemical elements that can be found in almost every place on earth, in all the geological layers and all water. In general, the existing iron in the water can be dissolved as a salt compound ferric (Fe^{3+}) or salt ferrous (Fe^{2+}); suspended as grains of colloidal (diameter <1 mm) or larger, such as $Fe(OH)_3$, and incorporated by organic substances or inorganic solids.

Floc formation (clot) will be faster progress if accompanied by the addition of a mechanical force such as stirring. Stirring will facilitate the occurrence of collisions, which will be followed by the incorporation of the particles forming large flocks. Large floc formation will facilitate the deposition process. This causes Iron and Manganese concentrations in river water decreased due to a number of material of Iron and Manganese has been settled.

CONCLUSION

1. Moringa coagulant ability (*Moringa oleifera*) in reducing waste levels at 80.7% Iron and 44, 71% Manganese on river water
2. Optimum coagulant dose and size measure of Moringa seeds (*Moringa oleifera*) in reducing the iron levels and manganese in the river water is the optimum dose of 2 mg / l while the optimum size measure is 140 mesh.

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THE EFFECTIVITY OF ETANOLIC EXTRACT SUNFLOWER LEAVES (*Helianthus annus*) AS ANTI-MALARIAL AGENT AGAINST *Plasmodium berghei*

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ABSTRACT

Malaria, an infectious disease with a high mortality rate, encountered efficacy on the decreasing level since its firstline drug that is combination between artemisin and amodiaquine. A traditional herbal medicine, sunflowers (*Helianthus annus*), in fact, have been empirically used as antimalarial agent in Indonesia. Sunflowers leaves contain sesquiterpen lacton that have physiological function as an antimalarial agent. This study aimed to reveal the antimalaria effect of sunflower leaves on the degree of parasitemia of mice infected by *Plasmodium berghei*. Mice were peritoneal infected by 10⁶ *Plasmodium berghei* ANKA and divided into 7 experimental groups: (1) negative control, (2) non-infection control, (3) positive control (chloroquine of dose 5.71 mg/kgBW), (4) Sunflower leaves of dose 0.1 mg /kgBW; (5) Sunflower leaves (henceforth ESF) of dose 1 mg/kgBW, (6) Sunflower leaves of dose 10 mg / kgBW, and (7) Sunflower leaves of dose 100 mg / kgBW. The treatments were started on day 0 when parasitemia degree reached 1-5% and continued for 5 days therapy. The parasitemia observation was carried out on day 0,1,2,3, and 4. The results showed that the extract of sunflower leaves could significantly inhibit the growth of *Plasmodium berghei* (p<0.05) with the result of ED₅₀ is 4.64 mg/kgBW.

Key word : Anti-malarial agent, etanolic extract, Sunflowers leaves, *Plasmodium berghei*

INTRODUCTION

Malaria is considered as one of the infectious diseases spreaded all over the world. Approximately, 2.57 billions or 41% residents in the transmission area are susceptible of being infected by *Plasmodium falciparum*, including Indonesia (Gething *et al*, 2011; WHO, 2011). On the basis of WHO data 2010, 37% Indonesian reside in malaria endemic area, such as: Bengkulu, Kepulauan Bangka Belitung, Central Kalimantan, North Sulawesi, Gorontalo, Central Sulawesi, Maluku, West Nusa Tenggara, East Nusa Tenggara Timur, West Papua, and Papua, with high infectious trasmission level. Other provinces, on the other hand, belong to low-endemic area as well as transmission level of malaria. In addition, the number of cases encountered in 2010 reached 229.829 cases which mortality level was 432 people (WHO, 2010).

The difficulty to overcome malaria problem is closely related to the *Plasmodium's* parasitic ability in forming self-defension toward antimalarial medicine, so that it will be the resistant toward malaria medicine. To be more specific, *Plasmodium falciparum*, the most number of species in Indonesia (53%) (WHO, 2011), have been found resistant toward klorokuin. In 1993, it is reported that *Plasmodium falciparum* was klorokuin resistant in 22 provinces, and even antimalarial drug resistant in 11 provinces (Tjitra, 2004; Fidock *et al*, 2004)

Recently, it is reported that *Plasmodium falciparum* is also resistant toward artemisin. The sensitivity test of dehydroartemisin toward *Plasmodium falciparum* in the form of *in vitro* revealed that there is increasing of IC₅₀ (Noedl, 2008; Pilai *et al*, 2012; Dondorp, 2009). Besides, the artemisin has half time which is very short so that there is recrudescence after the therapy (Sardjono and Fitri, 2007). Due to those recent facts, it is essential to conduct further research and development of antimalarial in order to solve the mentioned problems which one of them is by utilizing traditional *jamu*.

The sunflower leaves (*Helianthus annuus*) consist of sesquiterpen lacton compound (Macias *et al.*, 2002). In the previous study, it is known

that dichlorometan extract is effective in cutting-off the life cycle of *Plasmodium berghei* in 5, 50, 500 mg/kg BB and parasitemia degree reach 0% in the third and fourth day after having therapy in each dose (Muti'ah, *et al.*, 2011). The separation of sunflower leaves and KLTP, then, was continued by identifying isolate using spektrofotometer UV-Vis. FTIR contains steroid compound and sesquiterpen lacton (Bayyinah, 2013) in which sesquiterpen lacton was functionated against parasite in the erythrocyteal phase. The working mechanism of sesquiterpen lacton (artemisin) is through enzim ATPase inhibition depending on calsium (PfATP6). The free radical which is produced by artemisin tied and blocked PfATP6 irreversibly and specifically (Ridley, 2003). The function of ATPase in the complex system of pumping ion ion Na⁺/K⁺ is adjusting the degree of ion in the cell. The failure of PfATP6 function causes the drastic decrease of ion kalium in the cell which endangers the parasite. (Muti'ah, 2012). The current research, then, aimed to reveal the effect of anti-malaria from each ethanol extract of sunflower leaves.

MATERIAL & METHOD

Research Setting

This recent study was conducted in the laboratory of Chemistry Department (Organic Chemistry and Biotechnology Laboratory) and in the Biology laboratory (Animal Physiology, Biosystem, and Optical Laboratory) Faculty of Science and Technology, State Islamic University (UIN) Maulana Malik Ibrahim Malang in February to May 2013.

Plants

The leaves of sunflowers (*Helianthus annuus*) were taken from Punten, Sido Mulyo-Batu.. The powder of each sample was measured in 100 g. Then, it was macerated to get extract by using 80% ethanol solution. The filtrate was concentrated using *rotary evaporator* till the researcher achieved the concentrated extract which then was continued by giving N₂ gas.

Animal Model

This recent study employed animal testees which were forty-eight male mice

(*Mus musculus*), particularly Balb/C j48 species, weight of 21–25 g, age of 8–12 weeks. They were divided into seven experimental groups and fed using standard foods and drink of *ad libitum*.

Research Planning

The researcher used full random experimental planning. Mice were divided into 15 groups that had different treatment (for sunflower leaves and its combination); 8 treatment groups (for anting-anting plants with additional dosage 0,01 mg/kg BB), such as:

1. Negative control group was a group treated by giving *P. berghei* infection without therapy and solely given 0,5 mL CMC-Na 1%.
2. Positive control group was Chlorokuin group using 71 mg/kg BB dose once a day per oral (Sukandar, 2011).
3. ESF_1 was a group which was given infection and 80% ethanol extract sun flower leaves with 0,1 mg/kg BB dose once a day per-oral.
4. ESF_2 was a group which was given infection and 80% ethanol extract sun flower leaves with 1 mg/kg BB dose once a day per-oral.
5. ESF_3 was a group which was given infection and 80% ethanol extract sun flower leaves with 10 mg/kg BB dose once a day per-oral.
6. ESF_4 was a group which was given infection and 80% ethanol extract sun flower leaves with 100 mg/kg BB dose once a day per-oral.

The testing of antimalarial activity *in vivo* was conducted using Peter method (Phillipson and Wright, 1991 in Muti'ah, 2012). The therapy was conducted when parasitemia infection reached 1–5% that has been measured since the day 0. The therapy was given daily (morning/afternoon/evening depending on the % gaining of infection which was 1- 5% on the day of 0) in four days. The observation of parasitemia was started on the day 0, 1, 2, 3, and 4 (as the curative testing).

Donor Production

The treatment in producing donor was referred to the study conducted by Muti'ah *et al.* (2012). In producing donor system, erythrocyte which was infected by parasite was extruded to 200 μ L using PBS. Then, it was injected to the mice *intraperitoneally* (i.p). Furthermore, the researcher observed parasitemia degree in mice donor. If the percentage of parasitemia in a mouse donor reached 2,5 %, it means that it can infect other mice.

Freezing and Thawing Isolate *P. berghei*

The treatment of Freezing and Thawing isolate parasite in this current research referred to the study conducted by Coutrier (2009). The first thing done in freezing isolate parasite was by taking 0,8 mL heart blood from a mouse donor which had been infected, and then it was put in the vacum tube which contained EDTA. After that, the vacum tube containing heart blood and EDTA was added by 1,6 mL Alsever's solution containing 10 % glicerol. Next, vacum tube was covered and put in the liquid nitrogen tank about \pm 1 menit. Then, it was moved to the freezer of -70 °C. When its parasite infected blood was taken to give infection treatment, vacum tube containing isolate parasite was taken out from freezer (thawing process). Thus, parasite was thawed and ready to be infected to the animal testes. All works related to the isolate *P. berghei* were conducted in *Laminar air flow vertical* and asepticable condition.

Inoculation of *P. berghei*

The treatment of inoculation *P. berghei* referred to the study conducted by Muti'ah *et al.* (2012) which was *P. berghei* inoculation conducted *intraperitoneally* (i.p) with the number of parasite which was infected was 1×10^6 . In terms of mice examination which had been infected by the parasite, it was assumed that normal mencil had hematocrite level (a number which shows solid percentage in the blood toward blood liquid was 60 %, and mice donor had 6×10^9 eritocyte /mL in the blood. If the parasitemia degree of mice donor was 2,5%,

its blood will be taken as much as 6,7 µL, then it was re-extruded to 200 µL using PBS solution. After infection process, the researcher conducted daily observation till the parasitemia reach 1–5% since the day 0 of therapy. Next, the researcher gave medicational therapy or extract testing till the day 4.

Parasitemia Degree Measurement

Blazquez et al (2008) explained the technique in measuring parasitemia degree by creating blood deletion track. It was conducted by taking a drip of a mouse’s blood through cutting its tail and it is dripped to the glass object and dried. Next, the result of blood deletion was evenly spreaded by methanol and dried. The next process is coloring Giemsa by mixing Giemsa fluka and buffer Giemsa using 1:9 ratio. The Giemsa coloring was dripped to the deletion track and waited for 20 minutes. Next, it is washed using flowing water so that there is no remaining color and then dried. Next, the deletion of blood track which had been colored were examined its parasitemia using microscope with 1000x magnifition by calculating the number of erythrocyte which was infected by malaria out of 1000 erythrocyte. The parasitemia degree percentage was the number of erythrocyte infected by *P. berghei* in 1000 erythrocyte. The parasitemia degree percentage was measured using this formula:

$$\text{Percentage of parasitemia degree} = \frac{\text{The number of infected erythrocyte}}{1000 \text{ erythrocytes}} \times 100\%$$

On the other hand, the percentage of parasite growth inhibition was measured using the formula below:

$$\% \text{ inhibition} = \frac{(\text{negative control parasitemia} - \text{medicine/extract parasitemia})}{\text{negative control of parasitemia}} \times 100\%$$

After that, it was determined the value of ED₅₀ using probity analysis from % inhibition on the day 4.

RESULT AND DISCUSSION

The Effectiveness of 80% Ethanol Extract of sunflowers Leaves as the anti-malaria.

The observation of parasitemia degree was conducted on the day 0, 1, 2, 3, and 4. The deletion of blood was examined under microscope using 1000x magnifition by calculating the number of erythrocyte out of 1000 erythrocyte which was infected by malaria. The examination of parasitemia on the day 0 aimed to prove that all mice were in the equal parasitemia degree range on the day when the researcher gave medication. The result of parasitemia degree examination and deviation standard are shown in the Table 1, table 2, and table 3, below:

Table 1. The mean of parasitemia degree of 80% ethanol extract of sunflower and deviation standard

Experimental Group (mg/kg BB)	Mean of parasitemia degree (%) ± Deviation Standard				
	Day 0	Day 1	Day 2	Day 3	Day 4
Negative Control	1,5 ±0,31	4,3 ±1,03	5 ±1,24	5,5 ±1,46	5,6 ±1,14
Positive Control	2,9 ±0,86	1,4 ±0,33	1,2 ±0,3	0,38 ±0,31	0 ±0,00
ESF_1	1,7 ±0,38	2,5 ±0,4	3,33 ±0,99	3,5 ±0,47	3,90 ±0,8
ESF_2	3 ±1,09	3,1 ±0,5	2,9 ±1,18	3,8 ±0,54	3,90 ±0,26
ESF_3	1,9 ±0,66	3 ±0,74	3,4 ±0,49	3,5 ±1,12	2,4 ±0,48
ESF_4	2,6 ±1,51	2,7 ±0,86	3,20 ±0,38	3,20 ±0,71	2,1 ±0,74

Table 1 showed that the mean of parasitemia in the day 1, 2, 3, and 4 in all experimental groups which was treated by 80% ethanol extract was lower than negative

control group. Then, the deviation standard showed that the mean percentage of parasitemia degree deviated (Sulisetijono, 2006). Normally, the deviation standard for in

vivo test was not quite slight; however the upper and lower limit should not exceed the mean of the paracitemia degree percentage of the six mice on the daily treatment. The more deviation standard means the more various mice, in sum.

The deviation standard was categorized as the normal range as stated by

Pasaribu (1975). The range which was got by the mean was in the interval $\bar{x} - 2s$ and $\bar{x} + 2s$ which was using 95,45% percentage. The result of investigation result of parasitemia degree and the value of deviation standard in the **Table 1** above, was explained using **Fig. 1** below:

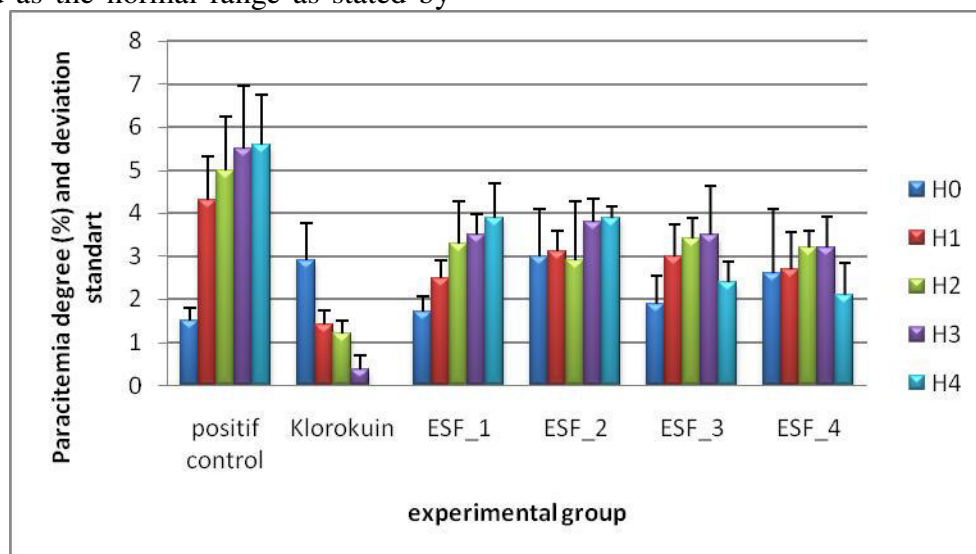


Figure 1. Graphic of Parasitemia Degree of 80% Ethanol Extract of Sunflower Leaves

The increasing degree of parasitemia in the negative control indicated that the number of erythrocyte infected by *Plasmodium berghei* is increasing as the more number of treatment days.

Next, the graphic of positive control group showed the opposite of the negative control one, in which it was concluded that the parasitemia degree on the day 4 is 0%. It showed that positive control is 100% effective for inhibiting the growth of parasite of *P.berghei*. It was also supported by deviation standard 0 which showed the high level of data validity and reliability.

After that, to the dosage of 80% ethanol extract of sunflowers leaves, alter in terms of parasitemia number in each mouse's body. However, it was clearly seen on the last day (test of Peter-The 4-day suppressive test of blood schizontocidal action) as stated by Philipson and Wright (1991), the number of parasitemia degree of sunflowers to the dosage of 1 and 2 increased on the day 4 after therapy treatment compared to the previous days. On the other hand, to the dosage of 3 and 4, it was shown that the parasitemia

degree decreased particularly on the day 4. Those two statements were not absolutely stated that the dosage of 3 and 4 gave effective therapy which was better than dosage of 1 and 2. Those are due to the extensive effects which were not related to the parasite which infected, some of them due to the existence of mice's immune system, consumption of food and drink, and wound got as the result of quarrelling among mice so that it affects the increasing of parasitemia degree among dosage.

To emphasize the dosage effectiveness of extraction toward parasite malaria growth infected mice's blood cell, it could be directly seen from the % inhibition in each treatment. The value of % of 80% ethanol extract inhibition of sunflowers leaves could be achieved using formula % of inhibition:

$$\% \text{ inhibition} = \frac{(\text{negative control of parasitemia} - \text{medicine/extract parasitemia})}{\text{negative control of parasitemia}} \times 100\%$$

The result of percentage calculation of parasite growth inhibition of 80% ethanol

extract of sunflowers leaves on the day of 4 after therapy are shown in the **Table 2**, below:

Table 2 Percentage and Probity of inhibition of parasite growth on the average of 80% ethanol extract of sunflowers leaves on the day 4

Dosage (mg/kg BB)	Percentage of Parasite Growth Inhibition	Probity % of Inhibition
0,1	30	4,48
1	30,7	4,49
10	57,9	5,18
100	61,8	5,31

Based on the percentage of parasite inhibition, it was known that the effectiveness of sunflowers extracts dosage is absolute. As showed on Table 4, the percentage of parasite inhibition increased as the increasing of dosage concentration given, the effectiveness in inhibiting the parasite growth is increasing. It is assumed that the more dosage given, the process of trophozoit changes to the early skizon is inhibited as well.

Then, in this current study, in order to decide the effective dosage to inhibit 50% of

parasite growth (ED₅₀) was by utilizing probity % inhibiting the parasite growth in four days which was then continued through regression analysis using Microsoft Office Excel.

Based on the Table 4, it was continued by determining the correlation between log dosage of 80% ethanol extract used and the percentage of probity value of the inhibition as shown in the **Fig. 2**

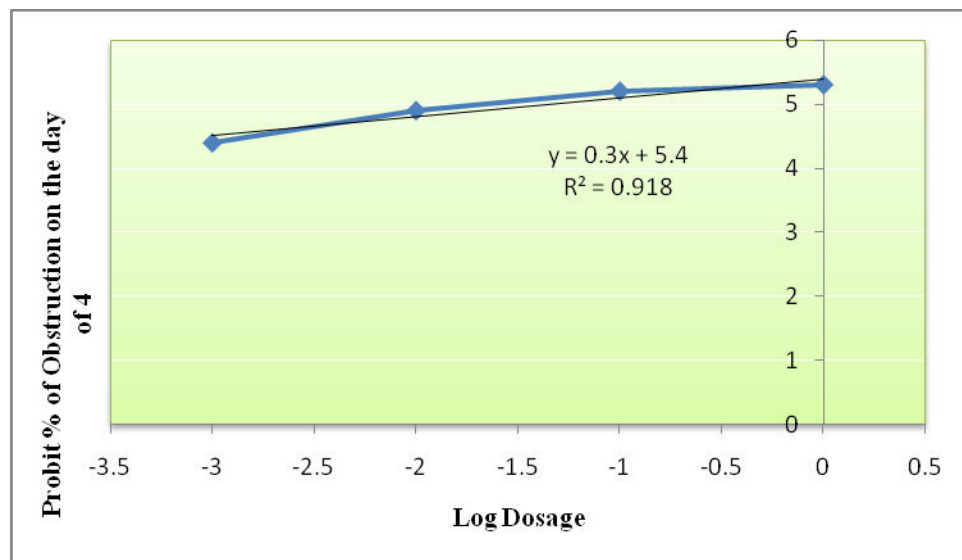


Figure 2. The curve of correlation between Log Dosage and Probity % of Inhibition of Sunflowers leaves

Figure 2 showed that the effective dosage which inhibits the growth of 50% parasite was in the dosage range of 1 mg/kg BB and 10 mg/kg BB, which was in the point of 4,64 mg/kg BB. The effectiveness point of 50% dosage was assumed as the effective

point in inhibiting 50% of parasite growth as stated by Herintsoa *et al.* (2005) which is < 10 mg/kg BB.

CONCLUSION

The 80% ethanol extract of sunflowers leaves is effective as the antimalarial agent toward the animal testees which are infected by *P.berghei* parasite on the ED₅₀ values of 4,64 mg/kg BB.

SUGGESTION

1. It is needed to separated and purify the compound used in the following step, for instance from KLTA which is then followed by KLTP and Kromatography coloumn, so that the researcher would get the pure isolate.
2. It is needed to have more identification using HPLC instruments to know the specification of active compound group and utilizing spectroscopy *Nuclear Magnetic Resonance* (NMR) to reveal the structure of antimalarial active compound.

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Spectrum of the Laplacian Matrix of Non-commuting Graph of Dihedral Group D_{2n}

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ABSTRACT

Let G be a graph with vertex set $V = \{v_1, v_2, \dots, v_p\}$, $A(G)$ is adjacency matrix of G and $D(G)$ is diagonal matrix with entry $d_{ii} = \deg_G(v_i)$, $i = 1, 2, \dots, p$. The Laplacian matrix of G is $L(G) = D(G) - A(G)$. Spectrum of the Laplacian matrix is obtained by finding of eigenvalues of $L(G)$ and their multiplicities. In this paper we study spectrum of the Laplacian matrix of non-commuting graph of dihedral group $\Gamma_{D_{2n}}$, and give results about characteristics polynomial of $L(\Gamma_{D_{2n}})$ and its spectrum of the Laplacian matrix. We obtained spectrum of the Laplacian matrix of $\Gamma_{D_{2n}}$ is

$$Spec_L(\Gamma_{D_{2n}}) = \begin{bmatrix} 2n-1 & n & 0 \\ n & n-2 & 1 \end{bmatrix}$$

Key-words: eigenvalues, eigenvector, spectrum, Laplacian matrix, non-commuting graph, dihedral group.

INTRODUCTION

A graph G is a finite nonempty set of objects called vertices together with a (possibly empty) set of unordered pairs of distinct vertices of G called edges (Cartrand & Lesniak, 1986). The vertex set of G is denoted by $V(G)$, while the edge set is denoted by $E(G)$.

Let G be a graph with vertex set $V(G) = \{v_1, v_2, \dots, v_p\}$. The adjacency matrix of G , denoted by $A(G)$, is $(p \times p)$ -square matrix where $a_{ij} = 1$ if $v_i v_j \in E(G)$ and $a_{ij} = 0$ if $v_i v_j \notin E(G)$, $1 \leq i, j \leq p$. The diagonal matrix of G , denoted by $D(G)$, is diagonal matrix where $d_{ii} = \deg_G(v_i)$. The Laplacian matrix of G is $L(G) = D(G) - A(G)$. Since the Laplacian matrix is real and symmetric, all its eigenvalues μ_i , $i = 1, 2, 3, \dots, p$ are nonnegative real numbers and can be labeled so that $\mu_1 \geq \mu_2 \geq \dots \geq \mu_p = 0$. If

$\mu_{i_1} \geq \mu_{i_2} \geq \dots \geq \mu_{i_g}$ are the distinct eigenvalues, then spectrum of $L(G)$ can be written as

$$Spec_L(G) = \begin{bmatrix} \mu_{i_1} & \mu_{i_2} & \dots & \mu_{i_g} \\ m_1 & m_2 & \dots & m_g \end{bmatrix}$$

where m_j indicates the algebraic multiplicity of the eigenvalue μ_{i_j} (Yin, 2008). Of course $m_1 + m_2 + \dots + m_g = p$ (Ayyaswamy & Balachandran, 2010). The multiplicity of μ_{i_j} as a root of characteristic equation $\det(\mu_{i_j} I - L(G)) = 0$ is equal to the dimension of the space of eigenvectors corresponding to μ_{i_j} (Bigg, 1974).

Let G be a non-abelian group with center $Z(G)$. Associate a graph Γ_G of G whose vertices are the non-central elements $G \setminus Z(G)$ and whose edges join those vertices $x, y \in G \setminus Z(G)$ for which $xy \neq yx$ in G . Then Γ_G is said to be the non-commuting graph of G (Abdollahi, et.al, 2006 and Abdollahi, et.al, 2010). Note that if G is abelian, then Γ_G is the null graph. Because G is non-abelian, the non-commuting graph Γ_G of G is always connected with diameter 2 and girth 3.

Some research about spectrum of the Laplacian matrix has been conducted. Yin (2008) investigated spectrum of the Laplacian matrix of graph G_l , where G_l obtained from complete graph K_l by adhering the root of isomorphic trees T to every vertex of K_l and d_{k-j+1} be the degree of vertices in the level j . Abdussakir, et.al (2012) determined spectrum of the Laplacian matrix of complete multipartite graph $K(\alpha_1, \alpha_2, \dots, \alpha_n)$. In this paper we determined spectrum of the Laplacian matrix of non-commuting graph of dihedral group order $2n$, where n is odd natural numbers and $n \geq 3$, because there are no research in this topic until the day.

RESULTS

In this section, we will give an example to determine spectrum of the Laplacian matrix of dihedral group for the cases of dihedral group

Dihedral group $D_6 = \{1, r, r^2, s, sr, sr^2\}$ with composition operation is non-abelian group. Using Cayley table

°	1	r	r ²	s	sr	sr ²
1	1	r	r ²	s	sr	sr ²
r	r	r ²	1	sr ²	s	sr
r ²	r ²	1	r	sr	sr ²	s
s	s	sr	sr ²	1	r	r ²
sr	sr	sr ²	s	r ²	1	r
sr ²	sr ²	s	sr	r	r ²	1

we can have center of D_6 is $Z(D_6) = \{1\}$. From the table, we have that non-commuting graph Γ_{D_6} has $\{r, r^2, s, sr, sr^2\}$ as its vertex set. Hence, we can picture Γ_{D_6} as following.

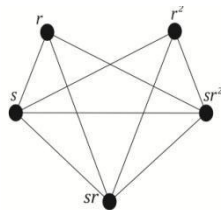


Figure non-commuting graph of D_6

Adjacency matrix for this graph is

$$A(\Gamma_{D_6}) = \begin{matrix} & r & r^2 & s & sr & sr^2 \\ \begin{matrix} r \\ r^2 \\ s \\ sr \\ sr^2 \end{matrix} & \begin{bmatrix} 0 & 0 & 1 & 1 & 1 \\ 0 & 0 & 1 & 1 & 1 \\ 1 & 1 & 0 & 1 & 1 \\ 1 & 1 & 1 & 0 & 1 \\ 1 & 1 & 1 & 1 & 0 \end{bmatrix} \end{matrix}$$

and its degree matrix is

$$D(\Gamma_{D_6}) = \begin{matrix} & r & r^2 & s & sr & sr^2 \\ \begin{matrix} r \\ r^2 \\ s \\ sr \\ sr^2 \end{matrix} & \begin{bmatrix} 3 & 0 & 0 & 0 & 0 \\ 0 & 3 & 0 & 0 & 0 \\ 0 & 0 & 4 & 0 & 0 \\ 0 & 0 & 0 & 4 & 0 \\ 0 & 0 & 0 & 0 & 4 \end{bmatrix} \end{matrix}$$

So, we have the Laplacian matrix $L(\Gamma_{D_6}) = D(\Gamma_{D_6}) - A(\Gamma_{D_6})$ as follows

$$L(\Gamma_{D_6}) = \begin{matrix} & r & r^2 & s & sr & sr^2 \\ \begin{matrix} r \\ r^2 \\ s \\ sr \\ sr^2 \end{matrix} & \begin{bmatrix} 3 & 0 & -1 & -1 & -1 \\ 0 & 3 & -1 & -1 & -1 \\ -1 & -1 & 4 & -1 & -1 \\ -1 & -1 & -1 & 4 & -1 \\ -1 & -1 & -1 & -1 & 4 \end{bmatrix} \end{matrix}$$

Now, we find eigenvalues of $L(\Gamma_{D_6})$ using formula

$$\det(L(\Gamma_{D_6}) - \lambda I) = 0.$$

Because

$$\det(L(\Gamma_{D_6}) - \lambda I) = (-1)(3 - \lambda)(-5 + \lambda)^2(-5\lambda + \lambda^2)$$

and $\det(L(\Gamma_{D_6}) - \lambda I)$ must equal to zero, we have

$$\lambda_1 = 5, \lambda_2 = 3, \lambda_3 = 0$$

as eigenvalues for $L(\Gamma_{D_6})$. Finally, we determine the algebraic multiplicity of each eigenvalue and we will have spectrum of the Laplacian matrix of non-commuting graph of dihedral group D_6 as follows

$$Spec_L(\Gamma_{D_6}) = \begin{bmatrix} 5 & 3 & 0 \\ 3 & 1 & 1 \end{bmatrix}.$$

By similar manner we have

$$Spec_L(\Gamma_{D_{10}}) = \begin{bmatrix} 9 & 5 & 0 \\ 5 & 3 & 1 \end{bmatrix}$$

and

$$Spec_L(\Gamma_{D_{14}}) = \begin{bmatrix} 13 & 7 & 0 \\ 7 & 5 & 1 \end{bmatrix}.$$

So, we will have final results for this investigation as the following.

Lemma 1.

Let D_{2n} be dihedral group order $2n$, where n is odd natural numbers and $n \geq 3$. Characteristic polynomial of the Laplacian matrix of non-commuting graph of D_{2n} is

$$p(\lambda) = (-1)^n (n - \lambda)^{n-2} ((-2n + 1) + \lambda)^{n-1} ((-2n + 1)\lambda + \lambda^2).$$

and the roots of $p(\lambda) = 0$ are $1, n$, and $2n - 1$.

Proof.

Adjacency matrix of $\Gamma_{D_{2n}}$ is $(2n - 1 \times 2n - 1)$ -square matrix

$$A(\Gamma_{D_{2n}}) = \begin{matrix} & \overbrace{\begin{matrix} n-1 \\ \dots & \dots & 0 \end{matrix}} & \overbrace{\begin{matrix} n \\ 1 & \dots & 1 \end{matrix}} & \overbrace{\begin{matrix} 1 \\ \dots & \dots & 1 \end{matrix}} \\ \begin{matrix} n-1 \\ \vdots \\ 0 \\ 1 \\ \vdots \\ 1 \\ \dots \end{matrix} & \left\{ \begin{matrix} \dots & \dots & 0 \\ \vdots & \ddots & \vdots \\ 0 & \dots & 0 \\ 1 & \dots & 1 \\ \vdots & \ddots & \dots \\ \dots & 1 & \dots \end{matrix} \right\} & \left\{ \begin{matrix} 1 & \dots & 1 \\ \vdots & \ddots & \vdots \\ 1 & \dots & 1 \\ \vdots & \ddots & \vdots \\ 1 & \dots & 1 \\ \dots & 1 & \dots \end{matrix} \right\} & \left. \begin{matrix} \vdots \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ \vdots \end{matrix} \right\} 2n-2 \end{matrix}$$

and its degree matrix is $(2n - 1 \times 2n - 1)$ -square matrix

$$D(\Gamma_{D_{2n}}) = \begin{matrix} & n & 0 & \dots & \dots & \dots & \dots & 0 \\ \begin{matrix} \vdots \\ 0 \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ 0 \end{matrix} & \left\{ \begin{matrix} \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\ 0 & 0 & n & \vdots & \vdots & \vdots & \vdots \\ \vdots & \vdots & \dots & 2n-2 & 0 & \vdots & 0 \\ \vdots & 0 & \dots & 0 & \vdots & \vdots & \vdots \\ \vdots & \vdots & 0 & \vdots & \vdots & \ddots & 0 \\ 0 & 0 & \dots & \dots & 0 & 0 & 2n-2 \end{matrix} \right\} \end{matrix}$$

So, its Laplacian matrix is $L(\Gamma_{D_{2n}}) = D(\Gamma_{D_{2n}}) - A(\Gamma_{D_{2n}})$

$$L(\Gamma_{D_{2n}}) = \begin{pmatrix} n & \dots & 0 & \dots & -1 & \dots & -1 & -1 \\ \vdots & \ddots & \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & \dots & n & \dots & -1 & \dots & -1 & \vdots \\ -1 & \dots & -1 & 2n-2 & -1 & \dots & -1 & -1 \\ \vdots & \ddots & \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ -1 & \dots & -1 & \vdots & -1 & \dots & -1 & \vdots \\ \dots & -1 & \dots & \vdots & -1 & \dots & 2n-2 & \vdots \end{pmatrix}_{2n-1 \times 2n-1}$$

We know that characteristic polynomial of $L(\Gamma_{D_{2n}})$ is

$$\det(L(\Gamma_{D_{2n}}) - \lambda I)$$

Apply the Gaussian elimination procedure we obtained this upper triangular matrix

$$\begin{vmatrix} (-1) & 0 & 0 & (n-1)-\lambda & \dots & -1 & -1 \\ \vdots & (n-\lambda) & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & \ddots & -1 & \dots & -1 & \vdots \\ 0 & 0 & 0 & (-2n+1)+\lambda & -(-2n+1)+\lambda & 0 & 0 \\ 0 & 0 & 0 & 0 & \vdots & \vdots & \vdots \\ 0 & 0 & 0 & 0 & 0 & \vdots & \vdots \\ 0 & 0 & 0 & 0 & 0 & 0 & (-2n+1)\lambda + \lambda^2 \end{vmatrix}$$

Because determinant for this matrix is multiplication of entry in main diagonal, so we obtained

$$p(\lambda) = (-1)(n-\lambda)^{n-2}((-2n+1)+\lambda)^{n-1}((-2n+1)\lambda + \lambda^2)$$

And, for $p(\lambda) = 0$ we obtained $\lambda_1 = 2n - 1$, $\lambda_2 = n$, and $\lambda_3 = 0$ as the roots of $p(\lambda) = 0$.

Theorem 1.

Let D_{2n} be dihedral group order $2n$, where n is odd natural numbers and $n \geq 3$. Spectrum of the Laplacian matrix of non-commuting graph of D_{2n} is

$$Spec_L(\Gamma_{D_{2n}}) = \begin{bmatrix} 2n-1 & n & 0 \\ n & n-2 & 1 \end{bmatrix}$$

Proof.

From Lemma 1 we have characteristic polynomial of $L(\Gamma_{D_{2n}})$ is

$$p(\lambda) = (-1)(n-\lambda)^{n-2}((-2n+1)+\lambda)^{n-1}((-2n+1)\lambda + \lambda^2).$$

and the roots of $p(\lambda) = 0$ are $1, n$, and $2n - 1$. So, we obtained $\lambda_1 = 2n - 1$, $\lambda_2 = n$, and $\lambda_3 = 0$ as eigenvalues of $L(\Gamma_{D_{2n}})$.

Now, we will determine multiplicities for each eigenvalues. Because the multiplicities are equal to number of basis for space of eigenvectors corresponding to $\lambda_i, i = 1, 2, 3$, we substitute λ_i to $L(\Gamma_{D_{2n}}) - \lambda_i I$ and apply Gauss-Jordan elimination to this matrix to obtain row-reduced eselon matrix and then see the number of zero rows of it.

For $\lambda_1 = 2n - 1$, after we eliminate $L(\Gamma_{D_{2n}}) - \lambda_1 I$, we obtained row-reduced eselon matrix with n

zero rows. So, for $\lambda_1 = 2n - 1$ we have its algebraic multiplicity is n .

For $\lambda_2 = n$, after we eliminate $L(\Gamma_{D_{2n}}) - \lambda_2 I$, we obtained row-reduced eselon matrix with $(n - 2)$ zero rows. So, for $\lambda_1 = 2n - 1$ we have its algebraic multiplicity is $(n - 2)$.

For $\lambda_3 = 0$, after we eliminate $L(\Gamma_{D_{2n}}) - \lambda_3 I$, we obtained row-reduced eselon matrix with 1 zero row. So, for $\lambda_3 = 0$ we have its algebraic multiplicity is 1.

We conclude that

$$Spec_L(\Gamma_{D_{2n}}) = \begin{bmatrix} 2n-1 & n & 0 \\ n & n-2 & 1 \end{bmatrix}$$

CONCLUSION

According to the result, we just determine spectrum of the Laplacian matrix of non-commuting graph of dihedral group D_{2n} , where n is odd natural numbers and $n \geq 3$. We have

$$Spec_L(\Gamma_{D_{2n}}) = \begin{bmatrix} 2n-1 & n & 0 \\ n & n-2 & 1 \end{bmatrix}$$

So, we suggest to the reader to investigate spectrum of the Laplacian matrix of non-commuting graph of dihedral group D_{2n} , where n is even natural numbers and $n \geq 3$. Investigation can also be done for spectrum of the Signless Laplacian matrix or detour matrix of non-commuting graph of dihedral group D_{2n} . Similar research can be conducted for symmetric group S_n .

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Analysis of Newton Gregory Polinomial for Numerical Solution of String Wave Model

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ABSTRACT

Discretized model is a continuous model transformation procedure to model discrete. Discretization is done using advanced finite difference method (forward finite difference), by analogy differential equations using limit rules, with the difference that using a different equation between discrete time points. The model used in this paper is to present wave models on the bridge causing the bridge to vibrate. Finite difference method used is the finite difference method of Newton Gregory explicit schemes, different forward and center for the time difference and central difference for the space. With explicit finite difference scheme is obtained settlement Gregory Newton stated in the form of discrete wave model homogeneous and wave non homogeneous. Comparison between the two models is the homogeneous wave bridge vibrate faster stable because the movement (or displacement) styles have changed over the value of x converge or remain stable with time at a distance of $4 \leq x \leq 8$ and the time in the 100th iteration. In the non-homogeneous equation of vibration that occurs at the beginning is very large then headed to the stability of the interval between $0,1 \leq x < 0,95$ was followed by a small vibration and headed back on the initial conditions. For the next examine, suggested for the next study is stability string wave model with used initial value, initial conditions, and difference of interval and variation, so that can be see minus of discrete models. And growth model of the wave with the variations method.

Keywords: Discretization, Model of The Wave, Finite Difference Methods Newton Gregory Explicit Scheme, The Model Continuous, Discrete Models.

INTRODUCTION

Vibration is a back and forth movement in a given time interval. Vibration associated with the oscillatory motion of objects and styles associated with the motion. All objects that have mass and elasticity capable of vibrating, so most machines and structural engineering (engineering) experience some degree of vibration and design usually requires consideration of the nature of oscillation. While the waves are vibrations that propagate through the medium system, means clear that the vibration is a small part of the wave. The basic model of one-dimensional waves can be written: $\frac{\partial^2 u}{\partial t^2} = c^2 \frac{\partial^2 u}{\partial x^2}$, where $c^2 = \frac{T}{\rho}$ with vibration acceleration parameters stated with $\frac{\partial^2 u}{\partial t^2}$, the mass density of the string (mass unity length) is expressed by ρ , the string tension is expressed with T , and $\frac{\partial^2 u}{\partial x^2}$ is the initial velocity. In this case the waves generated in the rope coming from the four parameters (Anonymous, 2013).

According to Wijayanto and Susatio (2010:5) wave model is stable if the movement (or displacement) converge or remain stable with time. On the other hand, if the displacement amplitude increases continuously (deviant) with time, is said to be dynamically unstable. Motion is distorted and the system becomes unstable if the energy put into the system through the addition of personnel to divert from its ground state to a state with higher energy (excitation).

THEORY STUDY

1. Theory of vibration

Vibration is alternating movements (repeatedly) in a certain time interval. Vibration associated with the oscillatory motion of objects and styles associated with the motion. All objects that have mass and elasticity capable of vibrating, so most machines and structural engineering (engineering) experience some degree of vibration and design usually requires consideration of the nature of oscillation.

Vibration is divided into two, namely:

- Free vibration occurs if the system oscillates due to the workings of styles that exist in the system itself (inherent), and if there is a broad style of working .
- Forced vibration is the vibration that occurs due to stimulation of external forces, if the stimulus is oscillating, the system is forced to vibrate at the frequency of stimulation (Thomson, 1986).

2. Partial Differential Equations String of Wave

An equation in which there are partial derivatives and there are two or more independent variables then the equation is called a partial differential equation (Ayres, 1992).

Under the conditions of partial differential equations is divided into three, namely:

- Partial differential equations linear.
- Partial differential equations kuasilinear.
- Partial differential equations nonlinear.

Based on the criteria of partial differential equations is divided into three, namely:

- Elliptic shape if $b^2 - 4ac < 0$
- Parabolic shape if $b^2 - 4ac = 0$
- Hyperbolic form if $b^2 - 4ac > 0$

3. Analysis Mathematical String of Wave Model

In this model it is assumed effects of friction and external forces are ignored. The origin of the one-dimensional wave model is (Ohene1, 2012:51).

$$m_1 \frac{\partial^2 v(x, t)}{\partial t^2} + T \frac{\partial^2 v(x, t)}{\partial x^2} - b_1 \frac{\partial v(x, t)}{\partial t} = 0 \quad (2.3.1)$$

where m_1 is the mass of each main cable, T is the tension in the main cable, b_1 is the attenuation coefficient of each main cable.

4. Newton Gregory Polynomial

Newton Gregory Polynomial is a special case of the Newton polynomial for points within alike. In most applications the values of x is the same as the table value function or the measurements taken at regular intervals (Munir, 2010).

If z is interpolated with polynomial two variables (two-dimensional interpolation), previously had determined how many degrees in the x direction and how many degrees in the y direction (Munir, 2010). Degree polynomial interpolation is the number of data points minus 1 data on an interpolation. For example, is a linear polynomial interpolation of two points, the mean linear polynomial is a polynomial of degree 1.

5. Finite Difference Methods Newton Gregory Explicit Scheme

From equation (2.3.1) obtained three forms of transformations, namely:

- a. Transformation center of difference Newton Gregory for the second derivative space at t according to equation (2.5.24) as follows:

$$v_{tt}(x_i, t_n) = \frac{v_i^{n+1} - 2v_i^n + v_i^{n-1}}{2\Delta t^2 q(q-1)}, \forall q = \frac{t - t_0}{\Delta t}$$

- b. Transformation center of difference Newton Gregory for the second derivative space at x according to equation (2.5.12) as follows:

$$v_{xx}(x_i, t_n) = \frac{v_{i+1}^n - 2v_i^n + v_{i-1}^n}{2\Delta x^2 s(s-1)}, \forall s = \frac{x - x_0}{\Delta x}$$

- c. Transformation forward of difference Newton Gregory for the time derivative (t) according to equation (2.5.17) as follows:

$$v_t(x_i, t_n) = \frac{v_i^{n+1} - v_i^n}{q\Delta t}, \forall q = \frac{t - t_0}{\Delta t}$$

6. Stability Analysis by the Finite Difference Methods Newton Gregory Explicit Scheme

Finite Difference Methods Newton Gregory Explicit Scheme called convergent if the solution finite difference approach and the analytic solution is called stable if the finite difference solution is not very sensitive to small changes (Flaherty, 1989).

$$\frac{\partial^2 u(x, t)}{\partial t^2} - c^2 \frac{\partial^2 u(x, t)}{\partial x^2} = 0$$

Here is a discrete form of the above equation:

$$y_j^{n+1} = \left(c \frac{\Delta t}{\Delta x}\right)^2 (y_{j+1}^n - 2y_j^n + y_{j-1}^n) + 2y_j^n - y_j^{n-1}$$

equation (2.6.2) can be rewritten as follows:

$$y_j^{n+1} = (1 - \lambda)2y_j^n + \lambda(y_{j+1}^n + y_{j-1}^n) - y_j^{n-1}$$

Discrete Fourier series can be used to analyze the stability of finite difference constant coefficient problems.

DISCUSSION

1. Analysis Newton Gregory Polinomial on the string Homogeneous of Wave Equation

Discrete form of the linear partial differential equation is:

$$v_i^{n+1} = \frac{-m_1 q \Delta t}{(-b_1 2\Delta t^2 q(q-1) + m_1 q \Delta t)} (-2v_i^n + v_i^{n-1}) - \lambda \frac{q^2(q-1)}{s(s-1)(-b_1 2\Delta t^2 q(q-1) + m_1 q \Delta t)} (v_{i+1}^n - 2v_i^n + v_{i-1}^n) + \frac{b_1 2\Delta t^2 q(q-1)(-v_i^n)}{(-b_1 2\Delta t^2 q(q-1) + m_1 q \Delta t)}$$

With the initial condition at the n^{th} time and distance to- i can be written as follows:

$$v_i^n = \exp[-10(4x_i - 1)^2],$$

$$\forall n = 0 \forall i = 0, 1, 2, \dots, l$$

With the value of s and q as follows:

$$s = \frac{x - x_0}{h} = \frac{1.95 - 1.92}{0.02} = 1.5$$

$$q = \frac{t - t_0}{h} = \frac{7.912 - 7.76}{0.08} = 1.9$$

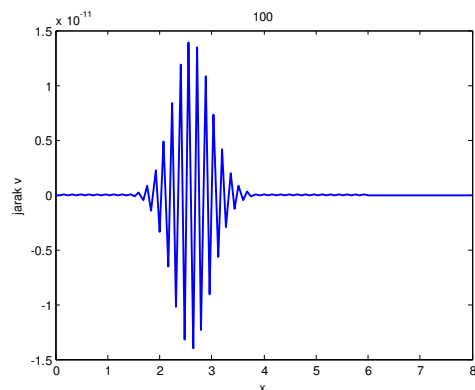


Figure 1. Graph Discret for model wave homogeneity equation $v(x, t)$

As can be seen in a comparison of images generated analytically matlab program are as follows:

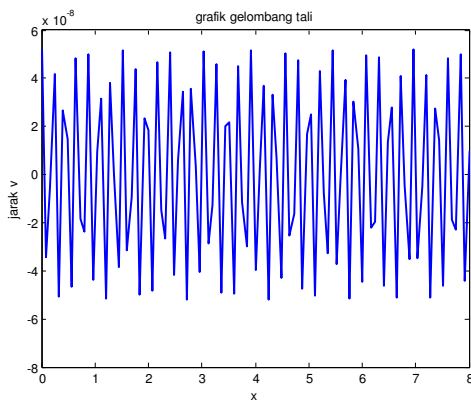


Figure 2. Graph Analytic for Model Wave Homogen Equation $v(x, t)$

2. Stability Analysis by the Finite Difference Methods Newton Gregory Explicit Scheme on the string Homogeneous of Wave Equation

Then the Courant number for wave equation of string (2.3.1) must satisfy the following inequality:

$$0 \leq \lambda \leq \frac{1}{4}$$

From the results of substitution λ value obtained in section 3.1

$$\begin{aligned} \lambda &= \frac{T\Delta t^3}{\Delta x^2} = 0.0000013 \frac{0.08^3}{0.02^2} \\ &= 0.0000013 \frac{0.000512}{0.0004} = 0.0000017 \end{aligned}$$

It can be concluded by using $\Delta t = 0,08$ with interval $0 \leq t \leq 8$ and $\Delta x = 0,02$ with interval $0 \leq x \leq 2$, λ value obtained qualified Courant stability number, namely:

$$1.7 \times 10^{-6} \leq \lambda \leq \frac{1}{4}$$

3. Analysis Newton Gregory Polinomial on the string nonHomogeneous of Wave Equation

Discrete form of the linear partial differential equation is:

$$\begin{aligned} v_i^{n+1} &= \frac{2\Delta t^3 q^2 (q-1)}{(-b_1 2\Delta t^2 q(q-1) + m_1 q \Delta t)} \\ &\quad - \frac{(-b_1 2\Delta t^2 q(q-1) + m_1 q \Delta t)}{m_1 q \Delta t} (-2v_i^n + v_i^{n-1}) \\ &\quad - \lambda \frac{q^2 (q-1) (v_{i+1}^n - 2v_i^n + v_{i-1}^n)}{s(s-1) (-b_1 2\Delta t^2 q(q-1) + m_1 q \Delta t)} \\ &\quad + \frac{b_1 2\Delta t^2 q(q-1) (-v_i^n)}{(-b_1 2\Delta t^2 q(q-1) + m_1 q \Delta t)} \end{aligned}$$

With the initial condition at the n^{th} time and distance to- i can be written as follows:

$$\begin{aligned} v_i^n &= \exp[-10(4x_i - 1)^2], \\ \forall n &= 0 \forall i = 0, 1, 2, \dots, l \end{aligned}$$

With the value of s and q as follows:

$$\begin{aligned} s &= \frac{x - x_0}{h} = \frac{1.95 - 1.92}{0.02} = 1.5 \\ q &= \frac{t - t_0}{h} = \frac{7.912 - 7.76}{0.08} = 1.9 \end{aligned}$$

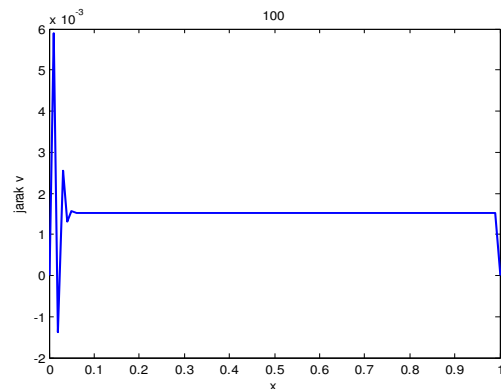


Figure 3. Graph Discret for Model Wave Non Homogen Equation $v(x, t)$

4. Stability Analysis by the Finite Difference Methods Newton Gregory Explicit Scheme on the string nonHomogeneous of Wave Equation

Then the Courant number for wave equation of string (2.3.1) must satisfy the following inequality:

$$0 \leq \lambda \leq \frac{1}{4}$$

From the results of substitution λ value obtained in section 3.1

$$\begin{aligned} \lambda &= \frac{T\Delta t^3}{\Delta x^2} = 0.0000013 \frac{0.01^3}{0.04^2} \\ &= 0.0000013 \frac{0.000001}{0.0016} = 0.000000008125 \end{aligned}$$

It can be concluded by using $\Delta t = 0,01$ with interval $0 \leq t \leq 8$ and $\Delta x = 0,04$ with interval $0 \leq x \leq 2$, λ value obtained qualified Courant stability number, namely:

$$8.125 \times 10^{-10} \leq D \leq \frac{1}{4}$$

CONCLUSION

Comparison between the two models is the homogeneous wave bridge vibrate faster stable because the movement (or displacement) styles have changed over the value of x

converge or remain stable with time at a distance of $4 \leq x \leq 8$ and the time in the 100^{th} iteration. In the non-homogeneous equation of vibration that occurs at the beginning is very large then headed to the stability of the interval between $0,1 \leq x < 0,95$ was followed by a small vibration and headed back on the initial conditions.

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COMPLETION CAMPER REAVER GAME SCENARIO IN 3D VIEW WITH UNINFORMED SEARCH METHODE USING NETLOGO SOFTWARE

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ABSTRACT

Search is the basis for intelligent behavior, not just a cognitive mechanism, but rather a fundamental process of Artificial Intelligence that contributes to our understanding of the intelligence in game scenario. This shows that the search method is not only a method of the many methods that can be used to achieve the goal, but is the most fundamental method of all. Informed Search has information on cost / cost to reach the goal state from the current state. With this information, Informed Search can do to develop or examine judgment collection node that leads to a goal state. This game in implementation for solving game with six camper and five reaver. They want everyone to get across a river using a boat that only fit two people, but the problem is that if the reaver outnumber camper at each stage, the reaver will eat where the role of camper quest to find an effective solution to the search problem using netlogo.

Keyword : *Game, Informed Search, Artificial Intelligence, camper and reaver*

INTRODUCTION

Background of this game is a game of strategy in which a full travel pitfalls and how to avoid them so they can survive until the goal. Character in this game scenario there are two of six people (in this case played by six camper) who travel where the trip across the river and had to cross over immediately if not wanting to be caught by the criminals in this case is characterized by reaver.

In this simulation game completion to reaver with six camper and five reaver. In this scenario the camper have come together on one side of the river and they all wanted to cross over to the other side. Provided a boat to cross enough to carry

two passengers in each crossing. Rules of the crossing if the number of passengers violated the camper will be eaten by the reaver.

The strategy in this crossing is divided into two stages, namely the first phase of which only two people can fit in the boat reaver at a time. Then the second phase of the reaver are not allowed to exceed the number of camper on every stage. If they do, they will beat the camper and then eat it. And the game will be Game Over.

Set of Problem

In formulating a strategy in this game is how to take across the camper

and reaver at the same time to secure the boat to the other side of the river. To solve this problem, search methods must be used to find different scenarios. Search methods used to find the scenario uninformed search algorithms.

The Purpose the Study

The most important thing in this game is how the algorithm incorporated in uninformed search like breadth-first search and depth-first search can be implemented into the software so that the solution netlogo search strategy in the game camper and reaver can be resolved properly.

The Design Model

In designing this model will use the agent-oriented approach that is relatively easy to be implemented in software netlogo, rather than using a queue data structure to implement a classical search algorithm. This agent do a search by transferring the information to another agent then forwards the search. Therefore, a queue data structure that is separate from what happened in the search is not needed in this search. The hope is that this could be the agent-oriented approach to provide a more intuitive solution that makes it easier to understand how the search strategy works. Using the design of the first-person perspective, so that it is easier to understand the differences in search strategy.

Search agent will maintain information about the current state as time is needed (path) and estimated (cost). Each seacher agents expand the search to see if this allows the following actions:

- Two camper get into the boat and cross to the other side;

- Two reaver get into the boat and cross to the other side;
- Two camper get into the boat and cross to the other side;
- Two reaver get into the boat and cross to the other side;
- Two of the camper and reaver get into the boat and crossed to the other side;
- Only one cannibal get into the boat and cross to the other side.

Note that the state of the search for these problems can be represented respectively by the two groups of characters: (# Camper at the start of the river, # reaver on the start side of the river, # boats on the start side of the river). Therefore, the start state is represented by the tuple (6,4, 1) and the goal state (0, 0, 0). In Interface, using parallel coordinates graph, is used to visualize the search.

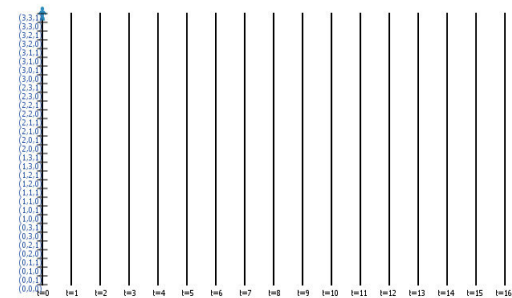


Figure 1. Graph coordinates to visualize search.

a. Interface Simulation Model

Interface Simulation models are defined as follows:

- Setup : to remove all environment variables and, re-animation and redraw the graph.
- Go-1 step: to make the search proceed one step at a time.
- Go-Finish: to create a continuous search continues until it reaches the destination or state is considered a success.
- Go-animation: to start the animation and displayed at the bottom center Interface. Voters sliders and switches model the

interface is defined as follows:
 Search-behavior: to determine the search strategy. Max-depth: to set the maximum depth of the search.

b. Uninformed Search

Searching uninformed or "blind searching" occurs when a search agent has no information about the environment is sought. A real analogy for this type of search is a blind man looking for a maze that had never entered before, who do not have prior knowledge. One approach is 'blind' search agent can make a decision to continue to make a choice at every intersection he met, and continues until he reaches the exit or central (purpose) or until it reaches a dead-end. can then backtracks to the last intersection that has not been visited, and then choose one of them. Repeatedly applying this behavior until the goal is reached.

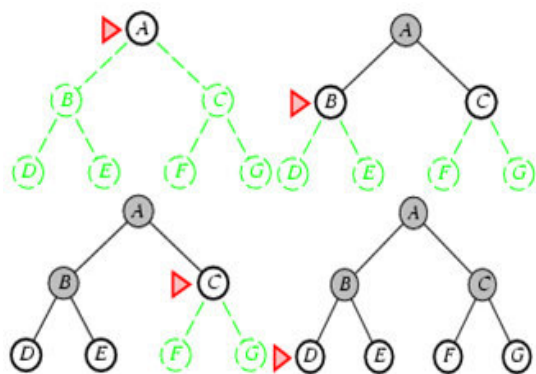


Figure 2. Tree Search Problem breath first search is relatively shorter.

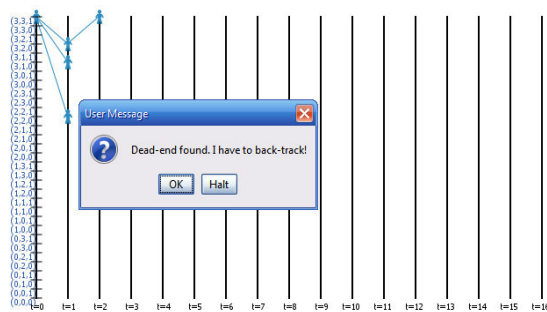


Figure 3. Graph shows DFS Dead-end that requires back-track the previous track

RESULTS AND DISCUSSION

Here are the results of the implementation of camper and reaver game where the generator engine to search using uninformed search strategies. In game has six camper and five reaver, then there is a boat and the river, where the camper had to find a strategy to safely cross the passengers in the boat where the balance must be maintained between the camper and reaver are.

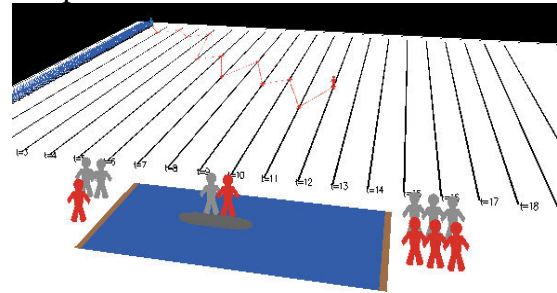


Figure 4 Camper and Reaver Display Game using NetLogo.



Figure 5. Animation shows the state 0-0-0 which means the search for solutions to the problems the game camper and reaver resolved.

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Educational 3D Games Using Finite State Machine to Increase Arabic Learning Quality

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ABSTRACT

This research discusses about behavioral change of scorpions NPC and scorpion hunter NPC using Finite State Machine (FSM) in 3D game so that there is interaction between the player character, scorpion and scorpion hunter. This game is intended as a medium of Arabic learning with learning content appropriate with questions of Test of Arabic as a Foreign Language (TOAFL). The Game is tested to 24 respondents at UIN Maulana Malik Ibrahim Malang. The result shows that, 87.5% of respondents stated that the game is very interesting and can increase their quality of learning Arabic and 91.6% of respondents find it easier to learn Arabic using game

Keywords: *Game, Education, AI, NPC, Finite State Machine, behavior*

INTRODUCTION

Learning the Arabic language is not an easy thing, especially for foreigners. This is due to the complexity of the Arabic language is quite complicated and limited media to facilitate learning. Arabic grammar known to the complexity and richness of its vocabulary. Even for students who study Arabic at the boarding school was having difficulty. (Gee, 2003)

The students usually make use of proficiency test for Arabic language or known as Test Of Arabic As a Foreign Language (TOAFL) to ascertain their ability in mastering the Arabic language. TOAFL is a test to measure proficiency in Arabic from various sides, such as the ability to read, listen, and analyze Arabic grammar. Obviously, to have adequate ability in Arabic, requires skill. Arabic

language skills can be achieved with a lot of reading Arabic texts. By a lot of reading Arabic text is useful for understanding Arabic grammatical thinking and increase Arabic language skills. (Barmawi : 2011).

Meanwhile, the use of technology, particularly game, for foreign language instruction has developed rapidly during the last decade. The consolidation of computers in education has been investigated widely and much attention has been dedicated to the role of computers in the classroom especially in finding a better way to implement effective ways to incorporate computers in the classroom (Greany, 2002).

Computer games have made a significant cultural, social, economic, political, and technological impact on society (Newman, 2004). Given the widespread popularity of video games,

their ability to sustain long-term player engagement with challenging tasks (Gee, 2003) and their tendency to elicit proactive player communities (Rheingold, 1994) it should come as no great surprise that educators have become increasingly interested in the potential of such games as learning tools.

Previous research on 3D educational game with implementing Finite State Machine (FSM) as the action of the changing face of the Non Playable Character (NPC) (Park *et al.*, 2007), is embeded education special learning elementary school. FSM is managed to provide intelligence on the changing face of appropriate design.

This research aims to realize the Arabic language proficiency test media in the form of an attractive and fun using game technology. 3D Adventure game genre is a form of media selected.

MATERIAL AND METHOD

Game

The game is a system in which players engage in artificial conflict, defined by rules, which generate quantitative results (Rheingold, 1994). The relationship between player actions and system outcome, which a player takes action within the designed system of a game and the system responds to the player action.

Adventure Game

Games can be classified among other Action Games, Combat Games, Adventure Games, Puzzle Games, Strategy Games, and Card Games (Gee, 2003). In this research, the game is built with the adventure game genre. Adventure games are often regarded as a form of interaction fiction. Interaction fiction is a term that refers to the medium in which the player can affect the outcome of the story.

Non Player Character (NPC)

Non-Player Character (NPC) usually called the autonomous agent. The agent that is used in computer animation and interactive media such as games and virtual reality, usually are autonomous. This agent represent a character in a story or game and has the ability to improvise their actions. Behavior of autonomous agent consist of several layers. The layers include: action selection, steering, and propulsion as shown in **Fig. 1**.

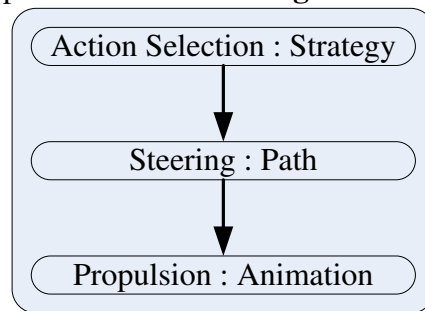


Figure 1. Autonomous Agent

Finite State Machine (FSM)

Finite State Machines (FSM) is a part of the Decision Support System on Artificial Intelligence (AI). Every NPC has a set of FSM to establish autonomous behavior. In FSM there are some state. State is the result of actions or behaviors desired. Each State are connected by transition. Transition is the action taken. Result of this action is a goal state. If the NPC is in a state for example state 1, then the NPC had a certain action, so the NPC will turn into the next state, for example state 2, as shown in **Fig. 2**.

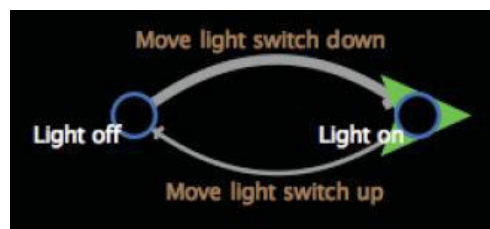


Figure 2. Finite State Machines

Test of Arabic as Foreign Language (TOAFL)

Test of Arabic as Foreign Language (TOAFL) is a standard test to measure a person's ability in mastering the Arabic language, both orally and in writing. Language skills tested in the Test of Arabic as Foreign Language (TOAFL) includes language (Arabic), Islamic sciences, and general knowledge. This content inserted in game.

DISCUSSION

Figure 3 shows the FSM design for NPC 1 or scorpions. The main state arranged in the FSM can be described as follows:

1. Spawn/start
State is the initial position of the NPC
2. Walk/run State patrol/patrol, NPC moves towards area player (enemy)
3. Attack
State NPC 1 engaged in combat, the player triggered NPC range. There are attack, chase and escape state in the attack state.
4. Dead
NPC health value = 0
List of state transition in the FSM can be collated in the following list:
 - a) Enemy in sight
 - b) Health = 0
 - c) Enemies die

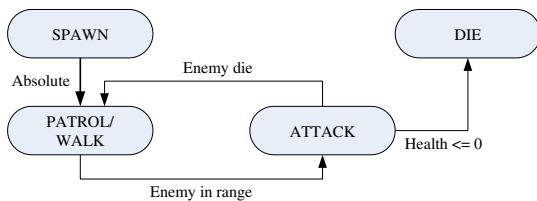


Figure 3. FSM for NPC 1

Fig. 4 shows the FSM design for NPC 2 or scorpions hunter. The main state arranged in the FSM can be described as follows:

1. Spawn/start
State is the initial position of the NPC
2. Stand by,
NPC wait for move trigger
3. Attack
State NPC 2 engaged in combat, the player triggered by NPC 1 range. There are run, attack, and chase state in this attack state.
4. Dead
List of state transition in the FSM can be collated in the following list:
 - a) Enemy in sight
 - b) Enemies die

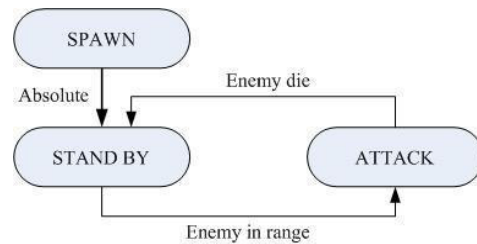


Figure 4. FSM for NPC 1

In this research, top level FSM for both NPC are shown in Fig. 5.

The results of questionnaires completed by 24 respondents from the class of Special Programs Learning Arabic (PKPBA) UIN Maulana Malik Ibrahim Malang after playing a 3D game are presented in table 1. The test is done by asking all respondents after playing 3D games to completion, then each respondent must fill out questionnaires that have been provided.

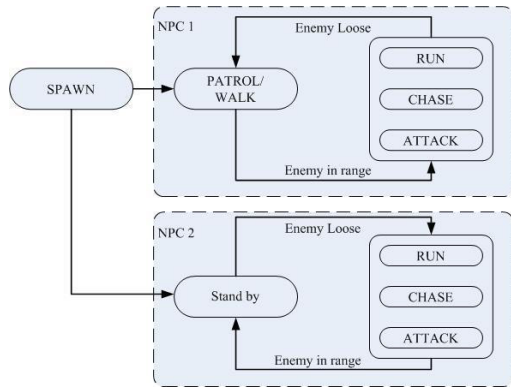


Figure 5. Top Level FSM for both NPC.

Table 1. Questionnaire Results

No	DESCRIPTION	ASSESSMENT	
		yes	no
1	I know the function of each menu	83%	16,6%
2	I understand menu guidance	87,5%	12,5%
3	I know how to choose the level	75%	25%
4	I know how to play	100%	0%
5	I know how to get help	91,6%	8,3%
6	I'm bored with this 3D games	12,5%	87,5%
7	3D games interesting and fun	87,5%	12,5%
8	I find it easier to learn Arabic with 3D gaming	91,6%	8,3%
9	I want to play 3D games again	91,6%	8,3%

From the data above game test results can be concluded in general that is easy to play 3D games with the percentage of respondents know the function of each menu by 83%, to understand the game of 87.5% instructions, knowing how to choose the level of 75% and know how to play at 100 %. This game can also be summed up as an interesting game and can improve the quality of learning Arabic. With the percentage of respondents said learning Arabic is not boring at 87.5%, learning interesting and fun Arabic 87.5%, of respondents find it easier to learn

Arabic using 3D games at 91.6%. The recapitulation of this questionnaire as a whole shows that the game is up and running properly.

CONCLUSION

The existing State pattern does not provide explicit representations for all the FSM concepts. This makes maintenance hard because it is not obvious how to apply a design change to the implementation.

Building a FSM requires the developer to extend classes rather than to configure them. Only FSM Actions need to be implemented in our framework. The resulting FSM Action objects can be reused in other FSMs. This opens the possibility to make a FSM Action component library.

There are also some disadvantages compared to the original State pattern: The context repository object possibly causes a performance penalty compared to directly accessing variables, since variables need to be obtained from a repository.

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Fresy Nugroho et al. * (2013) **-**

COMPARISON OF PEARSON CORRELATION AND CONDITIONAL MUTUAL INFORMATION TO CONSTRUCT MODEL OF TREE-AUGMENTED NETWORK (TAN) (Case study character handwriting recognition)

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ABSTRACT

One step in constructing a model of Tree-Augmented Network (TAN) is determining the dependency or the relationship between a pair of object features. Therefore, the accuracy in measuring the relationships between a pair of object features, it will have a significant impact on the classification or recognition. In this research, there are two methods to measure the closeness of the relationship between a pairs of object features. The two methods are the Pearson Correlation and Conditional Mutual Information in constructing a model structure Tree-Augmented Network. As a case study in testing the two methods are the case studies handwritten character recognition. The results of experiments involving 10 types of handwritten characters (A, B, C, D, E, F, G, H, I, J) which does not distinguish uppercase or lowercase letters with a total of 100 training data showed character. The first by using the correlation Pearson in measuring dependency relationships between a pairs of object features produced TAN models with highest system accuracy by 84%, while using Conditional Mutual Information TAN produce models with highest system accuracy of 97%. Difference in system accuracy is observed in more depth occur because of differences in the structure of TAN models produced, as a result of different measurement results dependencies. So it can be said that the value of Conditional Mutual Information corrected Pearson correlation value for forming the structure of TAN models better.

Keywords

Tree-Augmented Network, Character Handwriting, Conditional Mutual Information, Correlation Pearson, System Accuracy

INTRODUCTION

Tree-Augmented Network (TAN) is one of the reliable models in the classification or recognition, because the concept of the model is constructed with attention to relationships or dependencies between the pair object features. Because TAN models are constructed with attention to dependencies between a pair of features, so the accuracy in measuring the relationship between the object feature to be a very important issue. There are several methods commonly used to measure the closeness of the relationship between two variables or features. The methods commonly used are Correlation Pearson and Conditional Mutual Information.

In the concept of correlation is used to measure the closeness of two variables or features and the value indicated by the correlation coefficient. While Mutual information is built from information theory and the concepts used to measure the two-object dependencies based on its variables by considering uncertainty in the variables or features (Thomas, 1991), (Josien, 1999, 2003) (Battiti,1994), (Shan, 2011). Because the same measure the relationship two variables or futures, further in this study will be reviewed and tested two methods to construct structure of TAN models with cases handwriting character recognition (Irwan, 2012). From the results of the assessment and subsequent trial compared the structure of TAN models are generated and the accuracy of the system.

Tree-Augmented Network (TAN)

In concept, Tree-Augmented Network (TAN) is improvement of Naive Bayes classifier. In practice Naive Bayes classifier less realistic, because the assumed classifier Naive Bayes, probability of each attribute for each class are independent. Because that, then performed of Naive Bayes repairs on the augmented Naive Bayes so that it appears that in principle equivalent for Bayesian Network (Friedman, 1997). From the augmented Naive

Bayes resulted next classifier is Tree-Augmented Network as an efficient solution in finding the Bayesian network with the permissibility of the dependency between the features or variables.

If $D = \{X_1, \dots, X_n\}$ is a set of features of an object and C is a class of objects that exist, then the Bayesian network is formed Directed Acyclic Graph (G) which is the joint probability distribution D with network parameters (Θ) that is written in mathematical form $B = \langle G, \Theta \rangle$. While the joint probability distribution D (Friedman, 1997) when the class C is known and network parameter (Θ), written mathematically

$$P_B(X_1, \dots, X_n | C, \Theta) = \prod_{i=1}^n P_B(X_i | \Pi_{X_i} | C) = \prod_{i=1}^n \theta_{(X_i | \Pi_{X_i} | C)} \quad (1)$$

with $\theta_{x_i | \Pi_{x_i}} = P_B(x_i | \Pi_{x_i} | C)$, for each value of $x_i \in X_i$ and $\Pi_{x_i} \in \Pi_{X_i}$, where Π_{X_i} is a set of parent X_i within G .

Construct Tree-Augmented Network (TAN)

TAN models built by Chow and Liu procedure which has five stages (Amy, 2005) (Murphy, 2001). The first stage is determining the value of each pair of attribute dependencies (X_i and $X_j, i \neq j$). The second stage is building a complete undirected graph, with the nodes in the graph are all attributes or features X_1, \dots, X_n with the weight of each edge is the value of each pair attribute dependency (X_i and X_j). The third stage is building the spanning tree using the maximum weighted spanning tree (MWST) by Prim's algorithm (Levitin, 2003). Fourth stage, build tree trending of tree undirected that resulted on stage two and three by choosing a root feature and setting up direction edge of root. The fifth step, building a model of the structure of TAN by adding a vertex C and add C to each edge of object features.

Pearson Correlation

Pearson correlation is used to measure the closeness of the linear relationship between two objects features. The magnitude of the relationship ρ is indicated by the correlation coefficient for the population and symbolized by r for the sample (DeCoursey, 2003).

If known feature $X_i = \{x_{i1}, x_{i2}, \dots, x_{iN}\}$ and feature $X_j = \{x_{j1}, x_{j2}, \dots, x_{jN}\}$ the magnitude of the correlation coefficient is defined as follows:

$$\rho(X_i, X_j) = \frac{\sigma_{ij}}{\sqrt{\sigma_i^2 \sigma_j^2}} \tag{2}$$

where σ_i^2 , σ_j^2 and σ_{ij} are the elements of the covariance matrix X_i and X_j is written as follows:

$$\Sigma_{ij} = \begin{bmatrix} \sigma_i^2 & \sigma_{ij} \\ \sigma_{ji} & \sigma_j^2 \end{bmatrix} \tag{3}$$

The correlation coefficient is used to measure the closeness between the pair object features, to further as the weight on the edge of a graph.

Conditional Mutual Information

Used to measure the amount of dependencies between each pair of attributes that is X_i and X_j with $i \neq j$. For two continuous variables (X_i and X_j) assumed to follow a joint distribution (bivariate gaussian) and If known joint distribution X_i and X_j given $C = c$ the terms X_i and X_j follow the bivariate gaussian distribution with mean $\mu_{ij|c}$ and covariance $\sum_{ij|c}$ (Michael, 2006 then value of mutual information between variable X_i and X_j the condition C can be determined as in equation 4 (Aritz,2006).

$$I(X_i, X_j | C) = -\frac{1}{2} \sum_{c=1}^r P(c) \log(1 - \rho_c^2(X_i, X_j)) \tag{4}$$

$$\text{where } \rho_c(X_i, X_j) = \frac{\sigma_{ij|c}}{\sqrt{\sigma_{i|c}^2 \sigma_{j|c}^2}} \tag{5}$$

is the correlation coefficient between X_i and X_j

Parameter Estimation TAN Model

Estimation of the model parameters is done by looking at the structure of TAN models that have been constructed as well as attention to the features and his parent. From structure of models that has been constructed by using the rules of conditional probability can estimation parameter on each class (Friedman, 1997) (Jesus, 1999). If the joint feature and his parent in each class follow the bivariate gaussian distribution as well as distribution of feature his parent assumed follow a normal distribution then parameters of distribution can estimate using Maximum Likelihood estimator.

Recognition by TAN Model

Object recognition is done, after the structures of model constructed and the parameters of model are estimated. By using equation 1, will be determined on the probability of each class based on its parameters, and then using the rules of the recognized object based on the value of the biggest opportunities among others (Aitor, 2005).

METHODS

Constructing the Structure of TAN Models with Pearson correlation

Construct the structures of TAN models with Pearson correlation can be seen in Algorithm 1. Constructing the Structure of TAN Models with Pearson correlation

Algorithm 1. Constructing the Structure of

TAN Models TAN with Pearson correlation

```

StrukturMadel – TAN(D)
for setiap  $X_i, X_j$  do
    bobotij =  $\rho(X_i, X_j)$ 
UG ← GraphTB(bobot)
B ← MWST(UG)
{maximum weighted spanning tree}
TB ← GraphB(B,root)
TAN ← TambahK(TB)
return TAN
    
```

Based on the above algorithm, the Pearson correlation is used to determine the weight of edge in an undirected graph UG obtained with GraphTB function. By using the function MWST (maximum weighted spanning tree) which subsequently formed is B undirected tree (Bayesian Network). Furthermore B and root selected, the function GraphB constructed TB is directed tree and from B. Subsequently TB formed the model of TAN with TambahK function.

Constructing the Structure of TAN Models with Conditional Mutual Information

As for the constructing the structure of models TAN with Conditional Mutual Information, about the same as the steps in Constructing The Structure of TAN Models TAN with Pearson correlation, just a different way of getting weight. On its edge weights are obtained by using Conditional Mutual Information as in equation 4.

Experiments

Experiments to compare the two methods of correlation and mutual information in constructing TAN models is a data in the form of handwriting character (image) that consists of 10 classes (characters) is a/A, b/B, c/C ,d/D

,e/E ,f/F , g/G , h/H, i/I, j/J (regardless of the lowercase characters or large). Examples of characters handwriting as in **Fig. 1** with each type of character (class) has a sample size of 10 characters so that the total data for training is 100 characters .

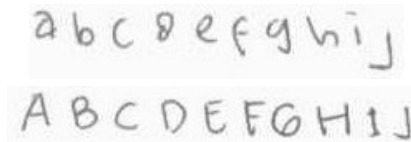


Figure 1. Real data with ten types of Object character handwriting (a/A, b/B, c/C, d/D, e/E, f/F, g/G,h/H, i/I, j/J) (Irwan, 2012)

While the experimental scenario against experimental data, can be seen in Table 1 that the experimental data scenarios with some dimensions or object features .

Table 1. The experimental data scenarios

Experiments	Dimension Object (Image)	Size feature Object
1	6x3	18
2	8x4	32
3	10x5	50
4	12x6	72
5	14x7	98

Dimensional object (image) of **Table 1** is the size of the image when used as training data should be the same, example in experiments 1 dimensions are 6 x 3 This means that every image character start character a/A to j/J made the same dimensions are 6 x 3.

Performance of an experiment to compare two methods of Pearson correlation and conditional mutual information in the TAN model building there are several stages. The experimental stages are follows:

1. Determine the dimensions of the features of training objects is many

- object features extracted from the object (image) training data
- 2. Dimensional object feature extraction is based on pre-defined features.
- 3. Construct a model structure TAN using Pearson Correlation and Conditional Mutual Information based algorithms construct the structure of TAN models as in the previous chapter.
- 4. Estimation of model parameters based on the structure of the model that has been constructed in step 3.
- 5. Perform classification or object recognition training data based on the structure of model has been built based on the results of step 3 and 4.
- 6. Determine the accuracy of the TAN model systems are built with the Pearson correlation method and system accuracy TAN models are built with Conditional Mutual Information in recognizing character handwriting.

The values that can be compared from the two methods of Pearson Correlation and Conditional Mutual Information in the TAN model are constructing structure of models which are generated for each scenario trial and the accuracy of the resulting system.

RESULTS

The experimental results using Pearson Correlation and Conditional Mutual Information to some dimensional image of characters handwriting as in the scenario can be seen in **Table 2**. In general, the results of the experiment showed that for all dimensions of the object (image), the structure of TAN models are constructed using Conditional Mutual Information shows the results better with average system accuracy is 87.4 %.

Table 2. The accuracy of system TAN model with Pearson Correlation and Conditional Mutual Information

no	Dimension Object (Image)	System Accuracy (%)	
		TAN with Pearson Correlation	TAN with Conditional Mutual Information
1	6x3(18 features)	77	83
2	8x4(32 features)	77	81
3	10x5(50 features)	80	85
4	12x6(72 features)	84	97
5	14x7(98 features)	75	91
average		78,6	87,4

The highest system accuracy is 97 %, obtained by using the structure of TAN models using Conditional Mutual Information on the dimensions of the object (image) 12 x 6 (72 features). Compared with the highest system accuracy generated by the structure of TAN models using the Pearson correlation is 84 %, it can be said the structure of TAN models with Conditional Mutual Information is much better .In more detail the results of the character handwriting recognition using the two methods can be seen in **Fig. 2**.

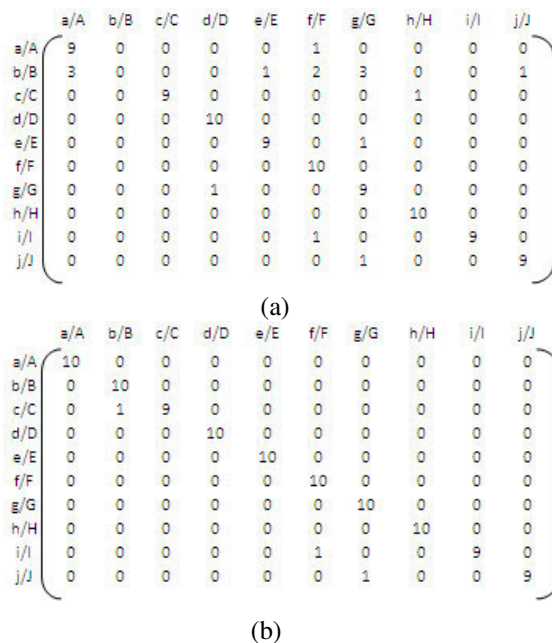


Figure 2. Confusion matrix character handwriting recognition: (a) TAN models with Pearson Correlation (b) TAN models with Conditional Mutual Information

Differences in the level of system accuracy of the two methods is caused by the structure of TAN models are constructed with use two methods is different (see **Fig. 3.**). The difference was evident in the resulting directed tree is features that act as a parent and not parent.

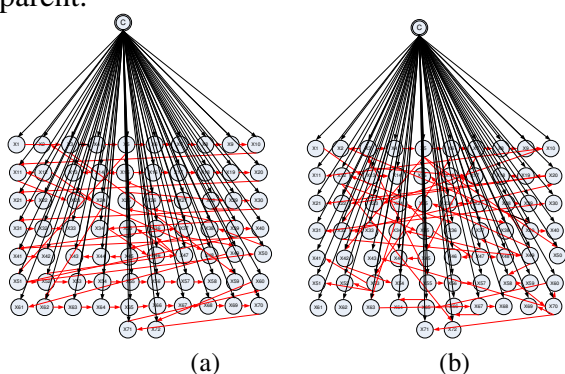


Figure 3. Structure of TAN models with dimensions of objects 12x6 (a) Pearson correlation (b) Conditional Mutual Information

By generated model structure of TAN and the level of accuracy of different systems demonstrate the value of Conditional Mutual Information corrected Pearson correlation values because the structure of TAN models is better.

CONCLUSION

Accuracy in measuring the magnitude of the dependency relationships between a pair of object features have effect in constructing the model as well as the effect on the accuracy of the TAN system. The results of experiments involving 10 types of characters handwriting (A, B, C, D, E, F, G, H, I, J) which does not distinguish uppercase or lowercase as training

data show that TAN models constructed using Pearson correlation produces highest accuracy of system is 84%. While the TAN models by using Conditional Mutual Information systems produce the highest accuracy of system is 97%. Differences accuracy of system occur because of differences in structure of TAN models as a result of the different measurement results dependencies between Pearson Correlation and Conditional Mutual Information. Experimental results also indicate that the value of Conditional Mutual Information corrected Pearson correlation values because the structure of TAN models is built better.

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SOLUTION OF VAN DER POL EQUATION USING ADAMS BASHFORTH MOULTON FOURTH ORDER METHODS

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ABSTRACT

Problems involving mathematical models, especially the form of differential equations often arise in the application. For example, the form of ordinary differential equations the process Van der Pol derived from RLC circuit problem. Van der Pol equation obtained from the research are studied Balthazar Van der Pol in 1920 for the same type with the RLC circuit, but with a passive resistor from Ohm's law is replaced by an active element formed from a closed triode tubes (semiconductor). This equation is a form of non-linear differential equations are difficult to solve analytically, so the solution can be done numerically, of whom can use method of fourth order Adams Bashforth Moulton (ABM). In this study, the completion of the Van der Pol equation using ABM fourth order methods where each of the three initial values x and y is obtained from the Runge Kutta (RK) method. Solution $x(t)$ is initially smaller gradually increased in amplitude and the solution $y(t)$ is also smaller increases gradually so that each oscillation solution reaches a certain limit. Further analysis of the dynamic behavior of the Van der Pol equation shows that the Van der Pol equation around the fixed point $(0,0)$ is an unstable spiral point. All trajectories move towards a single periodic orbit.

Key Words: Adams Bashforth Moulton (ABM) Fourth Order Methods, Van der Pol Equation.

INTRODUCTION

Mathematical model in the form of differential equations frequently arise in the application, for example, Van der Pol equation. Completion of the equation can not be solved analytically as the Van der Pol equation, then the solution can be done numerically. One of the numerical methods that can be used is ABM fourth order methods. It, first used the formula predictor to predict a value of y_{n+1} corrector formula is then used to correct the value of y_{n+1} better (Djojodihardjo, 2000). Then the behavior of solutions of Van der Pol equation is done by analyzing near fixed point and trajectory stability Van der Pol equation in phase plane.

BASIC THEORY

1. Van der Pol Equation

The research studied Balthazar Van der Pol in 1920 for the same type with the RLC circuit, but with a passive resistor from Ohm's law is replaced by an active element formed from a closed tube triode (semiconductor) as shown **Fig. 1** (Tsatsos, 2006). Van der Pol equation is shown as follows:

$$\frac{d^2x}{dt^2} + \mu(x^2 - 1) \frac{dx}{dt} + x = 0, \mu \in [0, \infty)$$

where μ is a parameter damped. So the Van der Pol equation system is obtained

$$\frac{dx}{dt} = y \text{ and } \frac{dy}{dt} = -x + \mu(1 - x^2)y \quad (2.1.3)$$

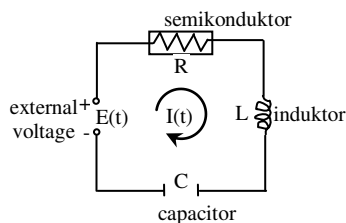


Figure 2. Circuit RLC of semiconductor

2. ABM Fourth Order Methods

ABM method fourth order formula for second order differential equations are as follows:

Predictor:

$$py_{n+1} = y_n + \frac{h}{24} (55y_n' - 59y_{n-1}' + 37y_{n-2}' - 9y_{n-3}')$$

$$pz_{n+1} = z_n + \frac{h}{24} (55z_n' - 59z_{n-1}' + 37z_{n-2}' - 9z_{n-3}')$$

Corrector:

$$y_{n+1} = y_n + \frac{h}{24} (9py_{n+1}' + 19y_n' - 5y_{n-1}' + y_{n-2}')$$

$$z_{n+1} = z_n + \frac{h}{24} (9pz_{n+1}' + 19z_n' - 5z_{n-1}' + z_{n-2}') \quad (2.2.1)$$

with $y_n' = f(x_n, y_n, z_n) \equiv z_n$ and $z_n' = g(x_n, y_n, z_n) \equiv y_n'', \forall n = 3, 4, \dots$ (Bronson and Costa, 2007).

3. Error of ABM Fourth Order Methods

The error predictor of Adams Bashforth (AB) and corrector of Adams Moulton (AM) in the order of $O(h^5)$ in the differential equation, that

$$E_{AM} = \frac{251}{720} h^5 y^{(5)}(\xi_1) \quad (2.3.1)$$

$$E_{AB} = -\frac{19}{720} h^5 y^{(5)}(\xi_2) \quad (2.3.2)$$

If Y_{n+1} is the exact value of y at x_{n+1} , then an error estimate from equation (2.3.1) and (2.3.2):

$$Y_{n+1} - py_{n+1} = \frac{251}{720} h^5 y^{(5)}(\xi_1) \quad (2.3.3)$$

$$Y_{n+1} - y_{n+1} = -\frac{19}{720} h^5 y^{(5)}(\xi_2) \quad (2.3.4)$$

Generally $\xi_1 \neq \xi_2$, but, if it considers that the relevant interval $y^{(5)}(t)$ remains close, then after reducing equation (2.3.3) from (2.3.4) obtained the following estimates for $y^{(5)}$ is $h^5 y^{(5)} = \frac{720}{270} (y_{n+1} - py_{n+1})$. If this is substituted into equation (2.3.2), so

$$E_{AB} = -\frac{1}{14} (y_{n+1} - py_{n+1}) = D_{n+1} \quad (2.3.5)$$

(Conte and Boor, 1993).

4. Dynamic Analysis in Autonomous Systems

4.1 Autonomous and Nonautonomous Systems

Let system of differential equations

$$\frac{dx}{dt} = F(x, y) \text{ and } \frac{dy}{dt} = G(x, y) \quad (2.4.1)$$

If F and G do not depend explicitly on t , it is called autonomous systems. Conversely, if F and G depend explicitly on t , it is called nonautonomous system (Hariyanto, *et al.*, 1992).

4.2 Fixed Point of Autonomous Systems

Fixed points of system (2.4.1) is (x^*, y^*) , such that $F(x^*, y^*) = 0$ and $G(x^*, y^*) = 0$ (Waluya, 2006).

4.3 Eigen Value, Eigen Vector, and Combination Linear of Solution

Let system of linear differential equations

$$\dot{x} = Ax \tag{2.4.2}$$

with $\dot{x} = \begin{bmatrix} \dot{x}_1 \\ \vdots \\ \dot{x}_n \end{bmatrix}$, $A = [a_{ij}]_{n \times n}$, and $x = \begin{bmatrix} x_1 \\ \vdots \\ x_n \end{bmatrix}$.

λ is a eigenvalues for matrix A if only if $(A - \lambda I)$ can't inversed (Conte dan Boor, 1993). There

$$\det(A - \lambda I) = 0 \tag{2.4.3}$$

For each eigenvalue λ_i , there is a corresponding nonzero solution $v^{(i)}$, called an eigenvector, with $i = 1, 2, \dots, n$, so obtained

$$(A - \lambda I)v = 0 \tag{2.4.4}$$

where I is the identity matrix and 0 is zero vector. For each eigenvalue-eigenvector pair there is a corresponding vector solution $x^i(t) = v^{(i)}e^{\lambda_i t}$ of Equation (2.4.2). A general solution of matrix A is combination linear of

$$x(t) = C_1 v^{(1)} e^{\lambda_1 t} + \dots + C_n v^{(n)} e^{\lambda_n t} \tag{2.4.5}$$

where C_1, C_2, \dots, C_n can obtained with given initial condition at Equation (2.4.2) (Boyce dan DiPrima, 1999). Assume that $M(t)$ is an $n \times n$ matrix solution of Equation (2.4.2). The determinant

$$W(t) = \det(M(t))$$

is called the Wronskian of the linear system. The solutions are called independent, provided that the corresponding matrix solution has $\det(M(0)) \neq 0$ (Robinson, 2004).

4.4 Analysis Phase Plane of Autonomous Systems

Let autonomous system is

$$\frac{dx}{dt} = ax + by \text{ and } \frac{dy}{dt} = cx + dy \tag{2.4.6}$$

with $a, b, c, d \in R$. Characteristic equation of Equation (2.4.6) is

$$\lambda^2 - (a + d)\lambda + (ad - bc) = 0 \tag{2.4.7}$$

Stability of fixed point for Equation (2.4.6) depended value λ_1 and λ_2 of Equation (2.4.7). This stability show in table as below:

Table 2.1 Stability of Equation (2.4.6)

Eigenvalues	Type of Critical Point	Stability
$\lambda_1 > \lambda_2 > 0$	Node	Unstable
$\lambda_1 < \lambda_2 < 0$	Node	Asymptotically stable
$\lambda_2 < 0 < \lambda_1$	Saddle point	Unstable
$\lambda_1 = \lambda_2 > 0$	Proper or improper node	Unstable
$\lambda_1 = \lambda_2 < 0$	Proper or improper	Asymptotically stable

$\lambda_{1,2} = \alpha \pm i\beta$	node	
$\alpha > 0$	Spiral point	Unstable
$\alpha < 0$		Asymptotically stable
$\lambda_{1,2} = \pm i\beta$	Center	Stable

(Boyce dan DiPrima, 2001).

4.5 Linearization

Let autonomous systems (2.4.1) with F and G is nonlinear, with using the Taylor expansion near (x^*, y^*) at the time $F(x^*, y^*) = G(x^*, y^*) = 0$, so obtained the linearized system at a fixed point (x^*, y^*) is given by

$$\begin{bmatrix} \dot{u} \\ \dot{v} \end{bmatrix} = \begin{bmatrix} \frac{\partial F}{\partial x} & \frac{\partial F}{\partial y} \\ \frac{\partial G}{\partial x} & \frac{\partial G}{\partial y} \end{bmatrix} \begin{bmatrix} u \\ v \end{bmatrix} \tag{2.4.8}$$

where all the partial derivatives in the matrix are evaluated at (x^*, y^*) with $(u, v) = (x - x^*, y - y^*)$ (Robinson, 2004).

5. Parameter Van der Pol Equation

Van der Pol equation has a relevant interest, particularly in the extreme cases when the parameter μ is either small or very large, which are associated with typical asymptotic behaviors of self-oscillating systems it describes. When μ is very large, in the limit tending to infinity, one obtains relaxation oscillations, i.e., strongly non linear oscillations exhibiting sharp periodic jumps. Typical examples of such systems are nearly sinusoidal electronic oscillators and multivibrators (Buonomo, 1998).

DISCUSSION

1. Solution of Van der Pol Equation Using ABM Fourth Order Methods

In Equation (2.1.3) show that

$$\begin{aligned} x' &= f(t, x, y) = y \\ y' &= g(t, x, y) = -x + \mu(1 - x^2)y \end{aligned}$$

and given $x_0 = 1, y_0 = 0, t = [0, 50]$, and take the value of $\mu = 1$ and $h = 0.04$. So many iterations that is $n = \frac{50-0}{0.04} = 1250$ iterations. Further find for value $x_{n+1}, y_{n+1}, \forall n = 3, 4, \dots, \Delta t/h$ using Equation (2.2.3) and (2.3.5) with $x'_n = f(t_n, x_n, y_n)$ and $y'_n = g(t_n, x_n, y_n)$, $\forall n = 3, 4, \dots$, with RK fourth order methods for starting iteration so obtained as table below:

Tabel 3.1 Solution of Van der Pol Equation Using ABM Fourth Order Methods

t_n	x_n	y_n	$ Dx_n $	$ Dy_n $
0	1	0	-	-
0.04	0.99920010	-0.03998997	-	-
0.08	0.99680154	-0.07992490	-	-
0.12	0.99280740	-0.11976378	-	-
0.16	0.98722205	-0.15948063	1.6×10^{-8}	0.9×10^{-8}
0.20	0.98005069	-0.19906410	1.6×10^{-8}	1.3×10^{-8}
⋮	⋮	⋮	⋮	⋮
50	-1.56707998	0.74400898	0.7×10^{-8}	5.3×10^{-8}

1. Plot.

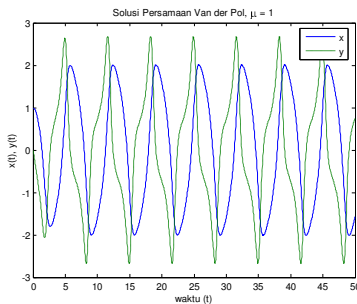


Figure 3. Plot Value x and y versus t

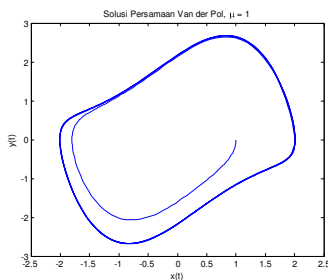


Figure 4. Plot Value y versus x

2. Dynamic Analysis at near Fixed Point Van der Pol Equation

A Van der Pol system (2.1.3), with $\mu = 1$ so Equation (2.1.3) to be

$$\begin{aligned} \frac{dx}{dt} &= y \\ \frac{dy}{dt} &= -x + (1 - x^2)y \end{aligned} \tag{3.2.1}$$

and obtained a fixed point (0,0), it is so the original point. For known behavior of solution of nonlinear Equation (3.2.1) then used approach linear system. Then using Equation (2.4.6) obtained the linearized system at a fixed point (0,0) is given by

$$\begin{bmatrix} \dot{u} \\ \dot{v} \end{bmatrix} = \begin{bmatrix} 0 & 1 \\ -1 & 1 \end{bmatrix} \begin{bmatrix} u \\ v \end{bmatrix} \tag{3.2.2}$$

where $u = x - x^* = x$ and $v = y - y^* = y$. Next look for the eigenvalues and eigenvectors of equation (2.4.4), and the eigenvalues are

$\lambda_{1,2} = \frac{1}{2} \pm \frac{\sqrt{3}}{2} i$. Eigenvalues in the form $\lambda_{1,2} = \alpha \pm i\beta$ with $\alpha = \frac{1}{2}$ and $\beta = \frac{\sqrt{3}}{2}$. This behavior is called a spiral point and for $\alpha = \frac{1}{2} > 0$, then the stability of the fixed point is unstable. Then with eigenvalues $\frac{1}{2} + \frac{\sqrt{3}}{2} i$, obtained eigenvectors $v^{(1)} = \begin{bmatrix} 1 \\ 2 \end{bmatrix} + \begin{bmatrix} -\sqrt{3} \\ 0 \end{bmatrix} i$. This

means that $v^{(1,2)} = u \pm iw$ with $u = \begin{bmatrix} A_1 \\ B_1 \end{bmatrix} = \begin{bmatrix} 1 \\ 2 \end{bmatrix}$ and $w = \begin{bmatrix} A_2 \\ B_2 \end{bmatrix} = \begin{bmatrix} -\sqrt{3} \\ 0 \end{bmatrix}$, and the general solution is

$$\begin{bmatrix} u(t) \\ v(t) \end{bmatrix} = e^{\frac{1}{2}t} \left(\begin{bmatrix} \frac{1}{2} c_1 \cos\left(\frac{\sqrt{3}}{2}t\right) + \frac{\sqrt{3}}{2} c_1 \sin\left(\frac{\sqrt{3}}{2}t\right) \\ c_1 \cos\left(\frac{\sqrt{3}}{2}t\right) \end{bmatrix} + \begin{bmatrix} 12c_2 \sin 32t - 32c_2 \cos 32t \\ c_2 \sin 32t \end{bmatrix} \right) \tag{3.2.3}$$

because $x(0) = 1$ and $y(0) = 0$ so $u(0) = 1$ and $v(0) = 0$, and obtained value $c_1 = 0$ and $c_2 = -\frac{2\sqrt{3}}{3}$. Then substituted into Equation (3.2.3) and obtained the particular solution for Equation (3.2.2) is

$$\begin{bmatrix} u(t) \\ v(t) \end{bmatrix} = \begin{bmatrix} e^{\frac{1}{2}t} \left(-\frac{\sqrt{3}}{3} \sin\left(\frac{\sqrt{3}}{2}t\right) - \cos\left(\frac{\sqrt{3}}{2}t\right) \right) \\ -\frac{2\sqrt{3}}{3} e^{\frac{1}{2}t} \sin\left(\frac{\sqrt{3}}{2}t\right) \end{bmatrix} \tag{3.2.4}$$

Then Wronskian of System (3.2.3) at $t = 0$ is $(0) = \det \left(\begin{bmatrix} 1 & -\sqrt{3} \\ 1 & 0 \end{bmatrix} \right) = \frac{\sqrt{3}}{2} \neq 0$. So solutions of system (3.2.3) is independent. Further system (3.2.2) obtained as shown figure (3) and (4). As for the Van der pol equation (3.2.1) obtained as shown figure (5) and (6).

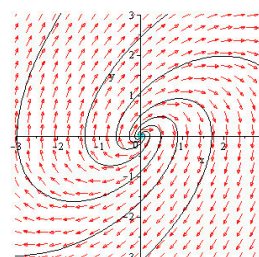


Figure 5. Phase portrait for Equation (3.2.2) with spiral point

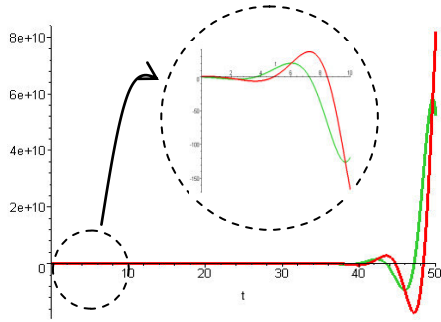


Figure 6. Plot of u and v versus t for Equation (3.2.2) with initial condition (1,0)

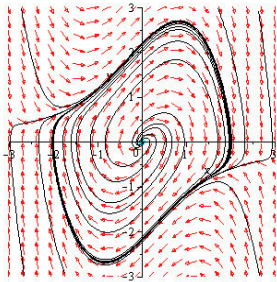


Figure 7. Phase portrait for Equation (3.2.1)

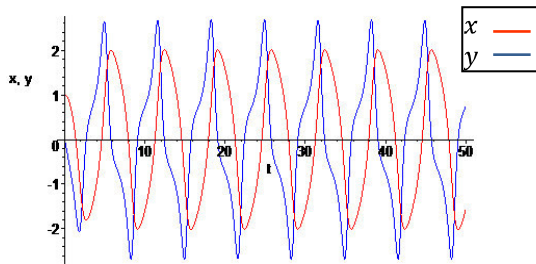


Figure 8. Plot x and y versus t for Equation (3.2.1) with initial condition (1,0)

3. Interpretation

Solutions of Equation (2.1.3) by using ABM fourth order methods, produced images (3.1) which is similar to the figure (3.6). Figure (3.3) shows that the trajectory moves in a clockwise direction. Behavior of this solution is called an unstable spiral. In the figure (3.4) shows that the value of u and v are unstable due to t infinite. Amplitude of the solutions u and v increased sharply at an interval of 40 to 50 and so did the period. Figure (3.5) show that all trajectory goes to a unique periodic orbit. Trajectory that moves from a fixed point to the periodic orbit similar moves with the movement trajectory on Van der Pol equations are linearized. Solution $x(t)$ and $y(t)$ is initially

smaller in amplitude gradually increased so that each oscillation solution reaches a certain limit. In addition, it was found that the graph $x(t)$ and $y(t)$ respectively have the $7\frac{1}{2}$ periods in the interval $t = [0,50]$. While the analysis of the dynamic behavior of the Van der Pol equation solutions around the fixed point (0,0) is an unstable spiral point as shown in the figure (5).

CONCLUSION

Based on the research conducted, it can be concluded that the settlement of Van der Pol equation using ABM method fourth order, it was found that at the time $t = 0.16$, and the value of $x = 0.98722205$ $y = -0.15948063$ with every step to error for x and y are respectively 1.6×10^{-8} and 0.9×10^{-8} . Further analysis of the dynamic behavior of the Van der Pol equation shows that the Van der Pol equation around the fixed point (0,0) is an unstable spiral point. All trajectories move towards a single periodic orbit.

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DOMINATION AND TOTAL DOMINATION CONTRACTION NUMBER FROM SOME SIMPLE CONNECTED GRAPH

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ABSTRACT

A set S of vertices of G is a dominating set of G if every vertex of G is dominated by at least one vertex of S . The minimum cardinality among the dominating sets of G is called the domination number of G and is denoted by $\gamma(G)$. A total dominating set T of G is a dominating set whose induced subgraph has no isolated vertex, namely every vertex of G has a neighbor in T . The total domination number γ_t and γ_t -set of G are defined similarly to $\gamma(G)$ and γ -set. Let $e = xy$ be an edge of a graph G , by G/e we denote the graph obtained from G by contracting the edge e into a new vertex v_e , which becomes adjacent to all the former neighbours of x and of y . In this study, the authors determined the number contraction domination and total domination from path graph, cycle graph, fan graph and star graph by way of : (1) determine the set of domination and total domination set, (2) contracting side based on the set of the set of domination and total domination, (3) create a theorem about the number contraction domination and total domination of the truth and prove theorems about numbers contraction domination and total domination in general.

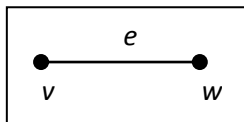
Keywords : Domination, total domination, domination contraction number, contraction

INTRODUCTION

A graph G is a pair set of (V, E) which V is a finite nonempty set of objects called vertex and E is a set (possibly empty) of unordered pair of different vertex of G called *edges*. The *vertex set* of G is denoted by $V(G)$, while the edge set is denoted by $E(G)$ (Chartrand and Lesniak, 1986).

Suppose that v and w is the vertex of a graph G . If v and w connected with an edge $e = vw$, then u and v called adjacent. Then v and w incident with edge e , vw incident with v and w , v and w called the endpoint of $e = vw$ (Wilson and Watkins, 1990).

From the definition above, we can draw a graph

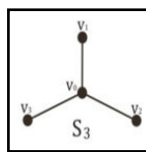


Figures 1. Adjacent and Incident

From figure 1, vertex v and edge e , edge e and vertex w are *incident*, but vertex v and w is *adjacent*.

A cycle of length n is an n -cycle, a 3-cycle is also called a triangle. A cycle graph in order n denoted by C_n (Chartrand and Lesniak, 1986).

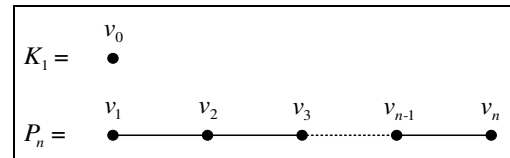
A star graph is a bipartite complete $K_{(1,n)}$ (Harary, 1969).



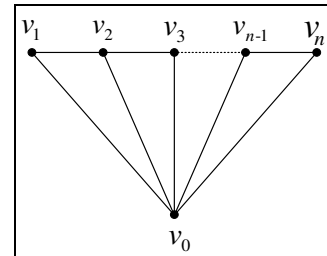
Figures 2. Star Graph S_3

A path graph is a graph that have one path. A path graph in order n denoted by P_n (Wilson and Watkins, 1990). In general, P_n have n edges and $n-1$ vertex.

A fan graph is defined as the graph join K_1 and P_n , where $F_n = K_1 + P_n$. So, a fan graph has $(n + 1)$ vertex and $(2n - 1)$ edge (Gallian, 2009). To draw a fan graph, look this picture:



Then a fan graph $F_n = K_1 + P_n$ is



Figures 3. Fan Graph F_n

Vertex v_0 called as centre of fan graph F_n .

The domination number of G , denoted by $\gamma(G)$, is the minimum cardinality of all dominating sets (Huang, Jia: 2010). A vertex v in a graph G is said to dominate itself and each of its neighbors, that is, v dominates the vertices in its *closed neighborhood* $N[v]$. A set S of vertices of G is a *dominating set* of G if every vertex of G is dominated by at least one vertex of S . equivalently, a set S of vertices of G is a dominating set if every vertex in $V(G) - S$ is adjacent to at least one vertex in S . the minimum cardinality among the dominating sets of G is called the *domination number* of G and its denoted by $\gamma(G)$. A dominating set of cardinality $\gamma(G)$ is then referred to as a *minimum dominating set* (Chartrand and Lesniak, 1996).

A total dominating set T of G is a dominating set whose induced subgraph has no isolated vertex, namely, every vertex of G has a neighbor in T . The total domination number γ_t and γ_t -set of G are defined similarly to $\gamma(G)$ and γ -set (Huang, Jia : 2010).

A set S of vertices in a graph $G(V, E)$ is called a total restrained dominating set if every vertex $v \in V$ is adjacent to an element of S and every vertex of $V - S$ is adjacent to a vertex in $V - S$. The total domination number of a graph G denoted

by $\gamma t(G)$ is the minimum cardinality of a total dominating set in G .

Let $e=xy$ be an edge of a graph $G(V, E)$. By G/e we denote the G/e graph obtained from G by contracting the edge into a new vertex v_e , contraction which becomes adjacent to all the former neighbours of x and of y . Formally, G/e is a graph (V', E') with vertex set $V': (V/\{x, y\}) \cup \{v_e\}$ (whereve is the 'new' vertex, i.e. $v_e \neq V \cup E$) and edge set $E' := \{vw \in E | \{v, w\} \cap \{x, y\} = \emptyset\} \cup \{v_e w | xw \in E/\{e\} \text{ or } yw \in E/\{e\}\}$ (Diestel, 2005).

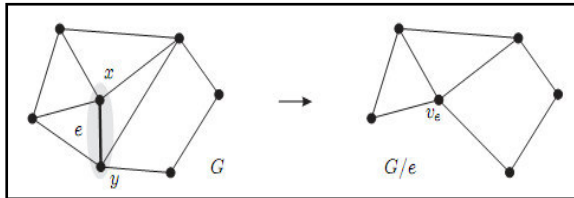


Figure 4. Edge Contraction $e = xy$

RESULTS AND DISCUSSION

Domination and Contraction Domination Numbers

To search for a pattern of domination and pattern of contraction numbers domination of the path graph n vertices, cycle graph n vertices, fan graph n vertices, and star graph n vertices then begins by determining the domination vertex set. Then determine the adjacent edge with domination vertex which would then be contracted. As the definition of (total) domination cantraction number of a graph as the minimum number of edges that must be contraction in order to decrease the (total) domination number.

Path Graph n Vertices (P_n) dan Cycle Graph n Vertices (C_n)

Name of graph	domination before the contracted ($\gamma(P_n)$) & ($\gamma(C_n)$)	contraction numbers domination ($ct_\gamma(P_n)$) & ($ct_\gamma(C_n)$)	domination after the contracted ($\gamma'(P_n)$) & ($\gamma'(C_n)$)
P_4 & C_4	2	1	1

P_5 & C_5	2	2	1
P_6 & C_6	2	3	1
P_7 & C_7	3	1	2
P_8 & C_8	3	2	2
P_9 & C_9	3	3	2
P_{10} & C_{10}	4	1	3
P_{11} & C_{11}	4	2	3
P_{12} & C_{12}	4	3	3
\vdots	\vdots	\vdots	\vdots
P_n & C_n	$\forall n \geq 4$ $\binom{n}{3}$, $\forall n \equiv 0(mod 3)$ $\frac{(n+2)}{3}$, $\forall n \equiv 1(mod 3)$ $\frac{(n+1)}{3}$, $\forall n \equiv 2(mod 3)$	$\forall n \geq 4$ $\frac{3n}{n}$, $\forall n \equiv 0(mod 3)$ $\frac{n}{n}$, $\forall n \equiv 1(mod 3)$ $\frac{2n}{n}$, $\forall n \equiv 2(mod 3)$	$\forall n \geq 4$ $\frac{(n-3)}{3}$, $\forall n \equiv 0(mod 3)$ $\frac{(n-1)}{3}$, $\forall n \equiv 1(mod 3)$ $\frac{(n-2)}{3}$, $\forall n \equiv 2(mod 3)$

Theorem 1

Domination before the contracted from path graph (P_n) and cycle graph (C_n) for $n \geq 4$ is

$$\gamma(P_n) = \gamma(C_n) = \begin{cases} \binom{n}{3}, \forall n \equiv 0(mod 3) \\ \frac{(n+2)}{3}, \forall n \equiv 1(mod 3) \\ \frac{(n+1)}{3}, \forall n \equiv 2(mod 3) \end{cases}$$

with n is many vertices from path graph (P_n) and cycle graph (C_n)

Proof of Theorem 1

a) $\gamma(P_n) = \gamma(C_n) = \binom{n}{3}, \forall n \equiv 0(mod 3)$

• $n = 6$ then $\gamma(P_6) = \gamma(C_6) = \binom{6}{3} = 2$ true

• $n = 3k$ then $\gamma(P_{3k}) = \gamma(C_{3k}) = \binom{3k}{3} = k$ true

• Will be proved : true for $n = 3k + 3$
 $n = 3k + 3$ then $\gamma(P_{3k+3}) = \gamma(C_{3k+3}) = \binom{3k+3}{3} = k + 1$

because $\gamma(P_{3k}) = \gamma(C_{3k}) = k$ is true for $n = 3k$ then $\gamma(P_{3k+3}) = \gamma(C_{3k+3}) = k + 1$ is true for $n = 3k + 3$

∴ so proven to be that $\gamma(P_n) = \gamma(C_n) = \binom{n}{3}, \forall n \equiv 0(mod 3)$

- b) $\gamma(P_n) = \gamma(C_n) = \frac{(n+2)}{3}, \forall n \equiv 1 \pmod{3}$
- $n = 4$ then $\gamma(P_4) = \gamma(C_4) = \frac{(4+2)}{3} = \frac{6}{3} = 2$ true
 - $n = 3k + 1$ then $\gamma(P_{3k+1}) = \gamma(C_{3k+1}) = \frac{((3k+1)+2)}{3} = \frac{(3k+3)}{3} = k + 1$ true
 - Will be proven : true for $n = 3k + 4$
 $n = 3k + 4$ then $\gamma(P_{3k+4}) = \gamma(C_{3k+4}) = \frac{((3k+4)+2)}{3} = \frac{(3k+6)}{3} = k + 2 = (k + 1) + 1$
 because $\gamma(P_{3k+1}) = \gamma(C_{3k+1}) = k + 1$ is true for $n = 3k + 1$ then $\gamma(P_{3k+4}) = \gamma(C_{3k+4}) = (k + 1) + 1$ true for $n = 3k + 4$
- \therefore so proven to be that $\gamma(P_n) = \gamma(C_n) = \frac{(n+2)}{3}, \forall n \equiv 1 \pmod{3}$

- c) $\gamma(P_n) = \gamma(C_n) = \frac{(n+1)}{3}, \forall n \equiv 2 \pmod{3}$
- $n = 5$ then $\gamma(P_5) = \gamma(C_5) = \frac{(5+1)}{3} = \frac{6}{3} = 2$ true
 - $n = 3k + 2$ then $\gamma(P_{3k+2}) = \gamma(C_{3k+2}) = \frac{((3k+2)+1)}{3} = \frac{(3k+3)}{3} = k + 1$ true
 - Will be proven : true for $n = 3k + 5$
 $n = 3k + 5$ then $\gamma(P_{3k+5}) = \gamma(C_{3k+5}) = \frac{((3k+5)+1)}{3} = \frac{(3k+6)}{3} = k + 2 = (k + 1) + 1$
 because $\gamma(P_{3k+2}) = \gamma(C_{3k+2}) = k + 1$ true for $n = 3k + 2$ then $\gamma(P_{3k+5}) = \gamma(C_{3k+5}) = (k + 1) + 1$ true for $n = 3k + 5$
- \therefore so proven to be that $\gamma(P_n) = \gamma(C_n) = \frac{(n+1)}{3}, \forall n \equiv 2 \pmod{3}$

Theorem 2

Contraction numbers domination from path graph (P_n) and cycle graph (C_n) for $n \geq 4$ is

$$ct_\gamma(P_n) = ct_\gamma(C_n) = \begin{cases} \frac{3n}{n}, \forall n \equiv 0 \pmod{3} \\ \frac{n}{n}, \forall n \equiv 1 \pmod{3} \\ \frac{2n}{n}, \forall n \equiv 2 \pmod{3} \end{cases}$$

with n is many vertexes from path graph (P_n) and cycle graph (C_n)

Proof of Theorem 2

- a) $ct_\gamma(P_n) = ct_\gamma(C_n) = \frac{3n}{n}, \forall n \equiv 0 \pmod{3}$
- $n = 6$ then $ct_\gamma(P_6) = ct_\gamma(C_6) = \frac{3 \cdot 6}{6} = 3$ true
 - $n = 3k$ then $ct_\gamma(P_{3k}) = ct_\gamma(C_{3k}) = \frac{3(3k)}{(3k)} = 3$ true
 - Will be proven : true for $n = 3k + 3$
 $n = 3k + 3$ then $ct_\gamma(P_{3k+3}) = ct_\gamma(C_{3k+3}) = \frac{3(3k+3)}{(3k+3)} = 3$
 because $ct_\gamma(P_{3k}) = ct_\gamma(C_{3k}) = 3$ true for $n = 3k$ then $ct_\gamma(P_{3k+3}) = ct_\gamma(C_{3k+3}) = 3$ true for $n = 3k + 3$
- \therefore so proven to be that $ct_\gamma(P_n) = ct_\gamma(C_n) = \frac{3n}{n}, \forall n \equiv 0 \pmod{3}$
- b) $ct_\gamma(P_n) = ct_\gamma(C_n) = \frac{n}{n}, \forall n \equiv 1 \pmod{3}$
- $n = 4$ then $ct_\gamma(P_4) = ct_\gamma(C_4) = \frac{4}{4} = 1$ true
 - $n = 3k + 1$ then $ct_\gamma(P_{3k+1}) = ct_\gamma(C_{3k+1}) = \frac{(3k+1)}{(3k+1)} = 1$ true
 - Will be proven : true for $n = 3k + 4$
 $n = 3k + 4$ then $ct_\gamma(P_{3k+4}) = ct_\gamma(C_{3k+4}) = \frac{(3k+4)}{(3k+4)} = 1$
 because $ct_\gamma(P_{3k+1}) = ct_\gamma(C_{3k+1}) = 1$ true for $n = 3k + 1$ then $ct_\gamma(P_{3k+4}) = ct_\gamma(C_{3k+4}) = 1$ true for $n = 3k + 4$
- \therefore so proven to be that $ct_\gamma(P_n) = ct_\gamma(C_n) = \frac{n}{n}, \forall n \equiv 1 \pmod{3}$

- c) $ct_\gamma(P_n) = ct_\gamma(C_n) = \frac{2n}{n}, \forall n \equiv 2 \pmod{3}$
- $n = 5$ then $ct_\gamma(P_5) = ct_\gamma(C_5) = \frac{2 \cdot 5}{5} = 2$ true

- $n = 3k + 2$ then $ct_\gamma(P_{3k+2}) = ct_\gamma(C_{3k+2}) = \frac{2(3k+2)}{(3k+2)} = 2$ true
- Will be proven : true for $n = 3k + 5$
 $n = 3k + 5$ then $ct_\gamma(P_{3k+5}) = ct_\gamma(C_{3k+5}) = \frac{2(3k+5)}{(3k+5)} = 2$
 because $ct_\gamma(P_{3k+2}) = ct_\gamma(C_{3k+2}) = 2$
 true for $n = 3k + 2$ then $ct_\gamma(P_{3k+5}) = ct_\gamma(C_{3k+5}) = 2$ true for $n = 3k + 5$
 \therefore **so proven to be that** $ct_\gamma(P_n) = ct_\gamma(C_n) = \frac{2n}{n}, \forall n \equiv 2 \pmod{3}$

Teorema 3

Domination after the contracted from path graph (P_n) and cycle graph (C_n) for $n \geq 4$ is

$$\gamma'(P_n) = \gamma'(C_n) = \begin{cases} \frac{(n-3)}{3}, \forall n \equiv 0 \pmod{3} \\ \frac{(n-1)}{3}, \forall n \equiv 1 \pmod{3} \\ \frac{(n-2)}{3}, \forall n \equiv 2 \pmod{3} \end{cases}$$

with n is many vertices from path graph (P_n) and cycle graph (C_n)

Proof of Theorem 3

- a) $\gamma'(P_n) = \gamma'(C_n) = \frac{(n-3)}{3}, \forall n \equiv 0 \pmod{3}$
- $n = 6$ then $\gamma'(P_6) = \gamma'(C_6) = \frac{(6-3)}{3} = \frac{3}{3} = 1$ true
 - $n = 3k$ then $\gamma'(P_{3k}) = \gamma'(C_{3k}) = \frac{(3k-3)}{3} = k - 1$ true
 - Will be proven : true for $n = 3k + 3$
 $n = 3k + 3$ then $\gamma'(P_{3k+3}) = \gamma'(C_{3k+3}) = \frac{(3k+3-3)}{3} = \frac{3k}{3} = k = (k - 1) + 1$
 because $\gamma'(P_{3k}) = \gamma'(C_{3k}) = k - 1$ true for $n = 3k$ then $\gamma'(P_{3k+3}) = \gamma'(C_{3k+3}) = (k - 1) + 1$ true for $n = 3k + 3$
 \therefore **so proven to be that** $\gamma'(P_n) = \gamma'(C_n) = \frac{(n-3)}{3}, \forall n \equiv 0 \pmod{3}$

- b) $\gamma'(P_n) = \gamma'(C_n) = \frac{(n-1)}{3}, \forall n \equiv 1 \pmod{3}$

- $n = 4$ then $\gamma'(P_4) = \gamma'(C_4) = \frac{(4-1)}{3} = \frac{3}{3} = 1$ true
- $n = 3k + 1$ then $\gamma'(P_{3k+1}) = \gamma'(C_{3k+1}) = \frac{(3k+1-1)}{3} = \frac{3k}{3} = k$ true
- Will be proven : true for $n = 3k + 4$
 $n = 3k + 4$ then $\gamma'(P_{3k+4}) = \gamma'(C_{3k+4}) = \frac{(3k+4-1)}{3} = \frac{3k+3}{3} = k + 1$
 because $\gamma'(P_{3k+1}) = \gamma'(C_{3k+1}) = k$ true for $n = 3k + 1$ then $\gamma'(P_{3k+4}) = \gamma'(C_{3k+4}) = k + 1$ true for $n = 3k + 4$
 \therefore **so proven to be that** $\gamma'(P_n) = \gamma'(C_n) = \frac{(n-1)}{3}, \forall n \equiv 1 \pmod{3}$

- c) $\gamma'(P_n) = \gamma'(C_n) = \frac{(n-2)}{3}, \forall n \equiv 2 \pmod{3}$

- $n = 5$ then $\gamma'(P_5) = \gamma'(C_5) = \frac{(5-2)}{3} = \frac{3}{3} = 1$ true
- $n = 3k + 2$ then $\gamma'(P_{3k+2}) = \gamma'(C_{3k+2}) = \frac{((3k+2)-2)}{3} = \frac{3k}{3} = k$ true
- Will be proven : true for $n = 3k + 5$
 $n = 3k + 5$ then $\gamma'(P_{3k+5}) = \gamma'(C_{3k+5}) = \frac{((3k+5)-2)}{3} = \frac{3k+3}{3} = k + 1$
 because $\gamma'(P_{3k+2}) = \gamma'(C_{3k+2}) = k$ true for $n = 3k + 2$ then $\gamma'(P_{3k+5}) = \gamma'(C_{3k+5}) = k + 1$ true for $n = 3k + 5$
 \therefore **so proven to be that** $\gamma'(P_n) = \gamma'(C_n) = \frac{(n-2)}{3}, \forall n \equiv 2 \pmod{3}$

Fan Graph n Vertices (F_n)

Name of graph	domination before the contracted ($\gamma(F_n)$)	contraction numbers domination ($ct_\gamma(F_n)$)	domination after the contracted ($\gamma'(F_n)$)
F_1	1	1	0
F_2	1	2	0
F_3	1	3	0
F_4	1	4	0
F_5	1	5	0
F_6	1	6	0
F_7	1	7	0
F_8	1	8	0
F_9	1	9	0
F_{10}	1	10	0
\vdots	\vdots	\vdots	\vdots
F_n	$\frac{n}{n}$	n	$\frac{n-n}{n}$

Theorem 4

Domination before the contracted from fan graph (F_n) is

$$\gamma(F_n) = \frac{n}{n}, \forall n \in N$$

with n is many vertexs from fan graph (F_n)

Proof of Theorem 4

- $n = 1$ then $\gamma(F_1) = \frac{1}{1} = 1$ true
- $n = k$ then $\gamma(F_k) = \frac{k}{k} = 1$ true
- Will be proven : true for $n = k + 1$
 $n = k + 1$ then $\gamma(F_{k+1}) = \frac{k+1}{k+1} = 1$
 because $\gamma(F_k) = 1$ true for $n = k$ then
 $\gamma(F_{k+1}) = 1$ true for $n = k + 1$
 \therefore so proven to be that $\gamma(F_n) = \frac{n}{n}, \forall n \in N$

Theorem 5

Contraction numbers domination from fan graph (F_n) is

$$ct_\gamma(F_n) = n, \forall n \in N$$

with n is many vertexs from fan graph (F_n)

Proof of Theorem 5

- $n = 1$ then $ct_\gamma(F_1) = 1$ true
- $n = k$ then $ct_\gamma(F_k) = k$ true
- Will be proven : true for $n = k + 1$
 $n = k + 1$ then $ct_\gamma(F_{k+1}) = k + 1$
 because $ct_\gamma(F_k) = k$ true for $n = k$
 then $ct_\gamma(F_{k+1}) = k + 1$ true for
 $n = k + 1$
 \therefore so proven to be that $ct_\gamma(F_n) = n, \forall n \in N$

Theorem 6

Domination after the contracted from fan graph (F_n) is

$$\gamma'(F_n) = \frac{n-n}{n}, \forall n \in N$$

with n is many vertexs from fan graph (F_n)

Proof of Theorem 6

- $n = 1$ then $\gamma'(F_1) = \frac{1-1}{1} = 0$ true
- $n = k$ then $\gamma'(F_k) = \frac{k-k}{k} = 0$ true

- Will be proven : true for $n = k + 1$
 $n = k + 1$ then $\gamma'(F_{k+1}) = \frac{(k+1)-(k+1)}{(k+1)} = 0$ because $\gamma'(F_k) = 0$ true
 for $n = k$ then $\gamma'(F_{k+1}) = 0$ true for
 $n = k + 1$
 \therefore so proven to be that $\gamma'(F_n) = \frac{n-n}{n}, \forall n \in N$

Star Graph n Vertexs (S_n)

Name of graph	domination before the contracted ($\gamma(S_n)$)	contraction numbers domination ($ct_\gamma(S_n)$)	domination after the contracted ($\gamma'(S_n)$)
S_3	1	3	0
S_4	1	4	0
S_5	1	5	0
S_6	1	6	0
S_7	1	7	0
S_8	1	8	0
S_9	1	9	0
S_{10}	1	10	0
\vdots	\vdots	\vdots	\vdots
S_n	$\frac{n \geq 3, n}{n}$	$\frac{n \geq 3, n}{n}$	$\frac{n \geq 3, n-n}{n}$

Theorem 7

Domination before the contracted from star graph (S_n) for $n \geq 3$ is

$$\gamma(S_n) = \frac{n}{n}, \forall n \in N$$

with n is many vertexs from star graph (S_n)

Proof of Theorem 7

- $n = 1$ then $\gamma(S_1) = \frac{1}{1} = 1$ true
- $n = k$ then $\gamma(S_k) = \frac{k}{k} = 1$ true
- Will be proven : true for $n = k + 1$
 $n = k + 1$ then $\gamma(S_{k+1}) = \frac{k+1}{k+1} = 1$
 because $\gamma(S_k) = 1$ true for $n = k$ then
 $\gamma(S_{k+1}) = 1$ true for $n = k + 1$
 \therefore so proven to be that $\gamma(S_n) = \frac{n}{n}, \forall n \in N$

Theorem 8

Contraction numbers domination from star graph (S_n) for $n \geq 3$ is

$$ct_\gamma(S_n) = n, \forall n \in N$$

with n is many vertexs from star graph (S_n)

Proof of Theorem 8

- $n = 1$ then $ct_\gamma(S_1) = n = 1$ true
- $n = k$ then $ct_\gamma(S_k) = n = k$ true
- Will be proven : true for $n = k + 1$
 $n = k + 1$ then $ct_\gamma(S_{k+1}) = n = k + 1$
 because $ct_\gamma(S_k) = k$ true for $n = k$
 then $ct_\gamma(S_{k+1}) = k + 1$ true for
 $n = k + 1$

∴ so proven to be that $ct_\gamma(W_n) = ct_\gamma(S_n) = n, \forall n \in N$

Theorem 9

Domination after the contracted from star graph (S_n) for $n \geq 3$ is

$$\gamma'(S_n) = \frac{n-n}{n}, \forall n \in N$$

with n is many vertexs from star graph (S_n)

Proof of Theorem 9

- $n = 1$ then $\gamma'(S_1) = \frac{1-1}{1} = 0$ true
- $n = k$ then $\gamma'(S_k) = \frac{k-k}{k} = 0$ true
- Will be proven : true for $n = k + 1$
 $n = k + 1$ then $\gamma'(S_{k+1}) = \frac{(k+1)-(k+1)}{(k+1)} = 0$

because $\gamma'(S_k) = 0$ true for $n = k$ then $\gamma'(S_{k+1}) = 0$ true for $n = k + 1$

∴ so proven to be that $\gamma'(S_n) = \frac{n-n}{n}, \forall n \in N$

Total Domination and Total Domination Numbers Contraction

To search for a pattern of domination and pattern of contraction numbers domination of the path graph n vertexs, cycle graph n vertexs, fan graph n vertexs, and star graph n vertexs then begins by determining the domination vertex set. Then determine the adjacent edge with domination vertex which would then be contracted. As the definition of (total) domination cantraction number of a graph as the minimum number of edges that must be contraction in order to decrease the (total) domination number.

Path Graph n Vertexs (P_n) dan Cycle Graph n Vertexs (C_n)

Name of graph	Total domination before the contracted ($\gamma t(P_n)$) & ($\gamma t(C_n)$)	contraction numbers total domination ($ct_{\gamma t}(P_n)$) & ($ct_{\gamma t}(C_n)$)	Total domination after the contracted ($\gamma' t(P_n)$) & ($\gamma' t(C_n)$)
P_5 & C_5	3	1	2
P_6 & C_6	4	1	3
P_7 & C_7	4	2	3
P_8 & C_8	4	3	3
P_9 & C_9	5	1	4
P_{10} & C_{10}	6	1	5
P_{11} & C_{11}	6	2	5
P_{12} & C_{12}	6	3	5
∴	∴	∴	∴
P_n & C_n	$\forall n \geq 5$ $\frac{n}{2}$, $\forall n \equiv 0(mod 4)$ $\frac{(n+1)}{2}$, $\forall n \equiv 1(mod 4)$ dan $\forall n \equiv 3(mod 4)$ $\frac{(n+2)}{2}$, $\forall n \equiv 2(mod 4)$	$\forall n \geq 5$ $\frac{3n}{n}$, $\forall n \equiv 0(mod 4)$ $\frac{n}{n}$, $\forall n \equiv 1(mod 4)$ dan n $\equiv 2(mod 4)$ $\frac{2n}{n}$, $\forall n \equiv 3(mod 4)$	$\forall n \geq 5$ $\frac{(n-2)}{2}$, $\forall n \equiv 0(mod 4)$ $\frac{(n-1)}{2}$, $\forall n \equiv 1(mod 4)$ dan $\forall n \equiv 3(mod 4)$ $\left(\frac{n}{2}\right)$, $\forall n \equiv 2(mod 4)$

Theorem 10

Total domination before the contracted from path graph (P_n) and cycle graph (C_n) for $n \geq 5$ is

$$\gamma t(P_n) = \gamma t(C_n) = \begin{cases} \left(\frac{n}{2}\right), \forall n \equiv 0(mod 4) \\ \frac{(n+1)}{2}, \forall n \equiv 1(mod 4) \\ \quad \forall n \equiv 3(mod 4) \\ \frac{(n+2)}{2}, \forall n \equiv 2(mod 4) \end{cases}$$

with n is many vertexs from path graph (P_n) and cycle graph (C_n)

Proof of Theorem 10

- a) $\gamma t(P_n) = \gamma t(C_n) = \left(\frac{n}{2}\right), \forall n \equiv 0(mod 4)$

- $n = 8$ then $\gamma t(P_8) = \gamma t(C_8) = \left(\frac{8}{2}\right) = 4$ true
- $n = 4k$ then $\gamma t(P_{4k}) = \gamma t(C_{4k}) = \left(\frac{4k}{2}\right) = 2k$ true
- Will be proven : true for $n = 4k + 4$
 $n = 4k + 4$ then $\gamma t(P_{4k+4}) = \gamma t(C_{4k+4}) = \left(\frac{4k+4}{2}\right) = 2k + 2$
because $\gamma t(P_{4k}) = \gamma t(C_{4k}) = 2k$ true for $n = 4k$ then $\gamma t(P_{4k+4}) = \gamma t(C_{4k+4}) = 2k + 2$ true for $n = 4k + 4$
 \therefore so proven to be that $\gamma t(P_n) = \gamma t(C_n) = \left(\frac{n}{2}\right), \forall n \equiv 0 \pmod{4}$

b) $\gamma t(P_n) = \gamma t(C_n) = \frac{(n+1)}{2}, \forall n \equiv 1 \pmod{4}$ and $n \equiv 3 \pmod{4}$

- $n = 5$ then $\gamma t(P_5) = \gamma t(C_5) = \frac{(5+1)}{2} = \frac{6}{2} = 3$ true
 $n = 7$ then $\gamma t(P_7) = \gamma t(C_7) = \frac{(7+1)}{2} = \frac{8}{2} = 4$ true
- $n = 4k + 1$ then $\gamma t(P_{4k+1}) = \gamma t(C_{4k+1}) = \frac{((4k+1)+1)}{2} = \frac{(4k+2)}{2} = 2k + 1$ true
 $n = 4k + 3$ then $\gamma t(P_{4k+3}) = \gamma t(C_{4k+3}) = \frac{((4k+3)+1)}{2} = \frac{(4k+4)}{2} = 2k + 2$ true
- Will be proven : true for $n = 4k + 5$ and true for $n = 4k + 7$
 $n = 4k + 5$ then $\gamma t(P_{4k+5}) = \gamma t(C_{4k+5}) = \frac{((4k+5)+1)}{2} = \frac{(4k+6)}{2} = 2k + 3 = (2k + 1) + 2$
because $\gamma t(P_{4k+1}) = \gamma t(C_{4k+1}) = 2k + 1$ true for $n = 4k + 1$ then $\gamma t(P_{4k+5}) = \gamma t(C_{4k+5}) = (2k + 1) + 2$ true for $n = 4k + 5$

 $n = 4k + 7$ then $\gamma t(P_{4k+7}) = \gamma t(C_{4k+7}) = \frac{((4k+7)+1)}{2} = \frac{(4k+8)}{2} = 2k + 4 = (2k + 2) + 2$
because $\gamma t(P_{4k+3}) = \gamma t(C_{4k+3}) = 2k + 2$ true for $n = 4k + 3$ then $\gamma t(P_{4k+7}) = \gamma t(C_{4k+7}) = (2k + 2) + 2$ true for $n = 4k + 7$

\therefore so proven to be that $\gamma t(P_n) = \gamma t(C_n) = \frac{(n+1)}{2}, \forall n \equiv 1 \pmod{4}$ and $n \equiv 3 \pmod{4}$

c) $\gamma t(P_n) = \gamma t(C_n) = \frac{(n+2)}{2}, \forall n \equiv 2 \pmod{4}$

- $n = 6$ then $\gamma t(P_6) = \gamma t(C_6) = \frac{(6+2)}{2} = \frac{8}{2} = 4$ true
- $n = 4k + 2$ then $\gamma t(P_{4k+2}) = \gamma t(C_{4k+2}) = \frac{((4k+2)+2)}{2} = \frac{(4k+4)}{2} = 2k + 2$ true
- Will be proven : true for $n = 4k + 6$
 $n = 4k + 6$ then $\gamma t(P_{4k+6}) = \gamma t(C_{4k+6}) = \frac{((4k+6)+2)}{2} = \frac{(4k+8)}{2} = 2k + 4 = (2k + 2) + 2$
because $\gamma t(P_{4k+2}) = \gamma t(C_{4k+2}) = 2k + 2$ true for $n = 4k + 2$ then $\gamma t(P_{4k+6}) = \gamma t(C_{4k+6}) = (2k + 2) + 2$ true for $n = 4k + 6$
 \therefore so proven to be that $\gamma t(P_n) = \gamma t(C_n) = \frac{(n+2)}{2}, \forall n \equiv 2 \pmod{4}$

Theorem 11

Contraction numbers total domination from path graph (P_n) and cycle graph (C_n) for $n \geq 5$ is

$$ct_{\gamma t}(P_n) = ct_{\gamma t}(C_n) = \begin{cases} \frac{3n}{n}, \forall n \equiv 0 \pmod{4} \\ \frac{n}{n}, \forall n \equiv 1 \pmod{4} \\ \frac{n}{n}, \forall n \equiv 2 \pmod{4} \\ \frac{2n}{n}, \forall n \equiv 3 \pmod{4} \end{cases}$$

with n is many vertexes from path graph (P_n) and cycle graph (C_n)

Proof of Theorem 11

- a) $ct_{\gamma t}(P_n) = ct_{\gamma t}(C_n) = \frac{3n}{n}, \forall n \equiv 0 \pmod{4}$
- $n = 8$ then $ct_{\gamma t}(P_8) = ct_{\gamma t}(C_8) = \frac{3 \cdot 8}{8} = 3$ true
 - $n = 4k$ then $ct_{\gamma t}(P_{4k}) = ct_{\gamma t}(C_{4k}) = \frac{3(4k)}{(4k)} = 3$ true
 - Will be proven : true for $n = 4k + 4$

$n = 4k + 4$ then $ct_{\gamma t}(P_{4k+4}) = ct_{\gamma t}(C_{4k+4}) = \frac{3(4k+4)}{(4k+4)} = 3$
 because $ct_{\gamma t}(P_{4k}) = ct_{\gamma t}(C_{4k}) = 3$ true for $n = 4k$ then $ct_{\gamma t}(P_{4k+4}) = ct_{\gamma t}(C_{4k+4}) = 3$ true for $n = 4k + 4$
 \therefore so proven to be that $ct_{\gamma t}(P_n) = ct_{\gamma t}(C_n) = \frac{3n}{n}, \forall n \equiv 0 \pmod{4}$

b) $ct_{\gamma t}(P_n) = ct_{\gamma t}(C_n) = \frac{n}{n}, \forall n \equiv 1 \pmod{4}$ and $n \equiv 2 \pmod{4}$

• $n = 5$ then $ct_{\gamma t}(P_5) = ct_{\gamma t}(C_5) = \frac{5}{5} = 1$ true

$n = 6$ then $ct_{\gamma t}(P_6) = ct_{\gamma t}(C_6) = \frac{6}{6} = 1$ true

• $n = 4k + 1$ then $ct_{\gamma t}(P_{4k+1}) = ct_{\gamma t}(C_{4k+1}) = \frac{(4k+1)}{(4k+1)} = 1$ true

$n = 4k + 2$ then $ct_{\gamma t}(P_{4k+2}) = ct_{\gamma t}(C_{4k+2}) = \frac{(4k+2)}{(4k+2)} = 1$ true

• Will be proven : true for $n = 4k + 5$ and true for $n = 4k + 6$

$n = 4k + 5$ then $ct_{\gamma t}(P_{4k+5}) = ct_{\gamma t}(C_{4k+5}) = \frac{(4k+5)}{(4k+5)} = 1$

because $ct_{\gamma t}(P_{4k+1}) = ct_{\gamma t}(C_{4k+1}) = 1$ true for $n = 4k + 1$ then $ct_{\gamma t}(P_{4k+5}) = ct_{\gamma t}(C_{4k+5}) = 1$ true for $n = 4k + 5$

$n = 4k + 6$ then $ct_{\gamma t}(P_{4k+6}) = ct_{\gamma t}(C_{4k+6}) = \frac{(4k+6)}{(4k+6)} = 1$

because $ct_{\gamma t}(P_{4k+2}) = ct_{\gamma t}(C_{4k+2}) = 1$ true for $n = 4k + 2$ then $ct_{\gamma t}(P_{4k+6}) = ct_{\gamma t}(C_{4k+6}) = 1$ true for $n = 4k + 6$

\therefore so proven to be that $ct_{\gamma t}(P_n) = ct_{\gamma t}(C_n) = \frac{n}{n}, \forall n \equiv 1 \pmod{4}$ and $n \equiv 2 \pmod{4}$

c) $ct_{\gamma t}(P_n) = ct_{\gamma t}(C_n) = \frac{2n}{n}, \forall n \equiv 3 \pmod{4}$

• $n = 7$ then $ct_{\gamma t}(P_7) = ct_{\gamma t}(C_7) = \frac{2 \cdot 7}{7} = 2$ true

• $n = 4k + 3$ then $ct_{\gamma t}(P_{4k+3}) = ct_{\gamma t}(C_{4k+3}) = \frac{2(4k+3)}{(4k+3)} = 2$ true

• Will be proven : true for $n = 4k + 7$
 $n = 4k + 7$ then $ct_{\gamma t}(P_{4k+7}) = ct_{\gamma t}(C_{4k+7}) = \frac{2(4k+7)}{(4k+7)} = 2$

because $ct_{\gamma t}(P_{4k+3}) = ct_{\gamma t}(C_{4k+3}) = 2$ true for $n = 4k + 3$ then $ct_{\gamma t}(P_{4k+7}) = ct_{\gamma t}(C_{4k+7}) = 2$ true for $n = 4k + 7$

\therefore so proven to be that $ct_{\gamma t}(P_n) = ct_{\gamma t}(C_n) = \frac{2n}{n}, \forall n \equiv 3 \pmod{4}$

Theorem 12

Total domination after the contracted from path graph (P_n) and cycle graph (C_n) for $n \geq 5$ is

$$\gamma' t(P_n) = \gamma' t(C_n) = \begin{cases} \frac{(n-2)}{2}, \forall n \equiv 0 \pmod{4} \\ \frac{(n-1)}{2}, \forall n \equiv 1 \pmod{4} \\ \forall n \equiv 3 \pmod{4} \\ \binom{n}{2}, \forall n \equiv 2 \pmod{4} \end{cases}$$

with n is many vertexes from path graph (P_n) and cycle graph (C_n)

Proof of Theorem 12

a) $\gamma' t(P_n) = \gamma' t(C_n) = \frac{(n-2)}{2}, \forall n \equiv 0 \pmod{4}$

• $n = 8$ then $\gamma' t(P_8) = \gamma' t(C_8) = \frac{(8-2)}{2} = \frac{6}{2} = 3$ true

• $n = 4k$ then $\gamma' t(P_{4k}) = \gamma' t(C_{4k}) = \frac{(4k-2)}{2} = 2k - 1$ true

• Will be proven : true for $n = 4k + 4$
 $n = 4k + 4$ then $\gamma' t(P_{4k+4}) = \gamma' t(C_{4k+4}) = \frac{(4k+4-2)}{2} = 2k + 2 - 1 = (2k - 1) + 2$

because $\gamma' t(P_{4k}) = \gamma' t(C_{4k}) = 2k - 1$ true for $n = 4k$ then $\gamma' t(P_{4k+4}) = \gamma' t(C_{4k+4}) = (2k - 1) + 2$ true for $n = 4k + 4$

\therefore so proven to be that $\gamma' t(P_n) = \gamma' t(C_n) = \frac{(n-2)}{2}, \forall n \equiv 0 \pmod{4}$

b) $\gamma' t(P_n) = \gamma' t(C_n) = \frac{(n-1)}{2}, \forall n \equiv 1 \pmod{4}$ and $n \equiv 3 \pmod{4}$

- $n = 5$ then $\gamma't(P_5) = \gamma't(C_5) = \frac{(5-1)}{2} = \frac{4}{2} = 2$ true
- $n = 7$ then $\gamma't(P_7) = \gamma't(C_7) = \frac{(7-1)}{2} = \frac{6}{2} = 3$ true
- $n = 4k + 1$ then $\gamma't(P_{4k+1}) = \gamma't(C_{4k+1}) = \frac{(4k+1-1)}{2} = \frac{4k}{2} = 2k$ true
- $n = 4k + 3$ then $\gamma't(P_{4k+3}) = \gamma't(C_{4k+3}) = \frac{(4k+3-1)}{2} = \frac{4k+2}{2} = 2k + 1$ true
- Will be proven : true for $n = 4k + 5$ and true for $n = 4k + 7$
- $n = 4k + 5$ then $\gamma't(P_{4k+5}) = \gamma't(C_{4k+5}) = \frac{(4k+5-1)}{2} = \frac{4k+4}{2} = 2k + 2$ because $\gamma't(P_{4k+1}) = \gamma't(C_{4k+1}) = 2k$ true for $n = 4k + 1$ then $\gamma't(P_{4k+5}) = \gamma't(C_{4k+5}) = 2k + 2$ true for $n = 4k + 5$

$n = 4k + 7$ then $\gamma't(P_{4k+7}) = \gamma't(C_{4k+7}) = \frac{(4k+7-1)}{2} = \frac{4k+6}{2} = 2k + 3 = (2k + 1) + 2$ because $\gamma't(P_{4k+3}) = \gamma't(C_{4k+3}) = 2k + 1$ true for $n = 4k + 3$ then $\gamma't(P_{4k+7}) = \gamma't(C_{4k+7}) = (2k + 1) + 2$ true for $n = 4k + 7$

∴ so proven to be that $\gamma't(P_n) = \gamma't(C_n) = \frac{(n-1)}{2}, \forall n \equiv 1(mod 4)$ and $n \equiv 3(mod 4)$

c) $\gamma't(P_n) = \gamma't(C_n) = \left(\frac{n}{2}\right), \forall n \equiv 2(mod 4)$

- $n = 6$ then $\gamma't(P_6) = \gamma't(C_6) = \left(\frac{6}{2}\right) = 3$ true
- $n = 4k + 2$ then $\gamma't(P_{4k+2}) = \gamma't(C_{4k+2}) = \left(\frac{4k+2}{2}\right) = 2k + 1$ true
- Will be proven : true for $n = 4k + 6$
- $n = 4k + 6$ then $\gamma't(P_{4k+6}) = \gamma't(C_{4k+6}) = \left(\frac{4k+6}{2}\right) = 2k + 3 = (2k + 1) + 2$ because $\gamma't(P_{4k+2}) = 2k + 1$ true for $n = 4k + 2$ then $\gamma't(P_{4k+6}) = \gamma't(C_{4k+6}) = (2k + 1) + 2$ true for $n = 4k + 6$

∴ so proven to be that $\gamma't(P_n) = \gamma't(C_n) = \left(\frac{n}{2}\right), \forall n \equiv 2(mod 4)$

Fan Graph n Vertices (F_n)

Name of graph	Total domination before the contracted ($\gamma t(F_n)$)	Contraction numbers total domination ($ct_{\gamma t}(F_n)$)	Total domination after the contracted ($\gamma' t(F_n)$)
F_1	2	1	0
F_2	2	2	0
F_3	2	3	0
F_4	2	4	0
F_5	2	5	0
F_6	2	6	0
F_7	2	7	0
F_8	2	8	0
F_9	2	9	0
F_{10}	2	10	0
⋮	⋮	⋮	⋮
F_n	$\frac{2n}{n}$	n	$\frac{n-n}{n}$

Theorem 13

Total domination before the contracted from fan graph (F_n) is

$$\gamma t(F_n) = \frac{2n}{n}, \forall n \in N$$

with n is many vertexes from fan graph (F_n)

Proof of Theorem 13

- $n = 1$ then $\gamma t(F_1) = \frac{2 \cdot 1}{1} = \frac{2}{1} = 2$ true
- $n = k$ then $\gamma t(F_k) = \frac{2 \cdot k}{k} = \frac{2k}{k} = 2$ true
- Will be proven : true for $n = k + 1$
- $n = k + 1$ then $\gamma t(F_{k+1}) = \frac{2 \cdot (k+1)}{(k+1)} = \frac{2(k+1)}{(k+1)} = 2$ because $\gamma t(F_k) = 2$ true for $n = k$ then $\gamma t(F_{k+1}) = 2$ true for $n = k + 1$
- ∴ so proven to be that $\gamma t(F_n) = \frac{2n}{n}, \forall n \in N$

Theorem 14

Contraction numbers total domination from fan graph (F_n) is

$$ct_{\gamma t}(F_n) = n, \forall n \in N$$

with n is many vertexes from fan graph (F_n)

Proof of Theorem 14

- $n = 1$ then $ct_{\gamma t}(F_1) = 1$ true
- $n = k$ then $ct_{\gamma t}(F_k) = k$ true
- Will be proven : true for $n = k + 1$
 $n = k + 1$ then $ct_{\gamma t}(F_{k+1}) = k + 1$
 because $ct_{\gamma t}(F_k) = k$ true for $n = k$
 then $ct_{\gamma t}(F_{k+1}) = k + 1$ true for
 $n = k + 1$

∴ so proven to be that $ct_{\gamma t}(F_n) = n, \forall n \in N$

Theorem 15

Total domination after the contracted from fan graph (F_n) is

$$\gamma' t(F_n) = \frac{n - n}{n}, \forall n \in N$$

with n is many vertexs from fan graph (F_n)

Proof of Theorem 15

- $n = 1$ then $\gamma' t(F_1) = \frac{1-1}{1} = 0$ true
- $n = k$ then $\gamma' t(F_k) = \frac{k-k}{k} = 0$ true
- Will be proven : true for $n = k + 1$
 $n = k + 1$ then $\gamma' t(F_{k+1}) = \frac{(k+1)-(k+1)}{(k+1)} = 0$
 because $\gamma' t(F_k) = 0$ true for $n = k$ then
 $\gamma' t(F_{k+1}) = 0$ true for $n = k + 1$

∴ so proven to be that $\gamma' t(F_n) = \frac{n-n}{n}, \forall n \in N$

Star Graph n Vertexs (S_n)

Name of graph	Total domination before the contracted ($\gamma t(S_n)$)	Contraction numbers total domination ($ct_{\gamma t}(S_n)$)	Total domination after the contracted ($\gamma' t(S_n)$)
S_3	2	4	0
S_4	2	5	0
S_5	2	6	0
S_6	2	7	0
S_7	2	8	0
S_8	2	9	0
S_9	2	10	0
S_{10}	2	11	0
∴	∴	∴	∴
S_n	$n \geq 3,$ $\frac{2n}{n}$	$n \geq 3,$ n	$n \geq 3,$ $\frac{n-n}{n}$

Theorem 16

Total domination before the contracted from star graph (S_n) for $n \geq 3$ is

$$\gamma t(S_n) = \frac{2n}{n}, \forall n \in N$$

with n is many vertexs from star graph (S_n)

Proof of Theorem 16

- $n = 1$ then $\gamma t(S_1) = \frac{2 \cdot 1}{1} = \frac{2}{1} = 2$ true
- $n = k$ then $\gamma t(S_k) = \frac{2 \cdot k}{k} = \frac{2k}{k} = 2$ true
- Will be proven : true for $n = k + 1$
 $n = k + 1$ then $\gamma t(S_{k+1}) = \frac{2 \cdot (k+1)}{(k+1)} = \frac{2(k+1)}{(k+1)} = 2$
 because $\gamma t(S_k) = 2$ true for $n = k$ then
 $\gamma t(S_{k+1}) = 2$ true for $n = k + 1$

∴ so proven to be that $\gamma t(S_n) = \frac{2n}{n}, \forall n \in N$

Theorem 17

Contraction numbers total domination from star graph (S_n) for $n \geq 3$ is

$$ct_{\gamma t}(S_n) = n, \forall n \in N$$

with n is many vertexs from star graph (S_n)

Proof of Theorem

- $n = 1$ then $ct_{\gamma t}(S_1) = n = 1$ true
- $n = k$ then $ct_{\gamma t}(S_k) = n = k$ true
- Will be proven : true for $n = k + 1$
 $n = k + 1$ then $ct_{\gamma t}(S_{k+1}) = n = k + 1$
 because $ct_{\gamma t}(S_k) = k$ true for $n = k$
 then $ct_{\gamma t}(S_{k+1}) = k + 1$ true for
 $n = k + 1$

∴ so proven to be that $ct_{\gamma t}(S_n) = n, \forall n \in N$

Theorem 18

Total domination after the contracted from star graph (S_n) for $n \geq 3$ is

$$\gamma' t(S_n) = \frac{n - n}{n}, \forall n \in N$$

with n is many vertexs from star graph (S_n)

Proof of Theorem 18

- $n = 1$ then $\gamma't(S_1) = \frac{1-1}{1} = 0$ true
- $n = k$ then $\gamma't(S_k) = \frac{k-k}{k} = 0$ true
- Will be proven : true for $n = k + 1$
 $n = k + 1$ then $\gamma't(S_{k+1}) = \frac{(k+1)-(k+1)}{(k+1)} = 0$
 because $\gamma't(S_k) = 0$ true for $n = k$ then
 $\gamma't(S_{k+1}) = 0$ true for $n = k + 1$
 \therefore so proven to be that $\gamma't(S_n) = \frac{n-n}{n}, \forall n \in N$

Conclusion

Based on the research that has been carried out, it can be concluded that by contracting the set of domination and total domination of the formulation can be obtained as follows :

- 1) The formula for the domination before contracted is
 - a) For $n \geq 4$
 $\gamma(P_n) = \gamma(C_n) = \begin{cases} \left(\frac{n}{3}\right), \forall n \equiv 0 \pmod{3} \\ \frac{(n+2)}{3}, \forall n \equiv 1 \pmod{3} \\ \frac{(n+1)}{3}, \forall n \equiv 2 \pmod{3} \end{cases}$
 - b) $\gamma(F_n) = \gamma(S_n) = \frac{n}{n}, \forall n \in N$
- 2) The formula for the contraction number domination is
 - a) For $n \geq 4$
 $ct_\gamma(P_n) = ct_\gamma(C_n) = \begin{cases} \frac{3n}{n}, \forall n \equiv 0 \pmod{3} \\ \frac{n}{n}, \forall n \equiv 1 \pmod{3} \\ \frac{2n}{n}, \forall n \equiv 2 \pmod{3} \end{cases}$
 - b) $ct_\gamma(F_n) = ct_\gamma(S_n) = n, \forall n \in N$
- 3) The formula for the domination after contracted is
 - a) For $n \geq 4$
 $\gamma'(P_n) = \gamma'(C_n) = \begin{cases} \frac{(n-3)}{3}, \forall n \equiv 0 \pmod{3} \\ \frac{(n-1)}{3}, \forall n \equiv 1 \pmod{3} \\ \frac{(n-2)}{3}, \forall n \equiv 2 \pmod{3} \end{cases}$

- b) $\gamma(F_n) = \gamma(S_n) = \frac{n-n}{n}, \forall n \in N$
- 4) The formula for the total domination before contracted is
 - a) For $n \geq 5$
 $\gamma t(P_n) = \gamma t(C_n) = \begin{cases} \left(\frac{n}{2}\right), \forall n \equiv 0 \pmod{4} \\ \frac{(n+1)}{2}, \forall n \equiv 1 \pmod{4} \\ \forall n \equiv 3 \pmod{4} \\ \frac{(n+2)}{2}, \forall n \equiv 2 \pmod{4} \end{cases}$
 - b) $\gamma t(F_n) = \gamma t(S_n) = \frac{2n}{n}, \forall n \in N$
- 5) The formula for the contraction number domination is
 - a) For $n \geq 5$
 $ct_{\gamma t}(P_n) = ct_{\gamma t}(C_n) = \begin{cases} \frac{3n}{n}, \forall n \equiv 0 \pmod{4} \\ \frac{n}{n}, \forall n \equiv 1 \pmod{4} \\ \forall n \equiv 2 \pmod{4} \\ \frac{2n}{n}, \forall n \equiv 3 \pmod{4} \end{cases}$
 - b) $ct_{\gamma t}(F_n) = ct_{\gamma t}(S_n) = n, \forall n \in N$
- 6) The formula for the domination after contracted is
 - a) For $n \geq 5$
 $\gamma't(P_n) = \gamma't(C_n) = \begin{cases} \frac{(n-2)}{2}, \forall n \equiv 0 \pmod{4} \\ \frac{(n-1)}{2}, \forall n \equiv 1 \pmod{4} \\ \forall n \equiv 3 \pmod{4} \\ \left(\frac{n}{2}\right), \forall n \equiv 2 \pmod{4} \end{cases}$
 - b) $\gamma't(F_n) = \gamma't(S_n) = \frac{n-n}{n}, \forall n \in N$

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DISCRETIZATION REACTION-DIFFUSION MODELS WITH FINITE DIFFERENCE METHOD

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ABSTRACT

Discretization model is a continuous model transformation procedure to model discrete. Discretization is done using advanced finite difference method, by analogy differential equations using limit rules, with different equations using the different between discrete time points. The model used in this paper is a model of reaction-diffusion (*Turing*) that represents the diffusion of fluid in the cells that cause the cells to move. Finite difference method is a numerical method that can be used to solve partial differential equations. Methods used explicit finite difference scheme developed for the time difference and central difference for the space to complete the reaction-diffusion equation (*Turing*). Based on the numerical solution obtained then the amount of domain growth does not affect the stability of reaction-diffusion models (*Turing*).

Keywords

Discretization, reaction-diffusion (*Turing*) model, forward finite differences method, continuous model, discrete model.

INTRODUCTION

Usefulness system system of nonlinear partial differential equations (PDE) being one of the exciting current study did not use the PDE system is a linear model of the reaction-diffusion (Turing). numerically. This procedure is done by discretizing the mathematical model of the reaction-diffusion (Turing).

Mathematically, the reaction-diffusion models are one-dimensional structures in the form system of nonlinear partial differential equations (PDE). This system consists of two partial differential equations and one ordinary differential equations.

Discretizing is a quantization process of continuous traits. Quantization is defined as the process of grouping continuous traits in certain intervals (step size). Usefulness discretization is to reduce and simplify the data, so that the discrete data obtained are more easily understood, used and described. Therefore, the form of discrete learning outcomes is seen as a fast and accurate results compared to the results of the continuous form. Discretization can be done by various methods, one of which is the method of finite difference.

Finite difference method is one of numerical methods that can be used to obtain the solution system of nonlinear partial differential equation. In principle this method is equation discretization in a coordinate system is continuous.

This paper has done model reaction diffusion discretization using the finite difference method eksplisist. process simulation using Matlab programs to display the behavior of each variables.

Partial Differential Equations

System is a system of differential equations containing partial derivatives and usually contains two or more independent variables. System of partial differential equations consisting of a system of linear differential equations and systems of nonlinear partial differential equations.

Numerical differential reaction-diffusion model

Numerical differential is used to approach the continuous differential forms into discrete form. Numerical differential is widely used to solve differential equations. The form can be derived based on the Taylor series.

If the function contains more than one independent variable, such as $f(x, y)$, the form of the Taylor series becomes:

$$f(x_{i+1}, y_{j+1}) = f(x_i, y_j) + \frac{\partial f}{\partial x} \Delta x + \frac{\partial f}{\partial y} \Delta y + \frac{\partial^2 f}{\partial x^2} \frac{\Delta x^2}{2!} + \frac{\partial^2 f}{\partial y^2} \frac{\Delta y^2}{2!} + \dots$$

Different methods Until Explicit Scheme for Reaction-Diffusion Model Finite difference method defines a region of independent variables in partial differential equations with a finite grid to approximate the dependent variable

Forward finite difference method for derivative with respect to time are as follows:

$$\frac{\partial u}{\partial t}(x_i, t_n) = \frac{u_i^{n+1} - u_i^n}{\Delta t}$$

and

$$\frac{\partial v}{\partial t}(x_i, t_n) = \frac{v_i^{n+1} - v_i^n}{\Delta t}$$

and central difference for the second derivative of the space are as follows:

$$\frac{\partial^2 u}{\partial x^2}(x_i, t_n) = \frac{u_{i+1}^n - 2u_i^n + u_{i-1}^n}{\Delta x^2}$$

and

$$\frac{\partial^2 v}{\partial x^2}(x_i, t_n) = \frac{v_{i+1}^n - 2v_i^n + v_{i-1}^n}{\Delta x^2}$$

The completion algorithm reaction-diffusion equations by the method of explicit finite difference scheme is as follows:

1. Specified explicit finite difference scheme for reaction-diffusion equations (Turing).
2. Specified parameters and limit the area to be completed.
3. Specified Courant number.
4. Substitution parameters and the Courant number on explicit finite difference scheme of the reaction diffusion equation.
5. Iteration for boundary conditions.
6. Iteration for the initial conditions
7. Iteration using an explicit finite difference scheme to obtain the value and each value over time.

RESULT AND DISCUSSION

Different methods Until Explicit Scheme Reaction-Diffusion Model. Reaction Diffusion Model is continuous as follows:

$$\frac{\partial u}{\partial t} = \frac{1}{\tau^2} \frac{\partial^2 u}{\partial x^2} + a - uv^2 - \rho u$$

$$\frac{\partial v}{\partial t} = \frac{d}{\tau^2} \frac{\partial^2 v}{\partial x^2} + b + uv^2 - v - \rho v$$

notation

$$u(x_i, t_n) = u_i^n$$

$$v(x_i, t_n) = v_i^n$$

Forward finite difference method for derivative with respect to time are as follows

$$\frac{\partial u}{\partial t}(x_i, t_n) = \frac{u_i^{n+1} - u_i^n}{\Delta t}$$

and

$$\frac{\partial v}{\partial t}(x_i, t_n) = \frac{v_i^{n+1} - v_i^n}{\Delta t}$$

and central difference for the second derivative of the space is as follows

$$\frac{\partial^2 u}{\partial x^2}(x_i, t_n) = \frac{u_{i+1}^n - 2u_i^n + u_{i-1}^n}{\Delta x^2}$$

and

$$\frac{\partial^2 v}{\partial x^2}(x_i, t_n) = \frac{v_{i+1}^n - 2v_i^n + v_{i-1}^n}{\Delta x^2}$$

Substituted form of the finite difference model of reaction-diffusion (Turing) then the discrete form of the model is obtained as follows:

$$\begin{cases} \frac{u_i^{n+1} - u_i^n}{\Delta t} = \frac{1}{\tau^2} \frac{u_{i+1}^n - 2u_i^n + u_{i-1}^n}{\Delta x^2} + a - u_i^n (v_i^n)^2 - \rho u_i^n \\ \frac{v_i^{n+1} - v_i^n}{\Delta t} = \frac{d}{\tau^2} \frac{v_{i+1}^n - 2v_i^n + v_{i-1}^n}{\Delta x^2} + b + u_i^n (v_i^n)^2 - v_i^n - \rho v_i^n \end{cases} \quad (3.1)$$

By simplifying the equation then obtained the following forms

$$\left\{ \begin{aligned} u_i^{n+1} &= \frac{1}{L^2} \frac{\Delta t}{\Delta x^2} u_{i+1}^n + \left(1 - 2 \frac{1}{L^2} \frac{\Delta t}{\Delta x^2}\right) u_i^n + \frac{1}{L^2} \frac{\Delta t}{\Delta x^2} u_{i-1}^n + \Delta t(\alpha - u_i^n (v_i^n)^2 - \rho u_i^n) \\ v_i^{n+1} &= \frac{d}{L^2} \frac{\Delta t}{\Delta x^2} v_{i+1}^n + \left(1 - 2 \frac{d}{L^2} \frac{\Delta t}{\Delta x^2}\right) v_i^n + \frac{d}{L^2} \frac{\Delta t}{\Delta x^2} v_{i-1}^n + \Delta t(b + u_i^n (v_i^n)^2 - v_i^n - \rho v_i^n) \end{aligned} \right.$$

defined

$$\lambda = \frac{1}{L^2} \frac{\Delta t}{\Delta x^2}$$

So that the previous equation can be written as follows:

$$u_i^{n+1} = \lambda u_{i+1}^n + (1 - 2\lambda)u_i^n + \lambda u_{i-1}^n + \Delta t(\alpha - u_i^n (v_i^n)^2 - \rho u_i^n)$$

$$v_i^{n+1} = d\lambda v_{i+1}^n + (1 - 2d\lambda)v_i^n + d\lambda v_{i-1}^n + \Delta t(b + u_i^n (v_i^n)^2 - v_i^n - \rho v_i^n)$$

If the iteration starts from the equation can be expressed in the following form

$$\left\{ \begin{aligned} u_i^n &= \lambda u_{i+1}^{n-1} + (1 - 2\lambda)u_i^{n-1} + \lambda u_{i-1}^{n-1} + \Delta t(\alpha - u_i^{n-1} (v_i^{n-1})^2 - \rho u_i^{n-1}) \\ v_i^n &= d\lambda v_{i+1}^{n-1} + (1 - 2d\lambda)v_i^{n-1} + d\lambda v_{i-1}^{n-1} + \Delta t(b + u_i^{n-1} (v_i^{n-1})^2 - v_i^{n-1} - \rho v_i^{n-1}) \end{aligned} \right.$$

Stencil finite difference form discrete reaction-diffusion models to the equation

is as follows:

$$u_i^n = \lambda u_{i+1}^{n-1} + (1 - 2\lambda)u_i^{n-1} + \lambda u_{i-1}^{n-1} + \Delta t(\alpha - u_i^{n-1} (v_i^{n-1})^2 - \rho u_i^{n-1})$$

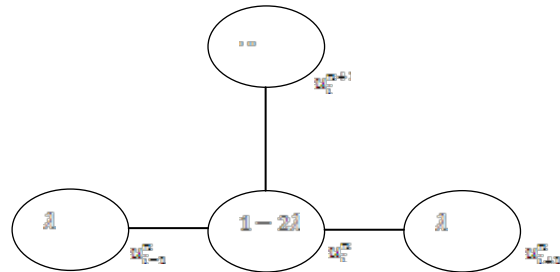


Figure 1. Different Methods To Stencil Explicit Schemes for Equations

$$u_i^n = \lambda u_{i+1}^{n-1} + (1 - 2\lambda)u_i^{n-1} + \lambda u_{i-1}^{n-1} + \Delta t(\alpha - u_i^{n-1} (v_i^{n-1})^2 - \rho u_i^{n-1})$$

Stencil finite difference form discrete reaction-diffusion models (Turing) to the equation

$$v_i^n = d\lambda v_{i+1}^{n-1} + (1 - 2d\lambda)v_i^{n-1} + d\lambda v_{i-1}^{n-1} + \Delta t(b + u_i^{n-1} (v_i^{n-1})^2 - v_i^{n-1} - \rho v_i^{n-1})$$

is as follows

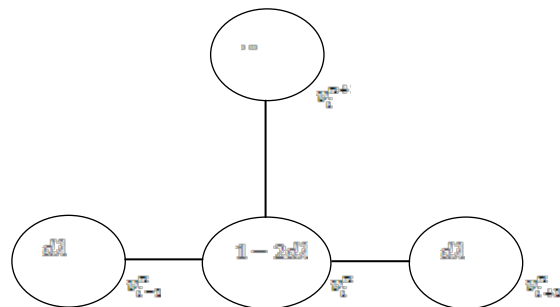


Figure 2. Different Methods To Stencil Explicit Schemes for Equations

$$v_i^n = d\lambda v_{i-1}^{n-1} + (1 - 2d\lambda)v_i^{n-1} + d\lambda v_{i+1}^{n-1} + \Delta t(b + u_i^{n-1}(v_i^{n-1})^2) \frac{L}{L_0} e^{\rho t} \rho v_i^{n-1}$$

The next iteration of boundary conditions, with the given boundary conditions, namely

$$u(x_0, t) = 0,9, \quad u(R, t) = 0,9, \quad v(x_0, t) = 1 \quad \text{and} \\ v(R, t) = 1 \quad \text{thus}$$

$$u_0^n = u_1^n = 0, \quad \forall n = 0,1,2,3, \dots, k$$

$$v_0^n = v_1^n = 0, \quad \forall n = 0,1,2,3, \dots, k$$

:

The next step is performed iterations with initial conditions, and use the following initial conditions

$$u_0^n = u_1^n = 0, \quad \forall n = 0,1,2,3, \dots, k$$

$$v_0^n = v_1^n = 0, \quad \forall n = 0,1,2,3, \dots, k$$

Having obtained the initial value and boundary value, then the iteration is done with discrete models previously obtained in accordance with the nets point count and the equations $\frac{dL}{dt} = \rho L$ can be written into the following form:

$$\frac{dL}{dt} = \rho L$$

$$\frac{dL}{L} = \rho dt$$

$$\ln|L| = \rho t + \ln C$$

$$\ln \frac{L}{C} = \rho t$$

$$\frac{L}{C} = e^{\rho t}$$

Because it is a time-dependent function is obtained,

$$L(t) = C e^{\rho t}$$

If given an initial value $N(0) = 0$ then,

$$L(t) = e^{\rho t}$$

Reaction-diffusion model (Turing) will be completed in the boundary region $0 < x < 1$ and $0 < t < 0,002$. Kinetic energy which is used in the model $a = 0,9$ and $b = 0,1$, diffusion coefficient ratio $d = 0,06$, domain length grows exponentially with time L with initial value $L(0) = 1$ and domain growth speed.

$\rho = 0,01$, so that the reaction-diffusion models can be written as follows

$$\begin{cases} \frac{\partial u}{\partial t} = \frac{1}{L(t)^2} \frac{\partial^2 u}{\partial x^2} + 0,9 - uv^2 - 0,01u \\ \frac{\partial v}{\partial t} = \frac{0,06}{L(t)^2} \frac{\partial^2 v}{\partial x^2} + 0,1 + uv^2 - v - 0,01v \end{cases}$$

Selected numbers and thus the value of the Courant number for this model i

$$\lambda = \frac{1}{L(t)^2} \frac{\Delta t}{\Delta x^2}$$

for value then to get,

$$\lambda = \frac{1}{L(0)^2} \frac{\Delta t}{\Delta x^2} = \frac{1}{1^2} \frac{0,00002}{(0,01)^2} = 0,2$$

Substituting the value of the method of finite difference explicit scheme for reaction-diffusion equation models to obtain,

for equations

$$u_j^n = \lambda u_{i+1}^{n-1} + (1 - 2\lambda)u_i^{n-1} + \lambda u_{i-1}^{n-1} + \Delta t(a - u_i^{n-1}(v_i^{n-1})^2)$$

$$u_j^n = 0,2u_{i+1}^{n-1} + 0,6u_i^{n-1} + 0,2u_{i-1}^{n-1} + 0,00002(0,9 - u_i^{n-1}(v_i^{n-1})^2 - 0,01u_i^{n-1})$$

and equations

$$v_j^n = d\lambda v_{i+1}^{n-1} + (1 - 2d\lambda)v_i^{n-1} + d\lambda v_{i-1}^{n-1} + \Delta t(b + u_i^{n-1}(v_i^{n-1})^2 - v_i^{n-1} - \rho v_i^{n-1})$$

$$v_j^n = 0,012v_{i+1}^{n-1} + 0,976v_i^{n-1} + 0,012v_{i-1}^{n-1} + 0,00002(0,1 + u_i^{n-1}(v_i^{n-1})^2 - v_i^{n-1} - 0,01v_i^{n-1})$$

:

Number of grid points on the axis is used with the following values:

$$l = \frac{R - x_0}{\Delta x} = \frac{1 - 0}{0,01} = 100$$

Analogously the number of grid points on the axis is used with the following values:

$$k = \frac{T - t_0}{\Delta t} = \frac{0,002 - 0}{0,00002} = 100$$

Stencils for these conditions are as follows

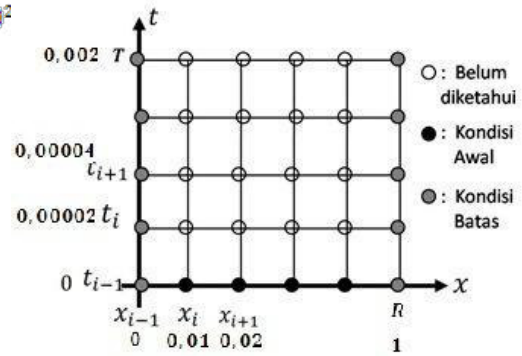


Figure 3. Network Point Count Up Scheme Explicit Difference Method 1 for Reaction-diffusion model.

The next iteration of boundary conditions as follows,

$$u(x_0, t) = u(0, t) = 0,9, u(R, t) = u(1, t) = 0,9, v(x_0, t) = v(0, t) = 1$$

and $v(R, t) = v(1, t) = 1, \forall 0 < t < 0,002$

then to get $u_i^n = 0,9$ and $v_i^n = 1, \forall n = 0,1,2,3, \dots, 100. \forall i = 0,1,2,3, \dots, 100$

which can be described as follows:

$u_0^0 = 0,9$	$v_0^0 = 1$
$u_0^1 = 0,9$	$v_0^1 = 1$
$u_0^2 = 0,9$	$v_0^2 = 1$
$u_{100}^2 = 0,9$	$v_{100}^2 = 1$
:	:
$u_0^{100} = 0,9$	$v_0^{100} = 1$
$u_{100}^0 = 0,9$	$v_{100}^0 = 1$
$u_{100}^1 = 0,9$	$v_{100}^1 = 1$
$u_{100}^2 = 0,9$	$v_{100}^2 = 1$

$$u_{100}^{100} = 0,9 \qquad v_{100}^{100} = 1$$

The next step is performed iterations with the following initial conditions.

$$u(x_0, t_i) = 0,9 + \text{random number}$$

$$v(x_0, t_i) = 1 + \text{random number}$$

Suppose then taken as the initial value and the value obtained boundary, iteration is done with discrete form count model according to the network. Calculation results can be seen by running the following program,

Results of the program are as follows:

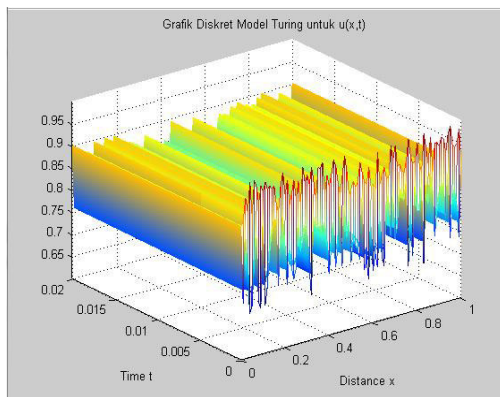


Figure 4. Graph Discrete Reaction-Diffusion Model for the Equation $\rho = 0,1$

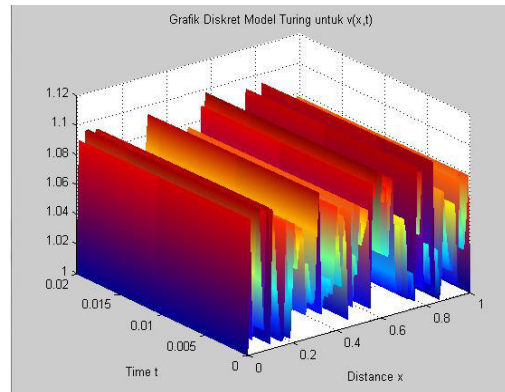


Figure 5. Graph Discrete Reaction-Diffusion Model for the Equation $\rho = 0,01$

The second simulation is done with the value so that the same program image obtained as follows:

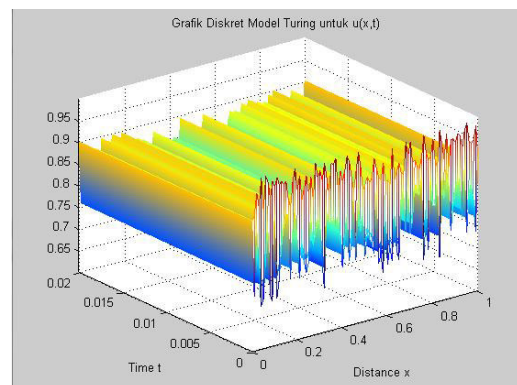


Figure 6. Graph Discrete Reaction-diffusion model (Turing) to the equation $\rho = 0,001$

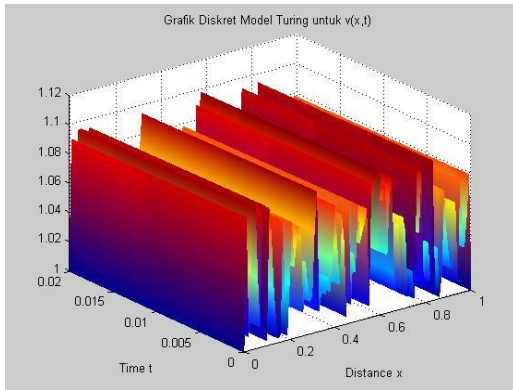


Figure 7. Graph Discrete Reaction-diffusion model (Turing) to the equation $p = 0,001$

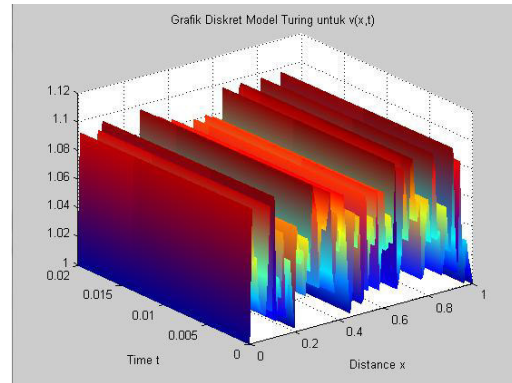


Figure 9. Graph Discrete Reaction-diffusion model (Turing) to the equation $v(x,t)$ with $p = 0,0001$

For the latter simulation is done by replacing the value in order to obtain the following picture:

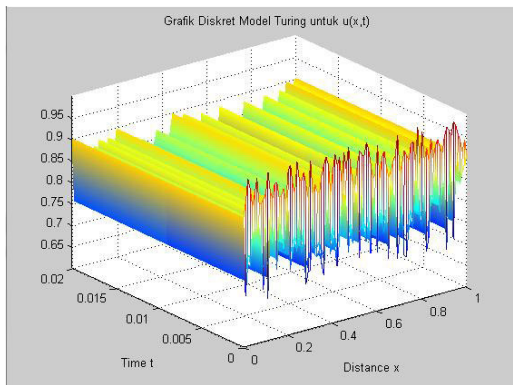


Figure 8. Graph Discrete Reaction-diffusion model (Turing) to the equation $p = 0,0001$

Interpretation of Results discretized reaction-diffusion model (Turing)

Boundary conditions used in this discussion is

$$u(x_0 = L, t) = 0,9, u(x_n = R, t) = 1, v(x_0 = L, t) = 0,9$$

and $v(x_n = R, t) = 1$. It is interpreted that the $x_0 = L$ and $x_n = R$ a border of non-dimensional kinetic energy are solved so that the change in value before $x_0 = L$ and after $x_n = R$ negligible. Boundary value $0,9$ can be interpreted that the non-dimensional kinetic energy at the point of $x_0 = L$ at $0,9$ and boundary value 1 can be interpreted that the non-dimensional kinetic energy at the point of $x_n = R$ by one for each species for all time.

With the given boundary conditions, it can limit the area to be giving out the completed

The parameters used in the model of reaction-diffusion (Turing) which p is the domain and the growth rate $-pu$ and $-pv$ illustrates the dilutive effect of the local expansion of the domain used

With parameters values $a = 0,9$ and $b = 0,1$, as well as the value of the parameter

$d = 0,06$, s the ratio of the value of the diffusion coefficient.

Initial conditions used in the discussion of reaction-diffusion models (Turing) is as follows:

$$u(x_0, t_1) = 0,9 + \text{random number}$$

$$v(x_0, t_1) = 1 + \text{random number}$$

The condition can be interpreted that the non-dimensional kinetic energy at the point in time for each species is affected by the addition of random numbers on the back of a constant.

CONCLUSION

Based on the above discussion it could be concluded:

Discrete form of reaction-diffusion models (Turing) is as follows:

$$u_i^{n+1} = \lambda u_{i+1}^n + (1 - 2\lambda)u_i^n + \lambda u_{i-1}^n + \Delta t(a - u_i^n(v_i^n)^2 - \rho u_i^n)$$

$$v_i^{n+1} = d\lambda v_{i+1}^n + (1 - 2d\lambda)v_i^n + d\lambda v_{i-1}^n + \Delta t(b + u_i^n(v_i^n)^2 - v_i^n - \rho v_i^n)$$

Discrete form of the model can represent the model kuntu and speed of growth of the value of the domain does not have any impact on the dynamic behavior of reaction-diffusion models (Turing).

For further research, it is recommended to continue the study discretized reaction-diffusion models (Turing) using the parameter values, initial values, boundary values and intervals are different and varied,

so apat seen shortages discrete models that have been built for other parameters.

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Implementation Of Multi Sensor System For MSR-H01 Hexapod Robot

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ABSTRACT

This research discusses about the system control model of hexapod robot. MSR-H01 is the hexapod robot kit, which equipped with pBrain system that controls every mechanics movement. Data communication between the microcontroller and pBrain using RS 232 serial communication standard. The hexapod robot use a lot of input sensors with microcontroller ATmega 128 as the main controller. The sensors are ultrasonics that serves to determine the environmental conditions and obstacle around the robot. The ultrasonic sensors also give reference to microcontroller to determine the motion commands that send to pBrain. The results shows that the robot can walk along the wall on a flat track with a maximum speed of 0.15 m / s.

Keywords

control, hexapod robot, ultrasonic sensors, microcontroller.

INTRODUCTION

Robot is an automated equipment that made to replace the functions that had been performed by humans . However, in the subsequent development , the robot is defined as a programmable multi- functional manipulator , that the programming was intended to do a specific task (Arif, 2011). In order to work automatically, robots need sensors to determine the condition of the environment . The robots that can migrate is referred as a mobile robot , generally also have sensors that are used to detect objects around the robot , especially the object in the movement path area.

The growth of technology also make more rapid progress towards sensor technology. Starting from the proximity sensor, metal, temperature, heat, light, and the image sensor can be obtained easily and inexpensively . To determine the distance of the object and the obstacle , the mobile robot use the proximity sensor . The proximity sensor is able to use this type of ultrasonic sensors or infrared sensors .

The proximity sensors that used in this research are type of ultrasonic sensors. Sensing process that performed on the sensor uses the reflection of sound methods for calculating the distance between the sensor with the target object (Hani, 2010). The ultrasonic sensor is a sensor that works by utilizing voice wave, so the bright and the dark interference from ambient light becomes smaller . This is different with the working principle of infrared sensors , which utilizes reflected light to determine the distance , making it more vulnerable to the interference of light in the environment .

Mobile robot system is expected to detect and determine the condition of the broader environment , not just in front of the robot but also the side of or behind the robot mechanics . Therefore, by knowing the wider environmental conditions , the system will be able to plan the movement of the robot path

toward the goal , find the shortest path and can plan the movement to avoid the obstacle . By using more sensors , the robot is expected environmental conditions to determine the wider environment condition and detail as expected .

MATERIALS AND METHODS

Function Oriented Robot

Robot systems that discussed in this study belongs to the function oriented robot model , which has the main components , such as: mechanical robots , sensors , actuators and controller system (Pitowarno, 2006).

Fig. 1 Illustrated the function -oriented robot system.

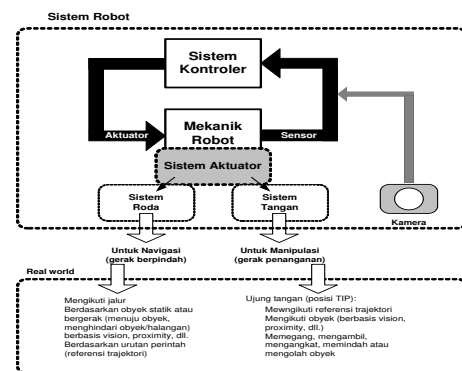


Figure 1 Function Oriented Robot System

MSR-H01 Hexapod robot

This research uses the MSR - H01 Hexapod robot as a mechanical system which is then controlled using a microcontroller ATmega 128 . MSR - H01 is a robot module that has 6 pieces each leg with 3 DOF (Degree Of Freedom) of every leg . Each DOF is driven by a servo motor -type xx as the actuators. Model MSR - H01 Hexapod robot is shown by **Fig. 2**.

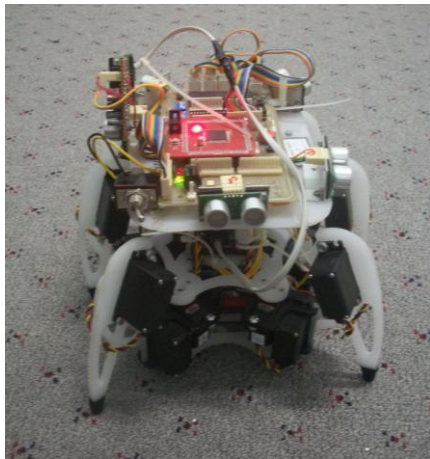


Figure 2 MSR-H01 Robot Kit

MSR - H01 is equipped with a hardware system that controls each mechanical servo system, the system is called the pBrain (Micromagic System, 2009). Furthermore pBrain can communicate with other systems such as a computer or other type of microcontroller minimum system.

Atmel AVR Microcontroller ATmega 128

AT Mega 128 is a control function input / output that used in this research. AVR is a series of 8-bit CMOS microcontroller Atmel artificial, based on RISC architecture (Reduced Instruction Set Computer). Almost all instructions are executed in one clock cycle. AVR has a 32 x 8 general-purpose registers, timer / counters flexible with compare modes, internal and external interrupts, a serial UART, programmable Watchdog Timer, and power saving mode. AVR also has In-System Programmable on-chip Flash allows the program memory to be reprogrammed in the system using the SPI serial connection. ATmega128 is an 8-bit CMOS microcontroller low-power RISC-based architecture is enhanced.

Most of instructions is done in one clock cycle, the ATmega128 has a throughput approaching 16 MIPS per MHz makes the system designer to optimize power

consumption versus processing speed (Datasheet ATmega128).

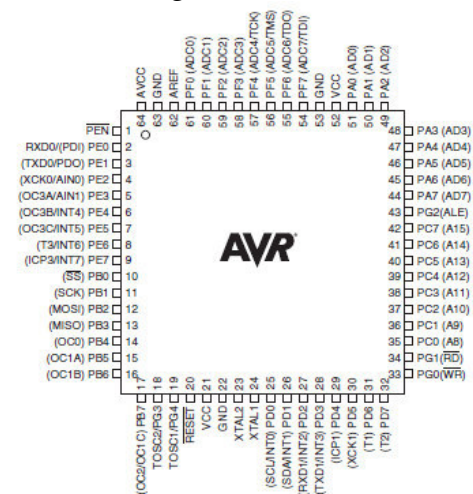


Figure 3 ATmega 128

ATmega16 pins on the 53-pin programmable I / O line package as shown in Figure 3. Software programs that are used to fill in CodeVision AVR microcontroller AVR is C programming language.

Ultrasonic sensors

The ultrasonic is a sensor that works on the principle of reflection of sound waves and used to detect the presence of a particular object in the area above the operating frequency 40 KHz to 400 KHz. Large amplitude of the electrical signal generated depending on the sensor unit receiver remote objects is detected nearby.

Sensing process that is performed on the sensor, uses the reflection method to calculate the distance between the sensor with the target object. The distance between the sensors is calculated by multiplying half the time spent by the ultrasonic signal travels from the sender circuit signal (Tx) to the signal received by the receiver circuit (Rx) with a propagation speed of the ultrasonic signal in the propagation medium digunakannya, namely air. Propagation speed of the ultrasonic signal in air is 342 m / s, equal to the propagation speed of sound in air. In this

research, ultrasonic sensors are used as robot sensor to determine the distance of objects around him (Suprpto).

System Design And Implementation

Design of hexapod robot is the main topic in this research that divided into several sections, ranging from sensors, microcontroller minimum, and actuators. There are illustrated by the block diagram that shown in **Fig. 4** .

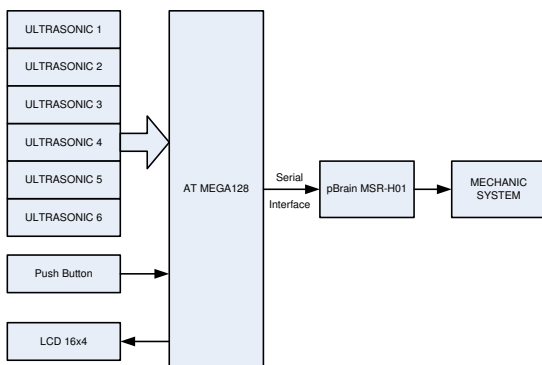


Figure 4 Block Diagram Sistem

Hexapod robot constructed using 6 ultrasonic sensors to detect objects. Each sensor is connected directly to the microcontroller ATmega128, as a reference microcontroller to give commands to the motion control of mechanical systems by pBrain MSR - H01 .

System that shown in **Fig. 5** is equipped using a pushbutton input that serves as the user interface between the robot and a human, especially for command start , reset , and other commands . 16x4 LCD is used as the output of a system which can display some information , especially relating to the condition of each sensor .

Control System Based ATmega 128

ATmega 128 in this study is used as the main control that regulates all forms of robot motion through pBrain , the condition of the ultrasonic sensor input and commands from the push button . Configuring PORT I / O ATMEGA 128 is shown through **Table 1** ..

Tabel 1 Configuration I / O PORT ATMEGA 128

PORT	Function
A.0, A.1	Triger and Echo Ultra 1
A.2, A.3	Triger and Echo Ultra 1
A.4, A.5	Triger and Echo Ultra 1
A.6, A.7	Triger and Echo Ultra 1
B.0, B.1	Triger and Echo Ultra 1
B.2, B.2	Triger and Echo Ultra 1
C.0	- LCD Display
C.7	
D.2, D.3	RX, TX serial communication
F.0 – F.3	Push button

Electronic circuit which includes of ATmega128 minimum system which becomes the main control hexapod robot in this research is shown in **Fig. 5**.

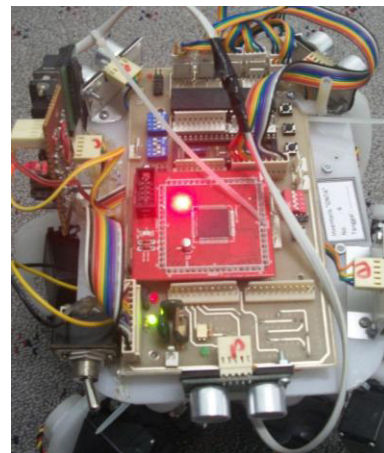


Figure 5. Electronic circuit of Hexapod robot

Ultrasonic Sensors Configuration

Ultrasonic sensors prepared by the concept of circular as shown in **Fig. 6**. The aim is that the robot can know all objects existing conditions in the surrounding environment .

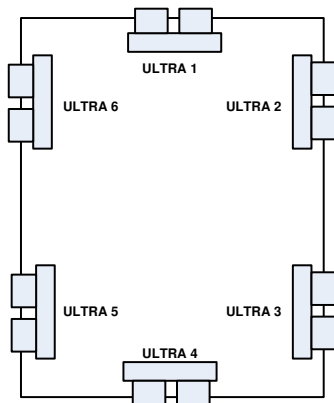


Figure 6. The Structure of ultrasonic sensor

RESULT AND DISCUSSION

Hexapod robot that became in this research can be automatically moves based on input from the ultrasonic sensors is controlled by ATmega 128 . Furthermore, the output of ATmega 128 associated with pBrain kit that can be translated into mechanical movement of the robot. pBrain has characteristics, waiting " @ @ @ " character from microcontroller before execute the commands through simkontrol. The Commands sent to the pBrain microcontroller is shown in **Table 2** . While examples of an initial order to be able to communicate with pBrain is shown in figure 7 which contains the source code in C language programs are created using AVR CodeVision .

```
File Edit Format View Help
while(datax[0]!=0);
while(hit<500)
{
    sprintf(lcd,"%3d %d",datax[0],hit);
    lcd_gotoxy(0,0);
    lcd_puts(lcd);
    hit++;
};

lcd_gotoxy(9,0);
lcd_putsf("@@@" );
rx_buffer1[0]=0;datax[0]=0;hit=0;

    for(x=0;x<3;x++)
    {
        putchar1('@'); //@
        delay_ms(10);
    };

while(datax[0]!=0);
putsf1("SIM CONTROL");
putchar1(13); //ENTER
```

Figure 7. SourceCode of initial communication with the microcontroller pBrain

Table 2 Microcontroller commands to pBrain

Key	Description
+	Raise Power Hexapod
-	Reduce Power Hexapod
SPACE	Stop hexapod
!	Emergency Stop (turn off the servo directly)
W	Forward
S	Backward
A	Turn left
D	Turn right
Q	Crab left (oblique way)
E	Crab right (slanted street)
1	Wave mode 1 (slow)
2	Wave mode 2
3	Wave 3 modes
4	Tripod mode (fast path)
5	Onroad modes (flat terrain , fast)
6	Offroad modes (slow , terrain obstacles)
7	Lowering the transfer rate 0.1second feet
8	Increase the transfer speed of foot 0.1second
9	Resetting the transfer rate to the default leg

R	Reset the position of the foot to normal
B	Enable 3D mode full balance
C	Full 3D mode disables balance

While the example command to generate mechanical motion robot sent to pBrain shown by **Fig. 8**.

```
File Edit Format View Help
while (1)
{
  while(PINF.1);
  lcd_clear();
  lcd_gotoxy(9,0);
  lcd_putsf("WAKE");
  putchar('+'); //berdiri
  delay_ms(6000);

  putchar('6'); //offroad mode
  hit=0;
  while(hit<6000)
  {
    sprintf(lcd,"%d",hit);
    lcd_gotoxy(0,0);
    lcd_puts(lcd);
    putchar('r'); //jalan ditempad
    if (!PINF.1) goto vv;
    hit++;
  };

  for(x=0;x<5;x++)
  {
    putchar('8'); //increase speed
    delay_ms(10);
  };
};
```

Figure 8. SourceCode motion commands to the microcontroller pBrain

Fig. 8 shows some of the basic commands are sent to pBrain. Among them is a standing order by sending the characters " + ", go into the off-road mode with the character " 6 ", the way place with character " r ", and increase the speed by sending the character " 8 ". Offroad mode in question is the movement of a robot with legs on tiptoe , so that the robot body can be lifted higher, with the aim to pass through irregular terrain / wavy.

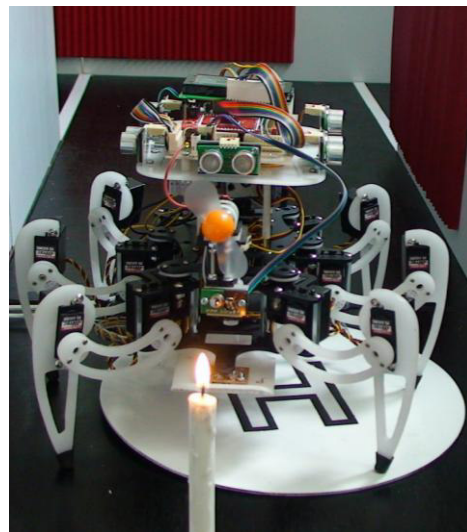


Figure 9 Trials the robot on the track

In the pilot phase, the hexapod robot models run on some tracks, flat, flat on the carpet and bumpy. **Fig. 9** shows the test robot when run on a flat track. Track corrugated referred to in this research is a flat track with some obstacle barrier , thus forming a bumpy track . Modes are tested in this study using off-road mode and the results are shown in **Table 3** .

Table 3. Test hexapod robot in 3 tracks

Trial	Track	Average speed
1.	Flat	7 m/s
2.	Carpet	13 m/s
3.	Wavy	16 m/s

According to the test results shown in **Table 3** , that is basically using offroad mode hexapod robot can still run on 3 different tracks . In terms of speed , hexapod robot can run faster on flat track to reach 7 m/s .

CONCLUSION

Conclusions and further research plans can be described from the results of this research are as follows :

1. Control systems using proven off-road mode can be used by the MSR - H01 hexapod robot to be able to move on a flat track , carpet , or wavy. With a maximum speed of 7 s / m on a flat track , and the latest on a bumpy track with a speed of 16 s / m.
2. Need more research on the use of other hexapod control modes, including onroad mode and a tripod mode . So further research directions can discuss automation system mode changes based on environmental conditions encountered .

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CHARACTERIZATION AND ANALYSIS OF CRYSTAL STRUCTURE MIXED KERATIN-MAGNESIUM COMPOSITE WITH RIETVELD METHOD

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ABSTRACT

Solid hydrogen storage is more profitable comparing with storage forms with regards to high efficiency. In this study, hydrogen storage is made from magnesium and keratin of feather mixings. Magnesium has a high ability to absorb hydrogen and keratin has a helical structure that is able to increase the capacity of hydrogen storage. Manufacturing of hydrogen storage aims to obtain mixed Mg-keratin composite X-ray diffraction characterization as well as to determine its crystal structure. Method in making hydrogen storage was started with pyrolysis process to obtain a keratin substance. The next step, it was mixed with Mg to create composite. The various compositions of mg-keratin composite are 2:8, 4:6, 6:4, and 8:2. These mixings then were milled for 5 hours at a speed of 100-110 rpm. X-ray Diffraction pattern showed that the composite samples 8 grams of Magnesium - 2 grams of keratin has the highest intensity while the composite sample 2 grams Magnesium - 8 grams of keratin has a hump at most. Hump showed the presence of carbon phase, which was pointed out by not having a high peak due to amorphous form. Based on Rietveld refinement data, Magnesium has hexagonal crystal structure and large density fractions. Atomic hydrogen can still be absorbed into magnesium because of the smallness of hydrogen radii and the cluster R- of helical keratin structure. In hydrogen storage applications, cluster R –it self is replaced by hydrogen atoms .

Keywords

Hydrogen Storage, Magnesium, Keratin, Crystal Structure, Rietveld Method

INTRODUCTION

One of alternative energy that can replace petroleum limitations is hydrogen. Hydrogen is the most abundant element on earth, which friendly and renewable. Hydrogen also has a weakness that is highly reactive and flammable. It is necessary for the safe storage of hydrogen.

Recent research of hydrogen storage systems for fuel cell vehicles is very intensive. There are three known methods of storage, which are in the form of gas, liquid and solid. Two of these methods are in form of gas (at high pressure 700 bar) and liquid (stable temperature at 253 °C) is not safe. Advanced storage system by using solid storage techniques is currently actively investigated. In this solid system, hydrogen is inserted in the certain material (Jalil, 2009). In this research, we made a composite of magnesium and keratin mixing. Keratin is capable in storing hydrogen because it has a helical structure and elements of the light. Therefore this study objectives to determine the results of X-ray diffraction patterns as well as to determine the crystal structure of mixed Mg-keratin composite. Hydrogen can be stored over the surfaces of solids (by adsorption) or within solids (by absorption). In adsorption (**Fig. 1(a)**), hydrogen attaches material surface either as hydrogen molecules (H_2) or as hydrogen atoms (H). In **Fig. 1(b)**, hydrogen molecules dissociate into hydrogen atoms which are incorporated into the solid lattice framework. This method make it possible to store larger quantities of hydrogen in smaller volumes at low pressure and room temperature. Finally, hydrogen can be strongly bound within molecular structures, as its chemical compounds contain hydrogen atoms (1.C, 1.D). Density increases from 1.A to 1.D (Departement of Energy, 2011).

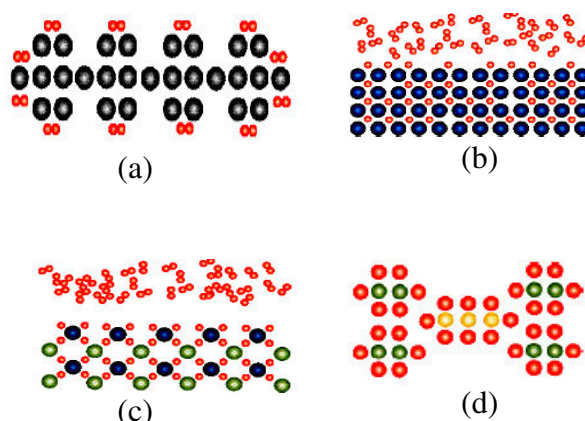


Figure 1.(a) Surface Adsorption, (b) Intermetallic Hydride, (c) Complex Hydride, (d) Chemical Hydride (Departement of energy, 2011)

Several types of materials (metals are generally lighter elements) are believed to have ability in absorbing hydrogen in large quantity. One of them is the magnesium which is considered as potential candidates for hydrogen storage materials. Magnesium, theoretically, has ability to absorb hydrogen in large quantity (7.6 wt %). This amount exceeds the maximum limit of the World Energy Agency which targeted at 5 wt % and it is able to work at temperatures below 100 °C (Zuettel, 2003).

In addition to magnesium, keratin is also capable storing hydrogen. It is product of epidermal tissue hardening. It is a fibrous protein, rich in sulfur and abundant in hair horn, hooves, fur, and all epidermal products (Haurowitz, 1984). According to Harraps and Woods (1964) protein range of chicken feather keratin is 85-90 % 14 % of this consists of a sistin disulfide bridging between two molecule bindings the molecules (Hill, 1982).

The results of X-ray Diffraction patterns is processed by Rietveld method to obtain lattice parameters for describing structure crystal. Rietveld analysis is a method of matching non-linear matching curve which is calculated from diffraction pattern (model) which is based on data of crystal structure and using the least-squares method. Suitability parameters (figures of merits) were used in Rietveld refinement (Hill, 1987):

(i) profile factors Rp

$$R_p = \frac{\sum_i |y_i - y_{ci}|^2}{\sum_i y_i} \quad (1)$$

(ii) weighted profile factor Rwp

$$R_{wp} = \left[\frac{\sum_i w_i |y_i - y_{ci}|^2}{\sum_i w_i y_i^2} \right]^{1/2} \quad (2)$$

(iii) index *Goodness-of-Fit* (GoF), commonly denoted by χ

$$GoF = \left[\frac{R_{wp}}{R_{exp}} \right] \quad (3)$$

with

$$R_{exp} = \left[\frac{N-P}{\sum_i w_i y_i^2} \right] \quad (4)$$

(iv) faktor Bragg R_B

$$R_B = \frac{\sum_i |I_i - I_{ic}|}{\sum_i I_i} \quad (5)$$

with I_i and I_{ic} are the measured intensities and a Bragg reflection.

$$D = \frac{k \lambda}{\beta \cos \theta} \quad (6)$$

Where D is crystallite size, K is a constant value (0.94), λ is the wavelength of $CuK\alpha$, β is the value of FWHM (Full-Width at Half Maximum), and θ is the Bragg angle.

Data of X-ray Diffraction (XRD) is saved with the file extension *.rd and *.cpi which are then processed with Match software to determine its phase. Lattice parameters of Rietica results used crystal structure model by using Powder Cell 2.4 software.

RESULTS

The characterization of these functional groups is done by using FTIR spectrophotometer brands Varian FTS type 1000 FT-IR.

MATERIALS AND METHODS

The steps in this research are as follows: feathers are cleaned then dried in the sun to remove moisture. After it is dried, then pyrolysed to get keratin substance. Pyrolysis temperature is done in two stages, first at or under temperature 215 °C for 15 hours. Second is at 450 °C for 1 h. Pyrolysed keratin results is sieved by using 140 mesh size. Then, it is purified by using 15 cm³ toluene and 45 cm³ distilled water, and then it is soaked for 1 hour and dried in the air. Pyrolyzed Keratin is tested by using FTIR spectrophotometer to determine the cluster function. To reduce its grain size it is used ball milling machine. The ball and material ratio are 5:1 with samples weighing 10 grams. Milling process lasted for 5 hours with a speed of 100-110 rpm. In this research, the ratios of Mg-keratin composite are: 2:8, 4:6, 6:4, and 8:2.

Obtaining the substance keratin - Magnesium composite, it is characterized by using analytical XRD at angle 0°- 90°. Scherrer equation is used to calculate crystal size of mixed Mg-keratin composite:



Figure 2. FTIR spectra of keratin

Keratin structure contained Carbon atoms (C) which binds to four other atoms (Fig. 3). One cluster is bound by a carbon that is R- cluster in the hydrogen storage. This cluster can be replaced by hydrogen atoms.

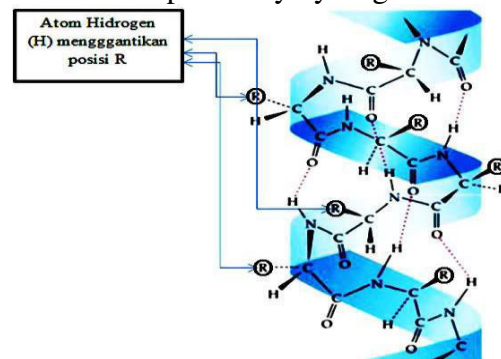


Figure 3. Possible position of hydrogen atoms in the substance keratin

Characterization of X-Ray Diffraction (XRD) Philips X'Pert performed using Diffractometer X-ray instruments with λ 1.54056 Å . Results of tested Mg-keratin composite showed by **Fig. 4** .

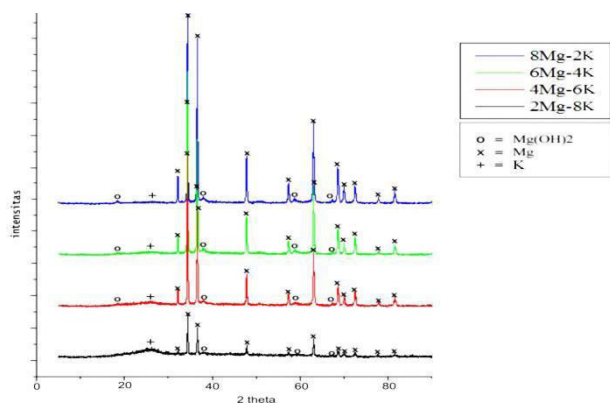


Figure 4. XRD pattern of composite keratin (K) and Mg

From equation 6 it is obtained crystal sizes of mixed Mg-keratin composite which are not much different (**Table 1**).

Table1.Crystal size of Mg and keratin composite

Composite	Crystal Size (nm)
2 Mg - 8 keratin	59,0619
4 Mg - 6 keratin	51,46642
6 Mg - 4 keratin	58,74761
8 Mg - 2 keratin	51,6242

From refinement result, the lattice parameter values are obtained (**Fig. 5 and 6**) and reliability values are shown in Table 2.

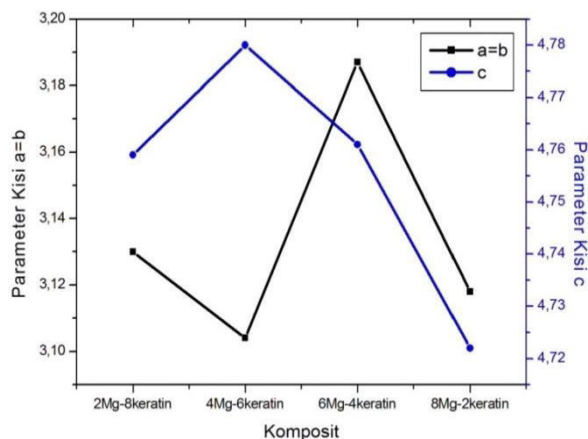


Figure 5. The lattice parameter of phase Mg(OH)₂

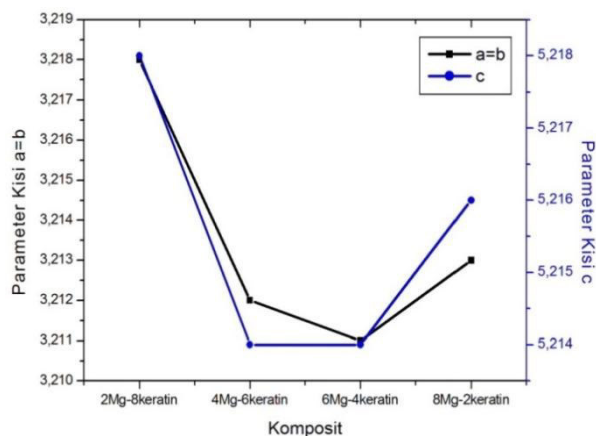


Figure 6. The lattice parameters at the phase Mg

Table 2 Reliability value data of Rietveldrefinement result

Composite	Reliability Value					GoF
	R _p	R _{wp}	R _{exp}	R _B		
				Mg(OH) ₂	Mg	
2Mg-8keratin	21.52	28.17	10.67	12.95	29.45	6.966
4Mg-6keratin	24.07	37.74	11.10	14.96	9.94	9.786
6Mg-4keratin	26.91	38.66	11.35	26.29	13.05	11.60
8Mg-2keratin	31.76	44.99	11.70	44.69	13.24	14.80

Crystal structure model for Mg(OH)₂ and magnesium are made from refinement result are shown in **Fig. 7 and 8**

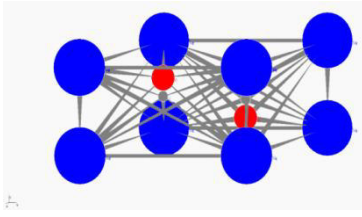


Figure 7. Model structure of crystalline $Mg(OH)_2$

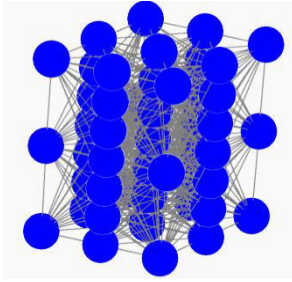


Figure 8. Model structure of crystalline Mg

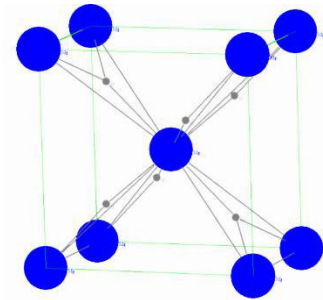
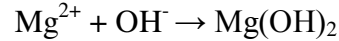


Figure 9. Model structure of crystalline MgH_2

DISCUSSION

Infrared analysis (Fig. 2) of the keratin at wave number 3437 cm^{-1} indicate the presence of N-H absorption. Aliphatic C-H absorptions are in wave numbers 2920 cm^{-1} , but it is difficult to interpret due to possibility as the absorption area of -CH₂-S-. Absorption area-CH₂-S is about $2950\text{ to }2920\text{ cm}^{-1}$ (Socrates, 1994). In addition, absorption occurs at 2361 cm^{-1} , 1054 cm^{-1} and 721 cm^{-1} which indicate vibration of $C \equiv N$, C-O and CH₂. Based on data of the existing uptake, the results of pyrolysis is keratin. The result of X-ray

diffraction (fig. 4) showed that there is Magnesium and $Mg(OH)_2$. Phase of $Mg(OH)_2$ (Fig. 7) appears because -OH cluster of keratin is lost. In keratin structure, the bonding OH cluster is weak, so that the bond can be broken. The OH cluster that reacts with Mg reaction:

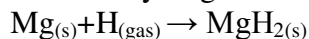


Carbon phase of keratin substances is not identified, because carbon has an amorphous structure. So that it appears in a hump structure. From the Fig. 4, it can be seen that the most hump at sample 2 Mg - 8 keratin. Hump. It is reduced at sample 4 Mg - 6 keratin and 6 Mg - 4 keratin, and almost nothing for sample 8 Mg - 2 keratin. Hump indicates that the presence of carbon is amorphous. Results of X-ray Diffraction (Fig. 4) can be seen that there are peaks which are different for each sample. The sequenced samples that have the highest peak are 8 Mg - 2 keratin, 6 Mg - 4 keratin, 4 Mg - 6 keratin, and the shortest 2 Mg - 8 keratin. This occurred because of the differences of magnesium concentration of each sample. The more magnesium concentration, the higher resulting peak. High concentration of material causing probability of excitation X-ray production is high, so intensity is tended to increase. (Phillips, 1995).

Reliability value (Tabel 2) smoothing results is much different from a predetermined value. This is due to the phase of iron (Fe) is not included in the model. Fe phase appears as granules used balls made of Fe. Thus, there is little possibility that Fe is separated from the ball. Because Fe is present in the sample is low, its phase is not included in the Rietica model.

From the results of the crystal structure of an existing picture, magnesium and $Mg(OH)_2$ has a hexagonal crystal structure. Magnesium has a large fraction of the density, that is 0,74. Density fraction is proportional to maximum volume that can be filled by the ball of atoms in the unit cell. The greater density fractions, causing the smaller cavity. But this is still a small cavity that can be filled by a

hydrogen atom, whose has a smaller radii around 25 pm . Hydrogen atoms interact with interstitial Mg. Reaction occurs between magnesium and hydrogen are:



Magnesium has a hexagonal crystal structure (fig. 8), but if magnesium is bonded to hydrogen , it will forma tetragonal structure. In one unit cell, Mg atoms bind to form MgH₂ six hydrogen atoms, as shown in Fig. 9. MgH₂ formation begins with adsorption and dissociation of hydrogen molecules on the surface of Mg, and continued with diffusion process and hydride formation. During this process, hydrogen atoms occupy tetrahedral interstitial position in order to form a solid solution in the Mg (Zulkarnain, 2011). Furthermore, the more extra hydrogen atoms in Mg, the more hydrogen atoms are localized and regularly placed in the formation Mg along formation of hydride phase and its crystal structure transforms into a tetragonal.

CONCLUSION

XRD characterization results indicate the composite samples 8 grams of Magnesium - 2 grams of keratin has the highest intensity, while the composite sample of 2 g Magnesium - 8 grams of keratin has a hump at most. Hump shows the presence of carbon phase/phase. This phase does not have a high peak due to the amorf form. From the XRD results, in addition to Mg phase there is a phase Mg(OH)₂. Phase Mg(OH)₂ appears because there is a part of the OH cluster which is disconnected. Keratin substances bind together with magnesium.

Rietveld refinement was executed obtaining lattice parameter a, b, and c. Magnesium has a hexagonal crystal structure that has a large density fractions. However, if it is applied to hydrogen storage, it can still be absorbed into Mg structure due to the smallness oh hydrogen atom radii. Bonding between magnesium and hydrogen are formed MgH₂ and it has tetragonal structure. Keratin

structures have -R- cluster. In hydrogen storage applications, -R- cluster can be replaced by hydrogen atoms.

ACKNOWLEDGMENT

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Review on Engineering Green-Materials and Applications from Tropical Plants Sources for Sustainable Future in Indonesia

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ABSTRACT

In the last decade, engineering green materials (EGM) showed report progress in research and applications. The EGM has been attracted by many researchers around the world in counter of man-made materials to obtain more efficient and eco-friendly engineering materials available in nature which are abundant resources to be explored in many engineering products. Indonesia, as one of a tropical plants resources in the world, has provided abundant resources from bast to non-bast fibers to boost up engineering green materials in research and applications, not including wood, rottan and bamboo which they have been utilised traditionally in bulk in many years as engineering applications. The EGM consists of natural plant fiber (NPF) and natural plant matrix (NPM) to build-up high performance natural fiber composite (HPNFC) materials. The EGM growth has caused global impacts in implementing material engineering domain and biotechnology. This paper reviews on the EGM in Indonesia, in their influence and development for engineering application, and the issues of recent technology also discussed briefly.

Keywords: tropical plants, EGM, NPM, HPNC

INTRODUCTION

Current development of material engineering has attracted researchers around the world to find new materials from natural fibers or plant besides bamboo and wood that has been known since long time ago as the ship's construction and building materials which are still commonly found in Indonesia. The United Nations has declared that 2009 is the year of the International Year of Natural Fibers that have been declared in December 2006 that will be facilitated by the FAO

(Food and Agriculture Organization), which contains a program for sustainable future [1]. It is necessary to expose and share the concept of the "Tropical plants review on the engineering green-material and applications for future sustainability in Indonesia" to the community which aims to encourage the agrotechnology development and enrichment engineering products in Indonesia. Realization of value-added utilization of fibers and matrix based on natural resources is an integrated into concepts amongs

materials and processes, farmer / businessman, academics / technologist /scientinst and government.

The specific objective of this presentation is to encourage and empower Indonesian stakeholders of the natural resources by using natural plant fibers (NPF) or cellulose fibers such as hemp, kenaf, pandan mat, coconut

fiber, pineapple, ramie, , banana etc. as a reinforcement material and also natural plant matrix (NPM) as an adhesive binder materials for building-up engineering green material (EGM) (Jamasri 2008). **Fig. 1** presents an example of the sources of tropical plant fiber sources material (Jamasri, 2008, Marsyahyo 2005, Rochardjo, 2009; Suryanto, 2013)



(a) Kenaf bast fiber



(b) Kapas seed fiber



(c) Rosela/yute bast fiber



(d) Rami bast fiber



(e) Pisang abaka bast fiber



(f) Nanas leaf fiber



(g) Mendong leaf fiber



(h) Ijuk fiber



(i) Abaca bast

Figure 1. Some abundant fiber resources for EGM

Empowerment through the cultivation as well as how the application of these materials can create new job opportunities for communities living close to forests or disadvantaged areas who have 'idle land' that directly assist the government in poverty reduction programs and create new job field and diversify the results agricultural products are more advanced in added value and utilization.

Indonesia is also rich in raw materials as a matrix binder fiber media. The matrix is extracted from roots of plants or trees such as pine gum, dammar or rubber. Quality of the natural matrix currently being investigated to determine the mechanical properties so they can be used for technical applications that have high economic value. Several types of natural matrix resources as shown in **Fig. 2** (Mujiyono 2010; Santhiarsa, 2012)

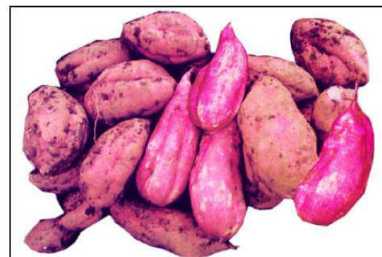


Figure 2. Raw material NPM for adhesive matrix obtain from gum and starch

Increasing consumables of the EGM worldwide shown on Table 1 and it can be predicted that the issues of eco/green

materials have been well-understood for sustainable future.

Tabel 1. World utilised natural fibers (x in thousands) (Santhiarsa, 2012)

Natural Fiber	1996	1999	2000	2005 (Estimated)	2010 (Estimated)
Flax	2100	15,900	20,000	–	–
Hemp	0	1700	3500	–	–
Jute	1100	2100	1700	–	–
Sisal	1100	500	100	–	–
Kenaf	0	1100	2000	–	–
Coir	0	0	1000	–	–
Total	4300	21,300	28,300	50,000–70,000	> 100,000

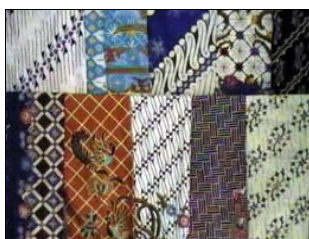
The emergence of a new passion in the use of textile fiber crop commodities supported by the stakeholders, especially in Indonesia as developed countries such as the support of researcher, engineer, businessman, farmer and society as an end user. This is in line with the issues of the world in recent year that are back to nature, eco-friendly, green environment, renewable, and biodegradable recycleability. The issues were growing due to the dominance of materials from plastic fibers (synthetics) that are not environmentally friendly and dependability of supply of petroleum for synthetic fiber

material as derivative hydrocarbons product for plastic materials that pose environmental and human health issues to be countered actively.

MATERIALS AND METHODS

Traditional Material and Methode

Most of EGM has been utilised as traditional handicrafts and furnitures, even bamboo and rattan also have used as structural building and ship applications and show reliability in many years. Figure 3 some examples the use of EGM.



(a) Textiles products



(b) Craft products



(c) Furniture products

Figure 3. Tradtional used of EGM

The EGM is need to be informed widely that can contribute more advantages to the people of Indonesia. The low application of this material is inseparable from the condition of a lack of scientific information and practice,

but there are several factors considered are: 1. Lack of information about the database utilization plant sources of local textile fibers in areas where people have not been inspired to innovate.

2. Lack of skills and the carrying capacity of science and technology, particularly the expertise and capital, which still need to be considered as well as intensive coaching through mentoring assistance and training.

Nowdays, EGM much more developes for engineering application which need some more advanced methode than traditionally achieved.

Advanced Material and Methode

Figure 4 is some flow of material to be processed, came from tropical plant cropped, preparation for pre-treatment, decortication, advanced treatment and designing woven structure. These step of processing methode continued to stacking methode to matrix materials as adhesive agent to build-up HPNFC.

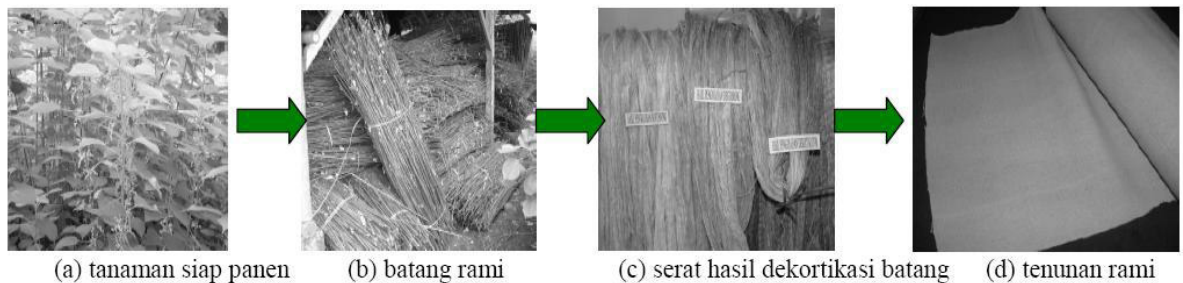


Figure 4. EGM to HPNFC processing steps

Composite processing shown at Figure 4 to build-up HPNFC, from cropped plant into raw fibers to end-woven structure. Figure 5

classified the methodes of processing composites that consists of fiber and matrix as a binding materials.

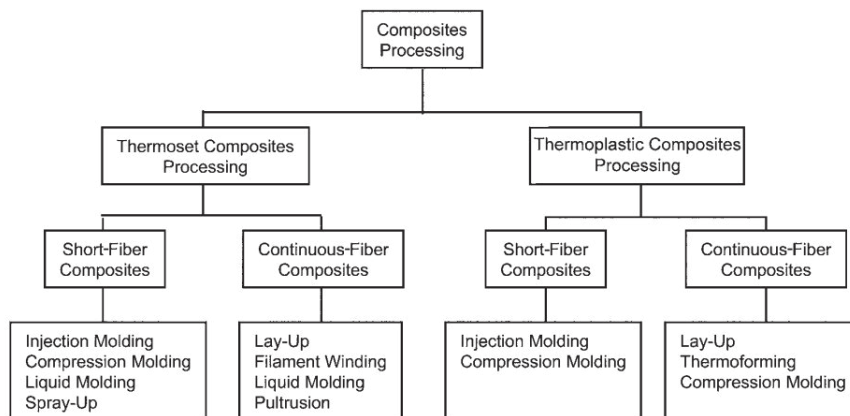


Figure 5. Classification of composite materials and processing

Processing HPNFC that consists of Ramie woven fibers and albasia natural matrix shown at Figure 6.

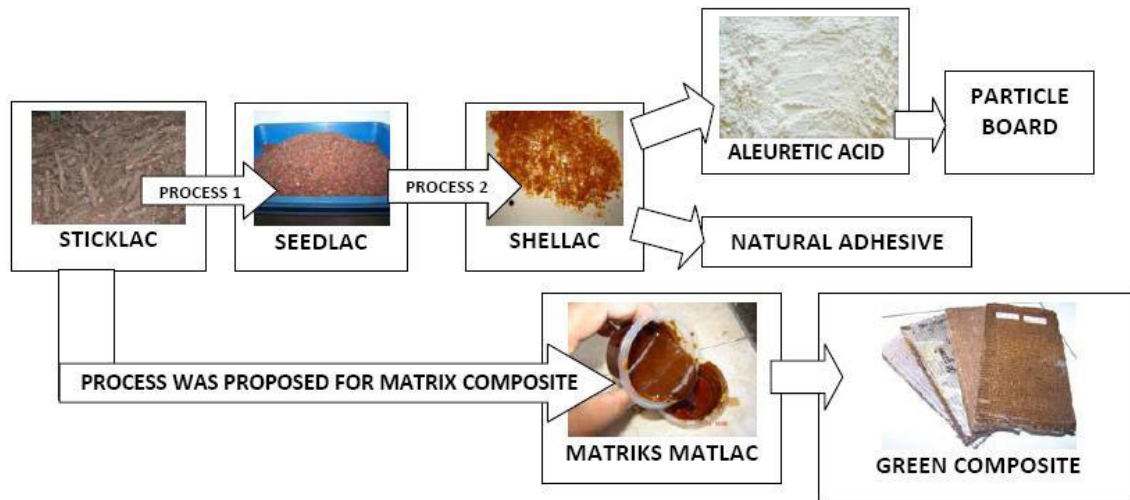


Figure 6. HPNFC processing routes [6]

I. THE ADVANCED APPLICATIONS OF EGM

Alternative usage of the EGM as developed for many purposes such as building materials, automotive interior components. HPNFC

shown having great opportunities for structural applications to fulfill engineering load dynamics such as lightweight structures, corrosion resistant parts and component, and heat resistant parts in certain environment, as seen on **Fig. 7.**



Figure 7. Automotive interior parts

In sport utility, lightweight shin decker from abaca bast composite ease to produce and has properties high impact resistant panels also

for military applications such as bulletproof panels in **Fig. 8.**



(a) Shin decker from abaca composite



(b) Bulletproof panels from ramie composite

Figure 8. Sport and military applications [9]

It can be noted that all possible engineering applications of the EGM more contributing on value added for engineering products compare to traditional products from the same resources.

CONCLUDING

In order realized the utilization and development of composite EGM plant-based sources for covering wide range engineering applications in Indonesia, then two things to note are:

1. Linkages between the science disciplines is needed to realize the development of textile

composite materials that have an impact on the welfare of the people especially in the side of economic value added and technologically synergy.

2. Community empowerment and natural resources are a necessity that should be supported by all parties in order to create the nation's independence, especially in the field of global diversity technopreneurship to develop agrotechnology from the abundant natural fiber sources for sustainable future in Indonesia.

3. Integrated collaboration between farmers / businessmen, academics / technologist and the key to the successful implementation of government concepts, materials and related processes with the development of natural sources of plant-based fibers for textile composites and accelerate the development of social economy in Indonesia

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Experimental Study Permeable Asphalt Pavement Used Domato Stone (Quarsite Dolomite) as Course Agregate for Surface Layer of Road Pavement

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ABSTRACT

The lot deposit of Domato Stone as local material from sea location in Banggai island in half Sulawesi of Indonesia. Was still not be exploited better. Some researach in the field of road construction showed that Domato Stone was powefull enough when mixtured asphalt structure. Permeable asphalt pavement or porous friction course is commonly knows as porous asphalt. The porous pavement used in japanes and europe. The pavement consists in a porous overlay allowing rainwater to flow down to the botton the overlay and then to drain on the edges of the pavement. Quality of porous asphalt was developed to drain pavement surface flow through it's pores, because of is specific propertis to mesure it's ability to drain the water (Permeability), a special measuring device is required. This study is aimed to measure the coefficient of permeability using the constanthead permeability test at transportation laboratory Hasanuddin University. The result were compared with the previous study. The test included horizontal and vertical permeability. Indirect Tensile Strength 0.0673 for asphalt quality 3% and Indirect Tensile Strength 0.2370 for asphalt quality 5%. Cantabro test, loss weight 77.10 for asphalt quality 3% and loss weight 9.70 for asphalt quality 5%. Vertikal test Permeability (binamarga 4.85 ml/s, Australia 5 ml/s, British 5.10 ml/s). Horizontal test Permeability (binamarga 4.89 ml/s, Australia 4.75 ml/s, British 4.81 ml/s). Based on the Scanning Electron Microscope (SEM) can be seen the microstructure and content of chemical elements present in the porous asphalt which prove that all elements of the liquid asbuton and concrete waste can blend and bind well.

Key words : Domato stone, Cantabro Loss, Indirect Tensile Strength, Permeability, X-RD and SEM.

INTRODUCTION

Permeable asphalt pavement or porous friction course is commonly known as porous asphalt. The porous pavement is commonly used in Europe and Japan. The pavement consists in a porous overlay and then to drain on the edges to the pavement (*Michael. E Barret. Ph.D*). The lot deposit of Domato stone in Indonesia was still not be exploited better. Among the existing utilization of it most of it was exploited for traditional needs fireplace material, some last research in the field of road construction showed that Domato stone was powerful enough when mixed material for pavement stabilization. Domato stone is local material from sea location in the island of Banggai half Sulawesi Indonesia. Its was kwarsit Dolomitan material Celebes (*Car Donald, 1985*). This Experimental be done for measuring properties permeability asphalt pavement with using Domato stone as Local material who was come from sea location at the Banggai Island half Celebes Indonesia with used Rice Hash as Filler.

As course aggregate on the surface layer Road Pavement. Capacity drain porous Asphalt were connecting correlation with spacing high and small porosity in structure Asphalt. Stability and Durability and Hydraulic conductivity its must be high test than 20% (*Ruz et. al, 1990*). Asphalt porous is open graded course Aggregate. Porosity asphalt porous (10%-15%) the structure made drain for flow water (*Nur Ali, et al. 2005*).



Figure 1. Permeable Friction Course

Aggregate was specimen mineral who was done for mixture road construction in the asphalt pavement it's must be 90%-95% for the total weight structure or 77%-85% for all volume (*Alkin, et. al 1997*).

Classification aggregate be measured by spacing at all : course aggregate it must be lost for filter No.8 it is higher than 2,36 mm. Fine aggregate it must be lost for filter No.8 and stoped to No. 200 or it is 2,36 mm and 75 μm . Filler it must be smaller than 75 μm and lost filter No. 200



Figure 2. Domato Stone (Local Containe of Banggai island in half celebes)

X-ray Computed Tomography

X-ray Computed Tomography (CT) is a nondestructive imaging technology, capable of acquiring a 3D or 2D image of the internal structure of a solid object, such as asphalt concrete. The directing planar x-rays pass through the specimen, along several different paths and from different directions and are captured by the detector. The attenuations of x-rays within a specimen are recorded for calculating the linear attenuation coefficients, which may be used to represent the spatial locations of the different components of the specimen.

After finishing the collection of attenuations for a full rotation of the specimen, it is vertically moved downward for scanning the next slice. X-ray CT was frequently applied in recent years to characterize cracks or any damage in asphalt mixtures by measuring the internal structure distribution of specimens, such as the locations of aggregates, as well as mastic and air voids. CT images are needed to reconstruct 3D visualization images of the specimens. 2D cross-sectional CT images were obtained to measure air void distribution and crack size at different depths with asphalt specimens. After capturing 2D cross-sectional images, the 3D visualization image of the sample can be reconstructed for importing to computer to simulate the performance of asphalt mixture under various loading and environmental conditions. Benefiting from this non-destructive technology, the intact sample may still be used for engineering properties tests such as the dynamic modulus test and the flow number test. Hence, X-ray CT is an effective technology to study the relationship between asphalt microstructure and engineering properties. (Masad and co-workers 1998) [5].

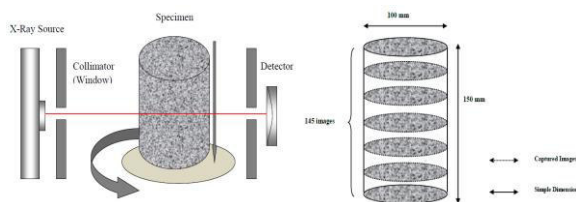


Figure 3. X-Ray Thomographi

Materials and Methods

Indirect Testing

Permeable asphalt pavement was produced with used domato stone as course aggregate. The domato stone broked in the spacing \varnothing 3/8" 1/2"-3/4" with

the BNA Blend Pertamina penetration 60/70. Briket at the Bitumen be done as the standard variation asphalt 3%, 3,5%, 4%, and 5% for testing experimental Indirect Tensile Strength (ITS) and Catambro Lost. We was controlling testing for composition asphalt permeable pavement with Standar National Indonesia (SNI) and American Association for Testing and Material (ASTM), Permeability and Marshal Test with asphalt variation 4-7% integral spacing 1% who use variation open gradation. Asphalt optimum standar is 4% be used to controlling variation asphalt. For optimum asphalt test be use variation asphalt 3% - 5% with spacing 5%.

For open gradation we use lost aggregate 3/4", 1/2" and lost filter by comparative 50 : 50. Fine aggregate we use filter number 4, finally number 200, we used 10%. BNA Blend Pertamina we use all variation asphalt category: 3%, 3.5%, 4%, 4.5% and 5%.

Test Indirect Tensile Strength (ITS) be controlling by ASTM D6931-07.



Figure 4. Test Indirect Tensile Strength

TESTING PERMEABILITY

Limitation Of Darcy's Law

In a porous media, the hydraulic conductivity K represents the specific discharge per unit hydraulic gradient, which means that the coefficient depends on both matrix and fluid properties (Bear, 1972). From a dimensional analysis, the

hydraulic conductivity can be derived as (Nutting, 1930):

$$K = \frac{k g}{v} \quad (1)$$

Where k is the intrinsic permeability, v the kinematic viscosity and g the gravity acceleration.

The intrinsic permeability is only a function of the matrix composing the porous media and its characteristics such as grain size distribution, tortuosity and porosity. For porous media, the Reynolds number (Re) can be defined as (Charbeneau, 2000):

$$Re = \frac{q d}{v} \quad (2)$$

Where q is Darcy's velocity and d is the average grain diameter or d10 of the size distribution profile of the porous media. Experiments have shown that Darcy's law remains valid as long as the Reynolds number doesn't exceed a value between 1 to 10 (Venkataraman, 1999). As the Reynolds number doesn't exceed this value, the flow remains laminar and is governed by viscous forces. However the inertial forces start to govern the flow through the porous media in transitional flow when Re becomes higher than the transition value. As the Reynolds number continues to increase, the flow becomes turbulent at some point (Bear, 1972).



Figure 5. Setup of the Testing Apparatus

Rain Fall – Run Off Relation For Porous Asphalt Testing

This experiment was carried out in Hasanuddin University experimental station Laboratory Structural and Transpiration Hasanuddin University, which specializes in runoff utilization. Three experimental steel cells were used briket asphalt, with each cell measuring 0,40m x 0,40m. There were drainage boards under the cells, which had p 1.0cm holes. Water outlets were set up under the holes to monitor the quantity of the collection water. Pipes were used to collect both surface runoff and subgrade infiltration. Surface runoff and subgrade drainage from each cell were measured with beakers and flasks at the rear of each cell through drainage pipes for each of the three types of porous pavements and the impervious cell. Precipitation and runoff rates were recorded every 5 min. An artificial rainfall apparatus was constructed on an adjustable shelf. It consisted of 36 Rainbird 1,800 sprinklers, with an operating pressure of 0.1 MPa, a discharge rate of 0.1m³ intensity is 0.905 and is controlled by sprayer combination.



Figure 6. Rain fall – run off relation for porous asphalt testing

- Treatment A, porous concrete asphalt (permeable asphalt pavement) with Hot Mix asphalt gradation Model Binamarga.
- Treatment B, porous concrete asphalt (permeable asphalt pavement) with permeable friction course gradation Model British
- Treatment C, porous concrete asphalt (permeable asphalt pavement) with porous asphalt gradation Australia.block paving with subbase of 5 cm thick

concrete lacking sand and 20cm thick gravel Model Australia

Mix Design Permeable Asphalt Pavement Testing

Mix design permeable asphalt pavement the used composition open graded sistem. Who was Mix Trial Gradation lost of material ¾”, 1/2 “ be stoped filter ½” and lost of material ½“ be stoped filter 3/8” with composition comparative 50-50 to course aggregate. The used fine aggregate lost filter number 4, and stoped filter number 200 all of 10% for mould capacity. Asphalt Blend Pertamina the use variation standard 3%, 3.5%, 4%, 4.5% and 5%. Briket make in for Ø 10 cm and depth ± 6.5cm and Briket make in □40x40 cm, depth ± 6.5cm.



(a) (b) (c)
Figure 7. Permeable asphalt pavement

Table 1. Total briket test indirect tensile strength and Cantabro

Item Testing	BNA Blend Pertamina (%)	Planning Briket (unit)
Indirect tensile strength	3	5
	3,5	5
	4	5
	4,5	3
	5	5
Cantabro	3	5
	3,5	5
	4	5
	4,5	5
	5	5
Total briket		50

Before briket test in cantabro, briket was plum to Los Angeles machine drum, speed (V) 30-33 rpm for rotation.

$$L = \frac{M_o - M_i}{M_o} \times 100$$



Figure 8. Test Cantabro Machine

X-RAY COMPUTED TOMOGRAPHY

X-ray computed tomography (CT) is an innovative, nondestructive technique used for obtaining digital information on the three-dimensional internal microstructure of solid materials. Different phases in solid materials may be distinguished using x-ray CT. In recent years, this imaging technique has been utilized to characterize the microstructure of asphalt mixes (Shashidhar 1999; Wang et al. 2001; Masad et al. 2002, 2004). In this study, the x-ray system in the Advanced Characterization of Infrastructure Materials laboratory at Universitas Negeri of Makassar was used to scan the test specimens. The setup includes two separate x-ray systems; the micro-focus and the mini-focus. The micro-focus system consists of a 225 kV x-ray source and an image intensifier detector, while the mini-focus has a 350 kV x-ray source and a linear detector. Due to the limited power of the micro-focus system, it is more applicable to scan small asphalt mixture specimens with better resolution. The mini-focus is more applicable to scan large asphalt mixture specimens with an adequate resolution of 0.17 mm/pixel. In this study, the mini-focus was used to scan the test specimens. The

three example cross-sectional images from each depth (top third, middle third and bottom third) of all the specimens are presented

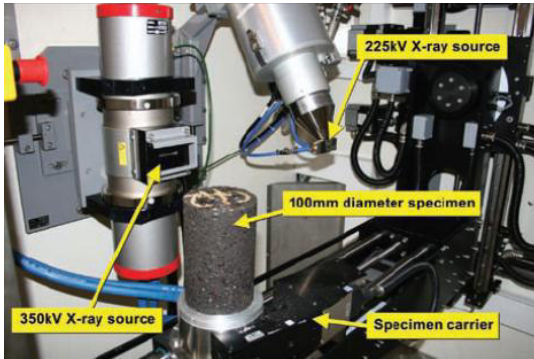


Figure 9. Components of the X-ray CT System at Texas A & M

RESULT AND DISCUSSION

Analysis Indirect Tensile Strength

Table 2. Outcome Indirect Tensile Strength test

Sam pel	Perce ntag e asphalt quality (%)	Diameter briket		Load Value (P) kgf	ITS Value Mpa
		mm	mm		
I	3.0	102.3	66.9		0
II		102.22	69.3	75.00	0.066134591
III		102	68.5	100.00	0.089401701
IV		102.4	67.7	75.00	0.067578595
V		102.4	67	125.00	0.113807734
Average					0.067384524
I	3.5	102.5	67.5	275.00	0.24827991
II		101.8	67.2	225.00	0.205448034
III		102.4	68.4	250.00	0.222956672
IV		102.1	68.8	200.00	0.177849373
V		102	68	275.00	0.247662431
Average					0.220439284
I	4.0	102.8	69	350.00	0.308221097
II		102.5	68.2	400.00	0.357427756
III		102.2	68.9	350.00	0.310480586
IV		102	69	375.00	0.332826983
V		102.3	67.4	350.00	0.317080137
Average					0.325207312
I	4.5	102.3	68.4	300.00	0.267809539
II		102.4	68.3	250.00	0.223283109
III		102.5	69.5	275.00	0.241135164
IV		102.6	67.6	325.00	0.292702092
V		102.2	68	275.00	0.247177769
Average					0.254421535
I	5.0	102.2	61.7	275.00	0.272416342
II		102.5	68	225.00	0.201644445
III		102.4	65	250.00	0.234619021
IV		102.4	66.1	225.00	0.207643158
V		102.2	68.2	300.00	0.268857717
Average					0.237036137

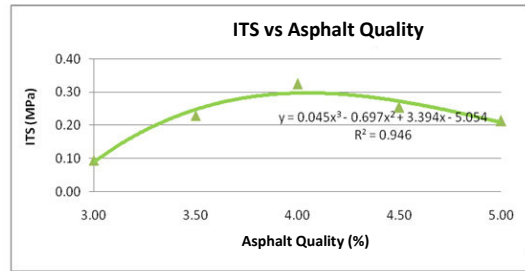


Figure 10. Correlation quality asphalt with Indirect Tensile Strength



Figure 11. Briket after Indirect Tensile Strength Test

Table 3. Recapitulation R_{maks} value

No.	Quality asphalt	Maximum Loading (Kgf)	ITS Value (MPa)	R Maks
1	3,00	125	0,1140	0,0180
2	3,50	275	0,2483	0,0234
3	4,00	400	0,3574	0,0283
4	4,50	325	0,2927	0,0253
5	5,00	250	0,2346	0,0225

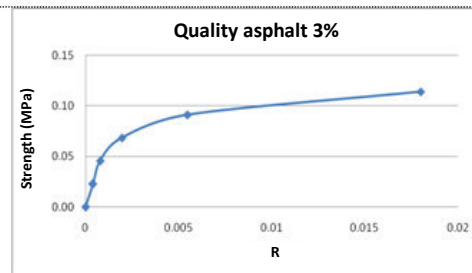


Figure 12. Correlation ITS Value and R value 3%

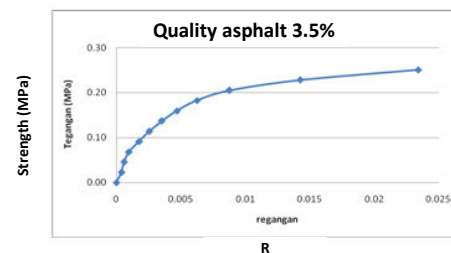


Figure 13. Correlation ITS Value and R value 3.5%

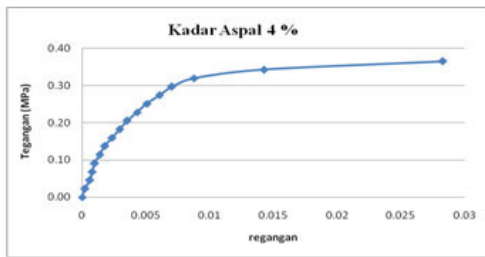


Figure 14. Correlation ITS Value and R value 4%

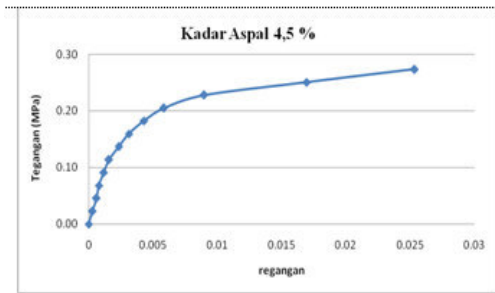


Figure 15. Correlation ITS Value and R value 4.5%

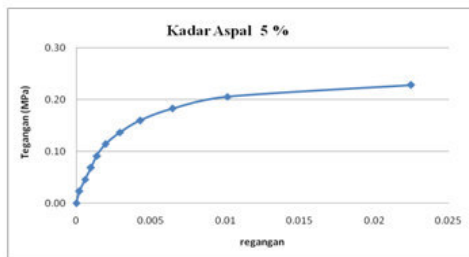


Figure 16. Correlation ITS Value and R value 5%

Analysis Cantabro

Table 4. Outcome Cantabro test

Sam pel	Perce ntag e quality asphalt (%)	Wei ght before test	Wei ght after test	Loss Weight	Loss Weight
		(Gram)	(Gram)	(Gram)	(%)
		M_0	M_i		L
I	3.0	1081	244	837.00	77.43
II		1083	248	835.00	77.10
III		1090	281	809.00	74.22
IV		1091	226	865.00	79.29
V		1070	241	829.00	77.48
Average					77.10
I	3.5	1085	731	354.00	32.63
II		1089	760	329.00	30.21
III		1071	748	323.00	30.16
IV		1069	711	358.00	33.49
V		1088	705	383.00	35.20
Average					32.34
I	4.0	1081	913	168.00	15.54
II		1082	936	146.00	13.49
III		1088	931	157.00	14.43
IV		1086	944	162.00	13.09

V		1090	913	177.00	16.24
Average					14.56
I	4.5	1084	959	125.00	11.53
II		1082	952	130.00	12.01
III		1086	940	146.00	13.44
IV		1088	961	127.00	11.67
V		1084	948	136.00	12.55
Average					12.24
I	5.0	1075	956	119.00	11.07
II		1084	968	116.00	10.70
III		1090	984	106.00	9.72
IV		1078	994	84.00	7.79
V		1105	1003	102.00	9.23
Average					9.70

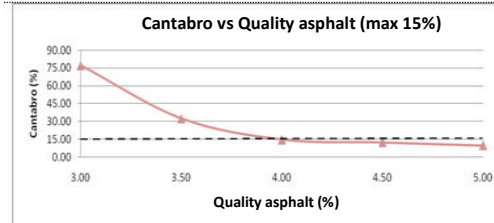


Figure 17. Correlation quality asphalt with value cantabro loss

Analysis Rainfal-Run Off

Effect of different treatments on runoff Surface runoff and infiltration rates were measured at the experimental station in April 2013 and September 2013. The experiment was carried out during the summer months, with most precipitation occurring in that season in South Celebes of Indonesia. A comparison of precipitation rates and surface runoff for the three permeable asphalt pavement and the impervious surface cell during a storm beginning at 16:30 on 26 April 2013. There was no measurable continuation of runoff on the Treatment A, B, C surface during the rainfall process. Runoff on the impervious surface cell closely followed precipitation rates during all rainfall events. Minor surface runoff from the porous surface cells beginning around 50 min into the event was attributed to leaks in the different sub-bases used to capture water, which would infiltrate into the subgrade soil. The phenomenon can be attributed to the storage capacity of the permeable asphalt pavement, which delays evacuation of water into the runoff outlet. This delaying effect also renders the evacuation a more gradual process, as reflected by both the reduction in

maximum runoff rates measured at the runoff outflow and by the increase in time required for discharge.

For the rainfall amount/duration of 118.72mm/2h, the runoff outflow percentages of Treatments A, B and C ranged from 22.4 to 68.5, but for Treatment D it was 92.9–97.8. For the rainfall amount/ duration of 94.09mm/2h, the incidence of runoff for Treatment B was 71 min later than the impervious pavement, with a recorded flow volume of just 23.00 mm. For the rainfall amount/duration of 59.36 mm/h, the incidence of runoff for the porous pavement surface was 45 min later than the impervious pavement, with the flood peaks reduced by 35–100%, especially for Treatment B, with a recorded flow of zero.

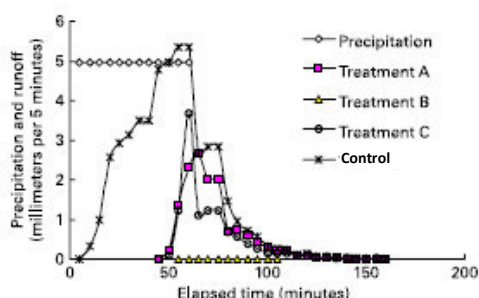


Figure 18. Comparison of precipitation rate, surface runoff of three porous pavement cells and the impervious surface cell.

The order of infiltration coefficients for the different media investigated in this study is KCLS. KPCBP. KS . KIS, where KCLS, KPCBP, KS and KIS are the infiltration coefficients for the materials of concrete lacking sand, permeable asphalt pavement, subgrade and impervious surface, respectively (see Picture 8). The factors discussed above explain the quantitative relationships of flood peak for the four surfaces FD . FC . FA . FB, where FD, FC, FA and FB signify the volume flood peaks for Treatments D, C, A and B, respectively.

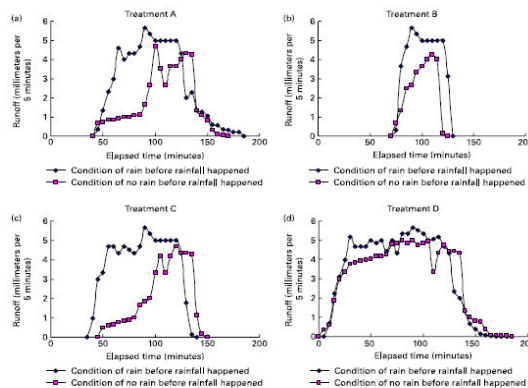


Figure 19. Runoffs of different treatments under conditions with rainfall and without rainfall before artificial rainfall happened

Table 5. Permeability test run-off

Item Test	Permeable Asphalt Pavement					
	Model Binamarga		Model Australia		Model British	
	V	t	V	t	V	t
Vertikal	4.58	5	5.00	5	5.08	5
	4.80	10	4.59	10	4.98	10
	4.60	15	4.75	15	4.93	15
	5.00	20	5.15	20	5.22	20
	4.70	25	4.95	25	5.05	25
	4.85	30	5.00	30	5.10	30
Horizontal	4.69	5	4.75	5	4.81	5
	4.90	10	4.95	10	5.00	10
	4.53	15	4.55	15	4.60	15
	4.65	20	4.70	20	4.82	20
	4.35	25	4.65	25	4.83	25
	4.36	30	4.45	30	4.56	30
Vertikal and Horizontal	4.76	35	4.82	35	4.89	35
	4.49	40	4.53	40	4.60	40
	4.51	5	4.61	5	4.64	5
	4.01	10	4.28	10	4.33	10
	3.98	15	4.08	15	4.10	15
	4.48	20	4.52	20	4.65	20
	3.85	25	3.95	25	4.05	25
	4.48	30	4.55	30	4.61	30
	4.15	35	4.26	35	4.50	35
	4.42	40	4.49	40	4.58	40
	4.18	45	4.27	45	4.38	45

Remarks : t = time (minute), V = speed flow (mL/s)

Analysis X-Ray - Tomography

The results of research indicates that porous asphalt mixture showed an influence on the value of the characteristics of porous asphalt particularly at concrete grading 50% retained 1/2" and 50% natural crushed stone retained 3/8" where the values obtained from the analysis of optimum binder content is 9.5%. Based on the Scanning Electron Microscope (SEM) can be seen the microstructure and content of chemical elements present in the porous asphalt which prove that all elements of the BNA Blend Pertamina

and concrete waste can blend and bind well.

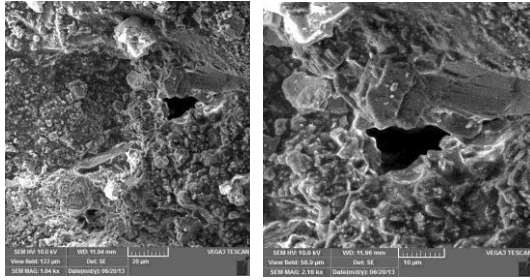


Figure 20. Photo X-Ray Permeable Asphalt Pavement (British Standard)

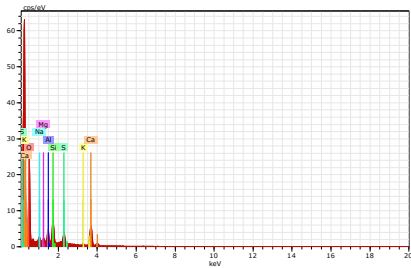


Figure 21. Tescan vega3SB

Element	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Compound	norm. Comp. [wt.%]	C Error (3 Sigma) [wt.%]
Oxygen	41.56	42.98	58.53		0.00	15.03
Silicon	10.48	10.83	8.41	SiO ₂	23.18	1.46
Aluminium	8.06	8.33	6.73	Al ₂ O ₃	15.74	1.29
Sodium	7.35	7.60	7.21	Na ₂ O	10.25	1.59
Magnesium	4.74	4.90	4.39	MgO	8.12	0.92
Potassium	1.50	1.55	0.86	K ₂ O	1.86	0.26
Calcium	16.39	16.95	9.21	CaO	23.71	1.58
Sulfur	6.64	6.86	4.66	SO ₃	17.14	0.84
Total:	96.71	100.00	100.00			

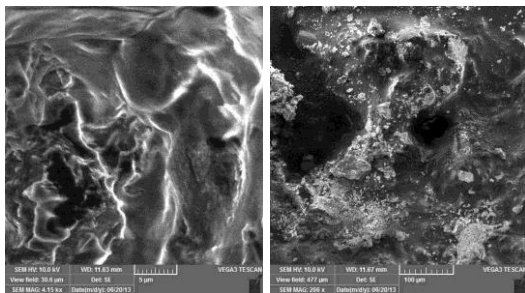


Figure 22. Photo X-Ray Permeable Asphalt Pavement (Australia Standard)

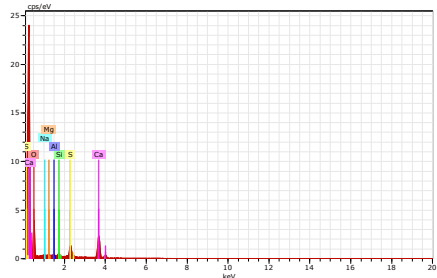


Figure 23. Tescan vega3SB

Spectrum: test

Element	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Compound	norm. Comp. [wt.%]	C Error (3 Sigma) [wt.%]
Oxygen	20.01	40.36	58.46		0.00	9.11
Silicon	1.56	3.15	2.60	SiO ₂	6.73	0.36
Aluminium	1.68	3.39	2.91	Al ₂ O ₃	6.40	0.42
Sodium	2.74	5.52	5.57	Na ₂ O	7.45	0.79
Calcium	16.39	33.05	19.11	CaO	46.25	1.68
Sulfur	5.37	10.83	7.83	SO ₃	27.05	0.76
Magnesium	1.83	3.69	3.52	MgO	6.12	0.50
Total:	49.57	100.00	100.00			

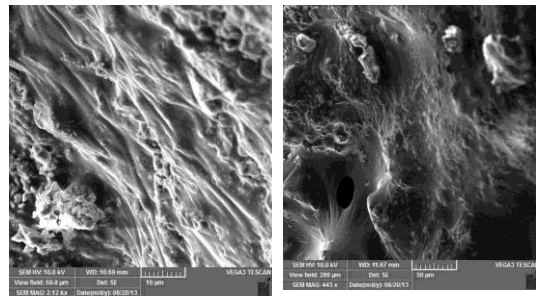
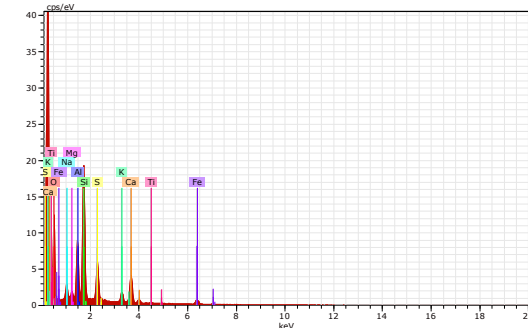


Figure 24. Photo X-Ray Permeable Asphalt Pavement (Binamarga Model)



Spectrum: test

Element	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Compound	norm. Comp. [wt.%]	C Error (3 Sigma) [wt.%]
Oxygen	31.75	46.16	61.82		0.00	12.30
Silicon	12.25	17.80	13.58	SiO ₂	38.09	1.66
Aluminium	6.29	9.15	7.27	Al ₂ O ₃	17.29	1.00
Sodium	3.26	4.74	4.42	Na ₂ O	6.39	0.75
Magnesium	1.29	1.87	1.65	MgO	3.10	0.32
Sulfur	4.69	6.82	4.56	SO ₃	17.03	0.60
Calcium	5.09	7.40	3.96	CaO	10.36	0.55
Potassium	1.64	2.39	1.31	K ₂ O	2.88	0.25
Iron	2.26	3.28	1.26	FeO	4.22	0.32
Titanium	0.27	0.40	0.18	TiO ₂	0.66	0.13
Total:	68.80	100.00	100.00			

CONCLUSIONS

1. Indirect tensile strength 0,1140 MPa for total load 125 Kgf, for the quality asphalt 3% R_{maks} 0,0180. Indirect tensile strength 0,2483 MPa for total load 275 Kgf, for the quality asphalt 3.5% R_{maks} 0,0234. Indirect tensile strength 0,3574 MPa for total load 400 Kgf, for the quality asphalt 4% R_{maks} 0,0283. Indirect tensile strength 0,2927 MPa for total load 325 Kgf, for the quality asphalt 4.5% R_{maks} 0,0253. Indirect tensile strength 0,2346 MPa for total load 250 Kgf, for the quality asphalt 5% R_{maks} 0,0225.
2. Permeable asphalt pavement mixture for Cantabro test we can see that optimum BNA Blend Pertamina for the coarse aggregate domato stone it was bigger porous when quality asphalt 3%. Loss weight Cantabro 77.10% correlation with quality asphalt 3%, loss weight Cantabro 32,34% correlation with quality asphalt 3.5%, loss weight Cantabro 14,56% correlation with quality asphalt 4%, Loss weight Cantabro 12,24% correlation with quality asphalt 4.5% and loss weight Cantabro 9,70% correlation with quality asphalt 5%.
3. This study evaluated the performance of three porous pavement systems from the perspective of infiltration and runoff, with very positive performance in comparison to a traditional impervious surface. All three porous pavement surfaces increased infiltration and decreased runoff. Larger porosity values, higher infiltration coefficients, thicker sub-base layers and lower initial water contents of the subgrade produce higher infiltration rates and smaller runoff coefficients. When rainfall infiltrates into a porous surface and its underlying sub-base, the outflow hydrograph will be influenced by the

way in which the construction materials retain or delay flow.

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Laboratorium Testing Gradation and X-Ray Themography to Properties Permeable Asphalt Pavement With Rice Hush Ash as Filler and Domato Stone as Course Agregate

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ABSTRACT

The lot deposit of paddy chaff (rice husk) in Indonesia was still not be exploited better. Among the existing utilization of it, most of it was exploited for traditional needs like fireplace material, dusty rub, combustion of brick making, mixture of brick material, etc. Quality of porous asphalt was developed to drain pavement surface flow through it's pores, because of is specific propertis to mesure it's ability to drain the water (Permeability) this research airme to identifiy behavior and elegtibility hask ash or foundation structure. Beside of infiltrative ability, ash has also like-cement nature which is able to improve coarseness among particles. Those two natures reveals that husk ash is suitable for compactor agent when it was treated as a filler. The laboratory test will involve four steps of test 1) Hot mix I, done for optimal water content subject to 75 % or more asphalt coated agregate, 2) Hot mix II, done for optimal water content subject to dry density at maximum, 3) Hot mix III, performed for asphalt content at optimum and 4) Hot mix IV, performed for filler content at optimum. The results of research indicates that porous asphalt mixture showed an influence on the value of the characteristics of porous asphalt particularly at concrete waste fraction grading 50% retained 1/2 " and 50% natural crushed stone retained 3/8" where the values obtained from the analysis of optimum binder content is 9.5%. Based on the Scanning Electron Microscope (SEM) can be seen the microstructure and content of chemical elements present in the porous asphalt which prove that all elements of the liquid asbuton and concrete waste can blend and bind well.

Key words : Domato stone, Cantabro Loss, Indirect Tensile Strength, Permeability, Rice Hush Ash, X-RD and SEM.

INTRODUCTION

Permeable asphalt pavement or porous friction course is commonly known as porous asphalt. The porous pavement is commonly used in Europe and Japan. The pavement consists in a porous overlay and then to drain on the edges to the pavement (*Michael. E Barret. Ph.D*). The lot deposit of Domato stone in Indonesia was still not be exploited better. Among the existing utilization of it most of it was exploited for traditional needs fireplace material, some last research in the field of road construction showed that Domato stone was powerful enough when mixed material for pavement stabilization. Domato stone is local material from sea location in the island of Banggai half Sulawesi Indonesia. It is a kwarsit Dolomitan material Celebes (*Car Donald, 1985*). This experimental was done for measuring properties permeability asphalt pavement with using Domato stone as local material which was come from sea location at the Banggai Island half Celebes Indonesia with used Rice Hash as Filler.

As course aggregate on the surface layer Road Pavement. Capacity drain porous Asphalt were connecting correlation with spacing height and small porosity in structure Asphalt. Stability and Durability and Hydraulic conductivity it must be high test than 20% (*Ruz. et. al, 1990*). Asphalt porous is open graded course Aggregate. Porosity asphalt porous (10%-15%) the structure made drain for flow water (*Nur Ali, et al. 2005*).

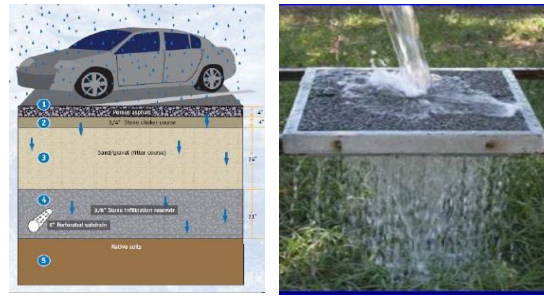


Figure 1. Permeable Friction Course

Aggregate was specimen mineral who was done for mixture road construction in the asphalt pavement it's must be 90%-95% for the total weight structure or 77%-85% for all volume (*Alkin, et. al 1997*).

Classification aggregate be measured by spacing at all : course aggregate it must be lost for filter No.8 it is higher than 2,36 mm. Fine aggregate it must be lost for filter No.8 and stopped to No. 200 or it is 2,36 mm and 75 μ m. Filler it must be smaller than 75 μ m and lost filter No. 200



Figure 2. Domato Stone (Local Containe of Banggai island in half celebes) and Rice Hush Ash as Filler

Table 1. Spesifikasi for gradation

Ukuran Saringan	Tipe I/50	Tipe II/37.5	Tipe III/25	Tipe IV/19	Tipe V/12.5
2" (50 mm)	100	-	-	-	-
1 1/2" (38.1 mm)	90-100	100	-	-	-
1" (25 mm)	-	90-100	100	-	-
3/4" (19 mm)	60-80	-	90-100	100	-
1/2" (12.5 mm)	-	60-80	-	90-100	100
3/8" (9.5 mm)	-	-	60-80	-	100
# 4 (4.75 mm)	20-55	25-60	35-65	45-75	60-80
# 8 (2.36 mm)	10-55	15-45	20-50	25-55	35-65
# 50 (0.3 mm)	2-16	3-18	3-20	5-20	6-25
# 200 (0.075 mm)	0-5	1-7	2-8	2-9	2-10
Kandungan pasir (%)	Min 35	Min 35	Min 35	Min 35	Min 35
Abrasi (%)	Min 40	Maks 40	Maks 40	Maks 40	Maks 40

Bina marga, 1991

Table 2. Asphalt spesification

No.	Jenis	Satuan	Syarat
1.	Kandungan air	%	-
2.	Stabilitas rendaman (24 jam)	%	0 - 1
3.	Viskositas	Cm ² /dt	20 - 100
4.	Homogenitas	%	0 - 0,1
5.	Residu	%	Min 57
6.	Penetrasi residu	(0.1 mm)	135 - 250
7.	Duktilitas residu	cm	Min 40

The asphalt institute, 1979

Table 3. Spesifikasi course aggregate

Saringan (mm)	Ukuran (ASTM)	% berat yang lewat
50	2"	100
37.5	1 1/2"	90 - 100
25	1"	20 - 100
12.5	1/2"	5 - 100
9.5	3/8"	0 - 100
4.75	# 4	0 - 30
2.36	# 8	0 - 10
0.075	# 200	0 - 5

Bina marga, 1991

Table 4. Spesifikasi fine aggregate

Saringan (mm)	Ukuran (ASTM)	% berat yang lewat
9.5	3/8"	100
4.75	# 4	90 - 100
2.36	# 8	20 - 100
0.600	# 30	5 - 100
0.075	# 200	1 - 100

Bina marga, 1991

Table 5. Spesifikasi Filler

Ukuran Saringan	% Lolos
No. 30 (0.59 mm)	100
No. 50 (0.279 mm)	95 - 100
No. 100 (0.149 mm)	90 - 100
No. 200 (0.075 mm)	65 - 100

Bina marga, 1995

Table 6. Spesifikasi chemistry rice hash ask

No.	Unsur	Kandungan (%)
1.	CaO	0.49
2.	K ₂ O	0.91
3.	MgO	0.22
4.	Na ₂ O	0.26
5.	TiO ₂	0.16
6.	Al ₂ O ₃	1.01
7.	P ₂ O ₅	0.01
8.	SiO ₂	96.70
9.	Fe ₂ O ₃	0.05
10.	MnO	0.19

Ceramic-material.com, 2004

X-ray Computed Tomography

X-ray Computed Tomography (CT) is a nondestructive imaging technology, capable of acquiring a 3D or 2D image of the internal structure of a solid object, such as asphalt concrete. The directing planar x-rays pass through the specimen, along several different paths and from different directions and are captured by the detector. The attenuations of x-rays within a specimen are recorded for calculating the linear attenuation coefficients, which may be used to represent the spatial locations of the different components of the specimen. After finishing the collection of attenuations for a full rotation of the specimen, it is vertically moved downward for scanning the next slice. X-ray CT was frequently applied in recent years to characterize cracks or any damage in asphalt mixtures by measuring the internal structure distribution of specimens, such as the locations of aggregates, as well as mastic and air voids. CT images are needed to reconstruct 3D visualization images of the specimens. 2D cross-sectional CT images were obtained to measure air void distribution and crack size at different depths with asphalt specimens. After capturing 2D cross-sectional images, the 3D visualization image of the sample can be reconstructed for importing to computer to simulate the performance of asphalt mixture under various loading and environmental conditions. Benefiting

from this non-destructive technology, the intact sample may still be used for engineering properties tests such as the dynamic modulus test and the flow number test. Hence, X-ray CT is an effective technology to study the relationship between asphalt microstructure and engineering properties. (Masad and co-workers 1998) [5].

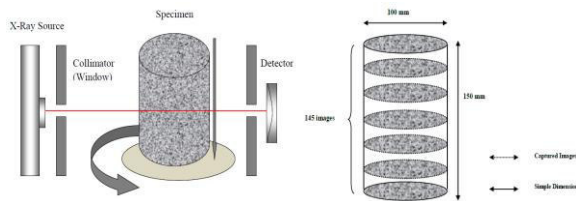


Figure 3. X-Ray Thomographi

4, finally number 200, we used 10%. BNA Blend Pertamina we use all variation asphalt category: 3%, 3.5%, 4%, 4.5% and 5%.

Test Indirect Tensile Strength (ITS) be controlling by ASTM D6931-07.



Figure 4. Test Indirect Tensile Strength

METHODOLOGY

Indirect Testing Tensile Strength

Permeable asphalt pavement was produced with used domato stone as course aggregate. The domato stone broked in the spacing $\text{Ø } 3/8'' \text{ } 1/2'' \text{ } 3/4''$ with the BNA Blend Pertamina penetration 60/70. Briket at the Bitumen be done as the standard variation asphalt 3%, 3,5%, 4%, and 5% for testing experimental Indirect Tensile Strength (ITS) and Catambro Lost. We was controlling testing for composition asphalt permeable pavement with Standar National Indonesia (SNI) and American Association for Testing and Material (ASTM), Permeability and Marshal Test with asphalt variation 4-7% integral spacing 1% who use variation open gradation. Asphalt optimum standar is 4% be used to controlling variation asphalt. For optimum asphalt test be use variation asphalt 3% - 5% with spacing 5%.

For open gradation we use lost aggregate $3/4''$, $1/2''$ and lost filter by comparative 50 : 50. Fine aggregate we use filter number

TESTING PERMEABILITY

Limitation Of Darcy's Law

In a porous media, the hydraulic conductivity K represents the specific discharge per unit hydraulic gradient, which means that the coefficient depends on both matrix and fluid properties (Bear, 1972). From a dimensional analysis, the hydraulic conductivity can be derived as (Nutting, 1930):

$$K = \frac{k g}{v} \quad (1)$$

Where k is the intrinsic permeability, v the kinematic viscosity and g the gravity acceleration.

The intrinsic permeability is only a function of the matrix composing the porous media and its characteristics such as grain size distribution, tortuosity and porosity. For porous media, the Reynolds number (Re) can be defined as (Charbeneau, 2000):

$$Re = \frac{q d}{v} \quad (2)$$

Where q is Darcy's velocity and d is the average grain diameter or d_{10} of the size distribution profile of the porous media. Experiments have shown that Darcy's law remains valid as long as the Reynolds number doesn't exceed a value between 1 to 10 (Venkataraman, 1999). As the Reynolds number doesn't exceed this value, the flow remains laminar and is governed by viscous forces. However the inertial forces start to govern the flow through the porous media in transitional flow when Re becomes higher than the transition value. As the Reynolds number continues to increase, the flow becomes turbulent at some point (Bear, 1972).



Figure 5. Setup of the Testing Apparatus

Rain Fall – Run Off Relation For Porous Asphalt Testing

This experiment was carried out in Hasanuddin University experimental station Laboratory Structural and Transpiration Hasanuddin University, which specializes in runoff utilization. Three experimental steel cells were used briquet asphalt, with each cell measuring 0,40m x 0,40m. There were drainage boards under the cells, which had p 1.0cm holes. Water outlets were set up under the holes to monitor the quantity of the collection water. Pipes were used to collect both surface runoff and subgrade infiltration. Surface runoff and subgrade drainage from each cell were measured with beakers and flasks at the rear of each cell through drainage pipes for each of the three types of porous pavements and the impervious cell. Precipitation and

runoff rates were recorded every 5 min. An artificial rainfall apparatus was constructed on an adjustable shelf. It consisted of 36 Rainbird 1,800 sprinklers, with an operating pressure of 0.1 MPa, a discharge rate of 0.1m^3 intensity is 0.905 and is controlled by sprayer combination.



Figure 6. Rain fall – run off relation for porous asphalt testing

- Treatment A, porous concrete asphalt (permeable asphalt pavement) with Hot Mix asphalt gradation Model Binamarga.
- Treatment B, porous concrete asphalt (permeable asphalt pavement) with permeable friction course gradation Model British
- Treatment C, porous concrete asphalt (permeable asphalt pavement) with porous asphalt gradation Australia block paving with subbase of 5 cm thick concrete lacking sand and 20cm thick gravel Model Australia

Mix Design Permeable Asphalt Pavement Testing

Mix design permeable asphalt pavement the used composition open graded sistem. Who was Mix Trial Gradation lost of material $\frac{3}{4}$ ", $\frac{1}{2}$ " be stoped filter $\frac{1}{2}$ " and lost of material $\frac{1}{2}$ " be stoped filter $\frac{3}{8}$ " with composition comparative 50-50 to course aggregate. The used fine aggregate lost filter number 4, and stoped filter number 200 all of 10% for mould capacity. Asphalt Blend Pertamina the use variation standard 3%, 3.5%, 4%, 4.5% and 5%. Briquet make in for \varnothing 10

cm and depth ± 6.5cm and Briket make in □40x40 cm, depth ± 6.5cm.

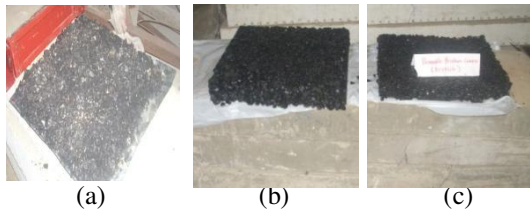


Figure 7. Permeable asphalt pavement

Table 1. Total briket test indirect tensile strength and Cantabro

Item Testing	BNA Blend Pertamina (%)	Planning Briket (unit)
Indirect tensile strength	3	5
	3,5	5
	4	5
	4,5	3
	5	5
Cantabro	3	5
	3,5	5
	4	5
	4,5	5
	5	5
Total briket		50

Before briket test in cantabro, briket was plum to Los Angeles machine drum, speed (V) 30-33 rpm for rotation.

$$L = \frac{Mo - Mi}{Mo} \times 100$$



Figure 8. Test Cantabro Machine

X-Ray Computed Tomography

X-ray computed tomography (CT) is an innovative, nondestructive technique used for obtaining digital information on the three-dimensional internal microstructure of solid materials. Different phases in solid materials may be distinguished using x-ray CT. In recent years, this imaging technique has been utilized to characterize the microstructure of asphalt mixes (Shashidhar 1999; Wang et al. 2001; Masad et al. 2002, 2004). In this study,

the x-ray system in the Advanced Characterization of Infrastructure Materials laboratory at Universitas Negeri of Makassar was used to scan the test specimens. The setup includes two separate x-ray systems; the micro-focus and the mini-focus. The micro-focus system consists of a 225 kV x-ray source and an image intensifier detector, while the mini-focus has a 350 kV x-ray source and a linear detector. Due to the limited power of the micro-focus system, it is more applicable to scan small asphalt mixture specimens with better resolution. The mini-focus is more applicable to scan large asphalt mixture specimens with an adequate resolution of 0.17 mm/pixel. In this study, the mini-focus was used to scan the test specimens. The three example cross-sectional images from each depth (top third, middle third and bottom third) of all the specimens are presented

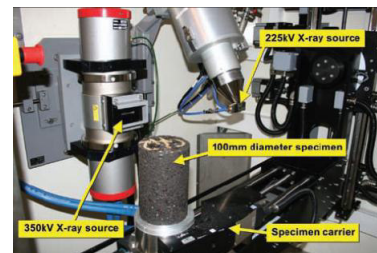


Figure 9. Components of the X-ray CT System at Texas A & M

RESULT AND DISCUSSION

Analysis Rice Hush Ash Gradation

Table 2. Outcome test for water content ductility (filler rice hush ash)

No	Weight of Briket	BNA Blend Pertamina	Time mix ture	Time pres sure	Water add	Water BNA Blend	Water Agre gate	Total water for mix ture	Volume Ductility
	gr	%	dt	jam	%	%	%	%	%
1	1000	9	60	15	1	3,53	0,7	5,23	50
2	1000	9	60	15	2	3,53	0,7	6,23	55
3	1000	9	60	15	3	3,53	0,7	7,23	65
4	1000	9	60	15	4	3,53	0,7	8,23	70
5	1000	9	60	15	5	3,53	0,7	9,23	85

Table 3. Outcome test for water content compaction (filler rice hush ash)

Water content %	Weight Briket			Volume Briket (5-6)-(5-4)/u	Waste Weight (4/7)	Water Content at test %	Dry Weight
	Air	+ Air	+ Water				
gr	gr	gr	cc	gr/cc	%	gr/cc	
4	5	6	7	8	9	10	
3	1069,8	1076,3	543,6	526,3	2,03	3,47	1,833
3	1064,6	1075,7	551,7	513,0	2,08	3,51	1,870
Average					2,054		1,851
4	1064,1	1069,5	569,5	494,7	2,15	3,33	1,942
4	1070,1	1077,0	576,2	494,0	2,17	4,69	1,931
Average					2,159		1,937
5	1071,8	1078,0	582,1	489,8	2,19	3,20	1,978
5	1062,0	1068,2	534,3	527,8	2,01	2,17	1,837
Average					2,100		1,907
6	1062,9	1069,9	559,6	503,4	2,11	4,35	1,888
6	1076,6	1086,0	574,0	502,7	2,14	4,35	1,915
Average					2,127		1,902
7	1065,3	1072,6	565,9	499,5	2,13	3,13	1,929
7	1062,1	1074,2	533,7	528,5	2,01	3,34	1,814
Average					2,071		1,872

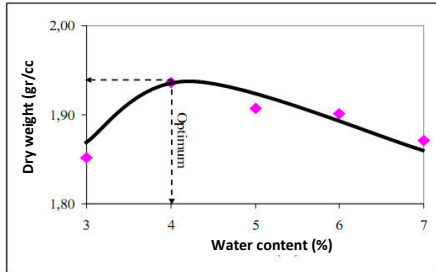


Figure 10. Correlation water content with dry weight (gr/cc) for filler rice hush ash

Table 4. Outcome test hot Mix (filler rice hush ash)

No.	Permeable Asphalt Pavement	BNA Blend Pertamina			
		I	II	III	IV
		8	9	10	11
1.	Water content (%)	3,60	3,12	2,66	2,88
2.	BD (gr/cc)	2,20	2,29	2,20	2,20
3.	Dry weight (gr/cc)	2,11	2,22	2,14	2,13
4.	VMA (%)	12,30	8,45	11,28	11,65
5.	Porous (%)	5,70	3,29	7,85	7,47
6.	Total Porous (%)	9,83	7,02	10,94	10,80
7.	Absorption (%)	2,91	2,20	2,74	3,03
8.	Dry Stability (kg)	887,06	865,25	850,71	807,08
9.	Weigth Stability (kg)	778,00	807,08	770,73	734,37
10.	Mish Stability (%)	87,70	93,28	90,60	90,99
11.	Flow (mm)	5,0	4,3	4,4	4,6

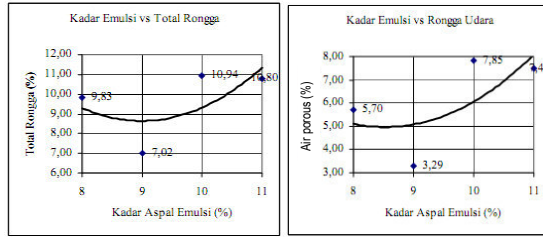
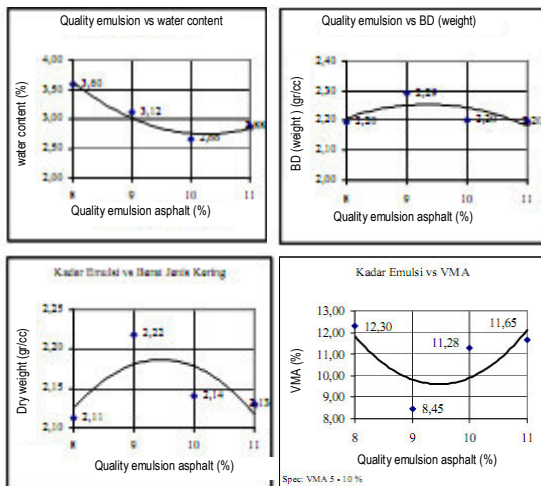


Figure 11. Parameter Uji Cold Mix III

Analysis Indirect Tensile Strength

Tabel 5. Outcome Indirect Tensile Strength test

Sam pel	Percentage asphalt quality (%)	Diameter briket	High Briket	Load Value (P)	ITS Value
		mm	mm	kgf	
		D	H		Mpa
I	3.0	102.3	66.9		0
II		102.22	69.3	75.00	0.066134591
III		102	68.5	100.00	0.089401701
IV		102.4	67.7	75.00	0.067578595
V		102.4	67	125.00	0.113807734
		Average			0.067384524
I	3.5	102.5	67.5	275.00	0.24827991
II		101.8	67.2	225.00	0.205448034
III		102.4	68.4	250.00	0.222956672
IV		102.1	68.8	200.00	0.177849373
V		102	68	275.00	0.247662431
		Average			0.220439284
I	4.0	102.8	69	350.00	0.308221097
II		102.5	68.2	400.00	0.357427756
III		102.2	68.9	350.00	0.310480586
IV		102	69	375.00	0.332826983
V		102.3	67.4	350.00	0.317080137
		Average			0.325207312
I	4.5	102.3	68.4	300.00	0.267809539
II		102.4	68.3	250.00	0.223283109
III		102.5	69.5	275.00	0.241135164
IV		102.6	67.6	325.00	0.292702092
V		102.2	68	275.00	0.247177769
		Average			0.254421535
I	5.0	102.2	61.7	275.00	0.272416342
II		102.5	68	225.00	0.201644445
III		102.4	65	250.00	0.234619021
IV		102.4	66.1	225.00	0.207643158
V		102.2	68.2	300.00	0.268857717
		Average			0.237036137

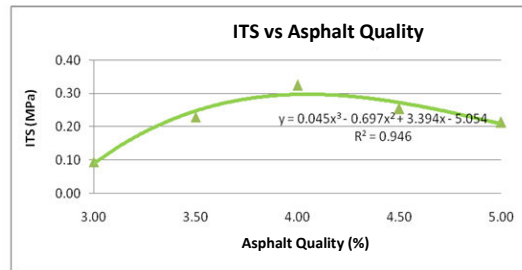


Figure 12. Correlation quality asphalt with Indirect Tensile Strength



Figure 13. Briket after Indirect Tensile Strength Test

Tabel 6. Recapitulation R_{maks} value

No.	Quality asphalt	Maximum Loading (Kgf)	ITS Value (MPa)	R Maks
1	3,00	125	0,1140	0,0180
2	3,50	275	0,2483	0,0234
3	4,00	400	0,3574	0,0283
4	4,50	325	0,2927	0,0253

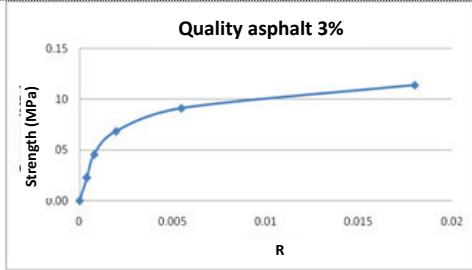


Figure 14. Correlation ITS Value and R value 3%

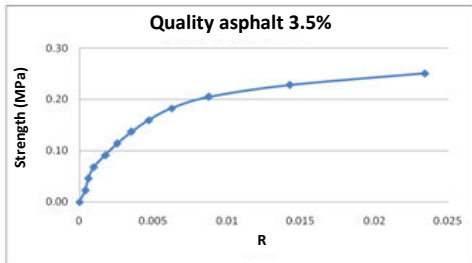


Figure 15. Correlation ITS Value and R value 3.5%

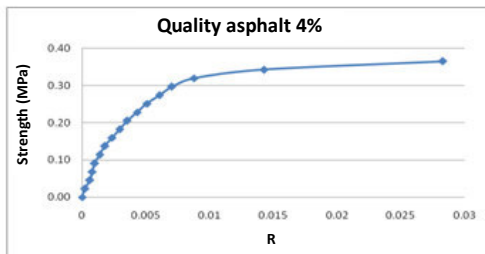


Figure 16. Correlation ITS Value and R value 4%

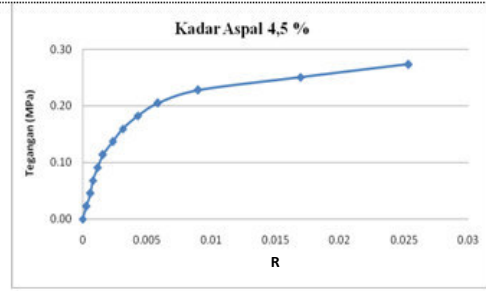


Figure 17. Correlation ITS Value and R value 4.5%

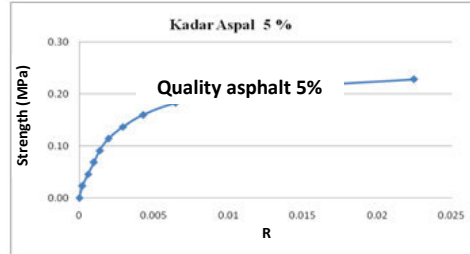


Figure 18. Correlation ITS Value and R value 5%

Analysis Cantabro

Table 7. Outcome Cantabro test

Sam pel	Percen tage quality asphalt (%)	Wei ght before test	Wei ght after test	Loss Weight	Loss Weight
		(Gram)	(Gram)	(Gram)	(%)
		M_o	M_i		L
I	3.0	1081	244	837.00	77.43
		1083	248	835.00	77.10
		1090	281	809.00	74.22
		1091	226	865.00	79.29
		1070	241	829.00	77.48
Average				77.10	
II	3.5	1085	731	354.00	32.63
		1089	760	329.00	30.21
		1071	748	323.00	30.16
		1069	711	358.00	33.49
		1088	705	383.00	35.20
Average				32.34	
I	4.0	1081	913	168.00	15.54
		1082	936	146.00	13.49
		1088	931	157.00	14.43
		1086	944	162.00	13.09
		1090	913	177.00	16.24
Average				14.56	
I	4.5	1084	959	125.00	11.53
		1082	952	130.00	12.01
		1086	940	146.00	13.44
		1088	961	127.00	11.67
		1084	948	136.00	12.55
Average				12.24	
I	5.0	1075	956	119.00	11.07
		1084	968	116.00	10.70
		1090	984	106.00	9.72
		1078	994	84.00	7.79
		1105	1003	102.00	9.23
Average				9.70	

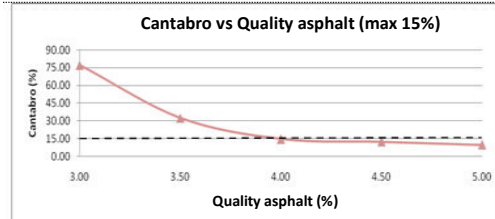


Figure 19. Correlation quality asphalt with value cantabro loss

Analysis Rainfal-Run Off

Effect of different treatments on runoff Surface runoff and infiltration rates were measured at the experimental station in April 2013 and September 2013. The experiment was carried out during the summer months, with most precipitation occurring in that season in South Celebes of Indonesia. A comparison of precipitation rates and surface runoff for the three permeable asphalt pavement and the impervious surface cell during a storm beginning at 16:30 on 26 April 2013. There was no measurable continuation of runoff on the Treatment A, B, C surface during the rainfall process. Runoff on the impervious surface cell closely followed precipitation rates during all rainfall events. Minor surface runoff from the porous surface cells beginning around 50 min into the event was attributed to leaks in the different sub-bases used to capture water, which would infiltrate into the subgrade soil. The phenomenon can be attributed to the storage capacity of the permeable asphalt pavement, which delays evacuation of water into the runoff outlet. This delaying effect also renders the evacuation a more gradual process, as reflected by both the reduction in maximum runoff rates measured at the runoff outflow and by the increase in time required for discharge. For the rainfall amount/duration of 118.72mm/2h, the runoff outflow percentages of Treatments A, B and C ranged from 22.4 to 68.5, but for Treatment D it was 92.9–97.8. For the rainfall amount/ duration of 94.09mm/2h, the incidence of runoff for Treatment B was 71 min later than the impervious pavement, with a recorded flow volume of just 23.00 mm. For the rainfall amount/duration of 59.36 mm/h, the incidence of runoff for the porous pavement surface was 45 min later than the impervious pavement, with the flood

peaks reduced by 35–100%, especially for Treatment B, with a recorded flow of zero.

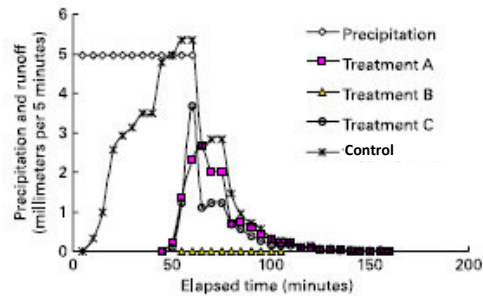


Figure 20. Comparison of precipitation rate, surface runoff of three porous pavement cells and the impervious surface cell.

The order of infiltration coefficients for the different media investigated in this study is KCLS. KPCBP. KS . KIS, where KCLS, KPCBP, KS and KIS are the infiltration coefficients for the materials of concrete lacking sand, permeable asphalt pavement, subgrade and impervious surface, respectively (see Picture 8). The factors discussed above explain the quantitative relationships of flood peak for the four surfaces FD . FC . FA . FB, where FD, FC, FA and FB signify the volume flood peaks for Treatments D, C, A and B, respectively.

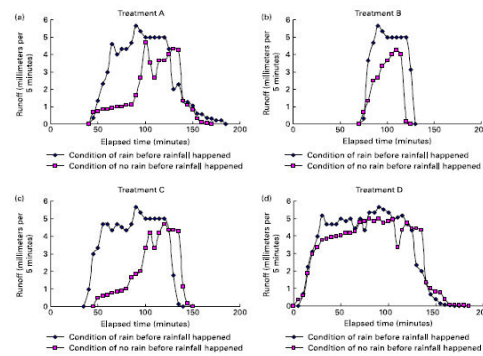


Figure 21. Runoffs of different treatments under conditions with rainfall and without rainfall before artificial rainfall happened

Table 8. Permeability test run-off

Item Test	Permeable Asphalt Pavement					
	Model Binamarga		Model Australia		Model British	
	V	t	V	t	V	t
Vertikal	4.58	5	5.00	5	5.08	5
	4.80	10	4.59	10	4.98	10
	4.60	15	4.75	15	4.93	15
	5.00	20	5.15	20	5.22	20
	4.70	25	4.95	25	5.05	25
	4.85	30	5.00	30	5.10	30
Horizontal	4.69	5	4.75	5	4.81	5
	4.90	10	4.95	10	5.00	10
	4.53	15	4.55	15	4.60	15
	4.65	20	4.70	20	4.82	20
	4.35	25	4.65	25	4.83	25
	4.36	30	4.45	30	4.56	30

	4.76	35	4.82	35	4.89	35
	4.49	40	4.53	40	4.60	40
Vertikal and Horizontal	4.51	5	4.61	5	4.64	5
	4.01	10	4.28	10	4.33	10
	3.98	15	4.08	15	4.10	15
	4.48	20	4.52	20	4.65	20
	3.85	25	3.95	25	4.05	25
	4.48	30	4.55	30	4.61	30
	4.15	35	4.26	35	4.50	35
	4.42	40	4.49	40	4.58	40
	4.18	45	4.27	45	4.38	45

Remarks : t = time (minute), V = speed flow (mL/s)

Analysis X-Ray - Tomography

The results of research indicates that porous asphalt mixture showed an influence on the value of the characteristics of porous asphalt particularly at concrete grading 50% retained 1/2" and 50% natural crushed stone retained 3/8" where the values obtained from the analysis of optimum binder content is 9.5%. Based on the Scanning Electron Microscope (SEM) can be seen the microstructure and content of chemical elements present in the porous asphalt which prove that all elements of the BNA Blend Pertamina and concrete waste can blend and bind well.

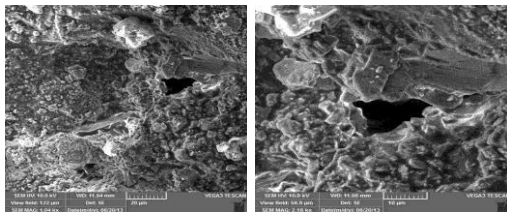


Figure 22. Photo X-Ray Permeable Asphalt Pavement (British Standard)

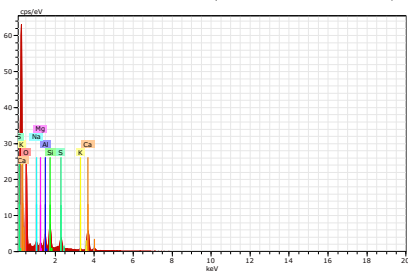


Figure 23. Tescan vega3SB

Spectrum: test

Element	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Compound norm. [wt.%]	Comp. C Error (3 Sigma) [wt.%]
Oxygen	41.56	42.98	58.53	0.00	15.03
Silicon	10.48	10.83	8.41	SiO2	1.46
Aluminium	8.06	8.33	6.73	Al2O3	1.29
Sodium	7.35	7.60	7.21	Na2O	1.59
Magnesium	4.74	4.90	4.39	MgO	0.92
Potassium	1.50	1.55	0.86	K2O	0.26
Calcium	16.39	16.95	9.21	CaO	1.58
Sulfur	6.64	6.86	4.66	SO3	0.84

Total: 96.71 100.00 100.00

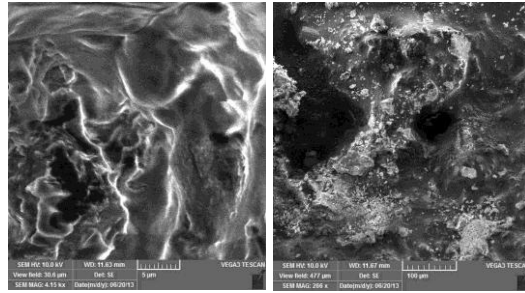


Figure 24. Photo X-Ray Permeable Asphalt Pavement (Australia Standard)

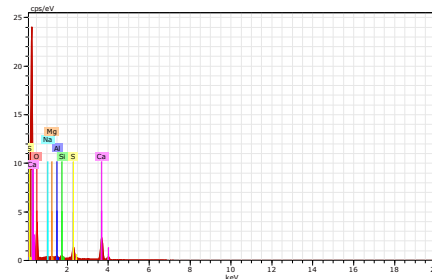


Figure 25. Tescan vega3SB

Spectrum: test

Element	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Compound norm. [wt.%]	Comp. C Error (3 Sigma) [wt.%]
Oxygen	20.01	40.36	58.46	0.00	9.11
Silicon	1.56	3.15	2.60	SiO2	0.36
Aluminium	1.68	3.39	2.91	Al2O3	0.42
Sodium	2.74	5.52	5.57	Na2O	0.79
Calcium	16.39	33.05	19.11	CaO	1.68
Sulfur	5.37	10.83	7.83	SO3	0.76
Magnesium	1.83	3.69	3.52	MgO	0.50

Total: 49.57 100.00 100.00

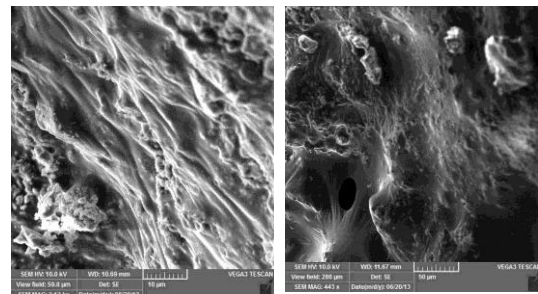
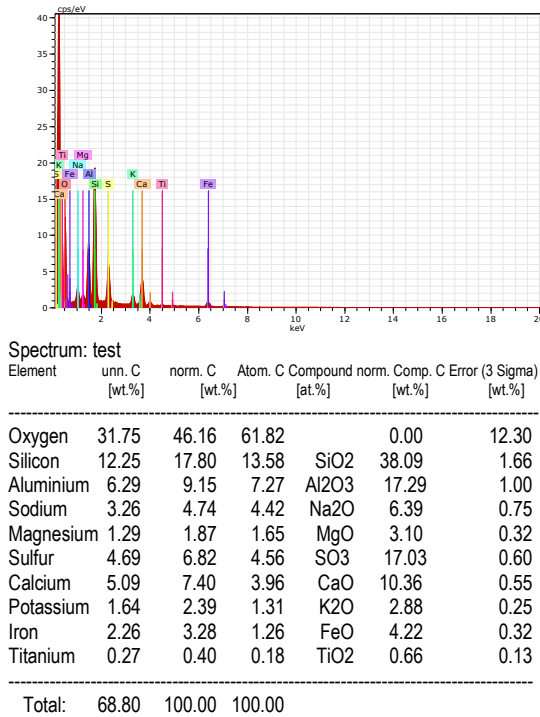


Figure 26. Photo X-Ray Permeable Asphalt Pavement (Binamarga Model)



CONCLUSIONS

1. Water content compaction for filler rice hush ash 3%, volume bricket 513,0, weight 2,054, water content test is 3,51, dry weight 1,833 gr/cc. Water content for correlation asphalt BNA bend pertamina: quality asphalt BNA 9%, water content agregate 0.7%, Bricket water content 5,23.
2. Indirect tensile strength 0,1140 MPa for total load 125 Kgf, for the quality asphalt 3% R_{maks} 0,0180. Indirect tensile strength 0,2483 MPa for total load 275 Kgf, for the quality asphalt 3.5% R_{maks} 0,0234. Indirect tensile strength 0,3574 MPa for total load 400 Kgf, for the quality asphalt 4% R_{maks} 0,0283. Indirect tensile strength 0,2927 MPa for total load 325 Kgf, for the quality asphalt 4.5% R_{maks} 0,0253. Indirect tensile strength 0,2346 MPa for total load 250 Kgf, for the quality asphalt 5% R_{maks} 0,0225.
3. Permeable asphalt pavement mixture for Cantabro test we can see that optimum BNA Blend Pertamina for the coarse agregate domato stone it was

bigger porous when quality asphalt 3%. Loss weight Cantabro 77.10% correlation with quality asphalt 3%, loss weight Cantabro 32,34% correlation with quality asphalt 3.5%, loss weight Cantabro 14,56% correlation with quality asphalt 4%, Loss weight Cantabro 12,24% correlation with quality asphalt 4.5% and loss weight Cantabro 9,70% correlation with quality asphalt 5%.

4. This study evaluated the performance of three porous pavement systems from the perspective of infiltration and runoff, with very positive performance in comparison to a traditional impervious surface. All three porous pavement surfaces increased infiltration and decreased runoff. Larger porosity values, higher infiltration coefficients, thicker sub-base layers and lower initial water contents of the subgrade produce higher infiltration rates and smaller runoff coefficients. When rainfall infiltrates into a porous surface and its underlying sub-base, the outflow hydrograph will be influenced by the way in which the construction materials retain or delay flow.

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ELECTRICAL PROPERTIES OF CaCO₃ FILLED CHITOSAN-PVA MEMBRANES

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ABSTRACT

Membrane technology is used in various applications, one of them is for fuel cell. The electrical properties of CaCO₃ filled chitosan-PVA membranes (conductivity, permittivity and impedance) have been analyzed. The membrane used in this study were synthesized via chitosan and PVA which is filled with various CaCO₃ concentration : 0, 0.1 0.2 0.3 and 0.4 gr. The purpose of CaCO₃ filling is for increasing capacity of membrane in releasing proton. CaCO₃ has ionic bonding with negative charged compounds. From experiment result, it is obtained impedance (Z) and permittivity (ϵ) values from which showed dielectrical material inside membrane. It also has two kind polarization orientations that are space charge and dipolar. Proton conductivity was increased around 1.32×10^{-4} S/cm⁻¹ when 0.4 g CaCO₃ concentration was added

Keywords

Alternative energy, membrane, fuel cell, CaCO₃, Chitosan, Polyvinyl alcohol

INTRODUCTION

Fuel Cell has discovered more than 150 years ago by Schoenbein and William Robert Grove (1839). William was a trial judge, inventor and physicist, was born on 11 July 1811, in Swansea, South Wales and died in London on August 1896 (Muliawati, 2008). The membrane is a thin layer that serves as a solid electrolyte and an anode and a cathode separator selectively control the transport of protons from the anode to the cathode in a fuel cell. PEMFC containing a platinum catalyst to produce energy, PEMFC requires only hydrogen and oxygen from the air, water to operate. In addition, the fuel cell does not use corrosive fluid (Jamal *et al.*, 2007).

Development of technology membrane in kind of applications this time is various, especially on the development in the field of energy. This study was conducted as an alternating of the existing membrane is Nafion[®] due to the current price of Nafion[®] in the market is still very expensive, so a constraint if developed in Indonesia. Therefore, many new materials are developed and are expected to replace the function of Nafion[®].

PEM Fuel Cell can directly convert chemical energy into electrical energy by electrochemical process. The functions of membrane as an electrolyte and a separator between the two happened transport gas reactants and hydrogen ions from the anode to the cathode of an electrochemical events are then generated electrical energy.

Chitosan is a linear polymer with a high molecular weight of 2-deoxy-2-amino glucose, has the chemical name of (1-4)-2-amino-deoxy- β -D-glucose (Rha, 1984). And is one of the chitin derivative obtained by deacetylation or omission COCH₃ group (Purwatiningsih, 1993). Also includes a natural polysaccharide comprising copolymers of glucosamine and N-acetylglucosamine, which is derived from industrially processed exoskeleton of crustaceans, and is a natural polymer materials

second most abundant after cellulose (Illum, 1998).

Chitosan is non-toxic, readily biodegradable and cationic polyelectrolyte because it has a functional group that is causing an amino group positively charged chitosan and other polysaccharides contrast to (Ornum, 1992), in addition to the amino group, there are also primary and secondary hydroxyl groups. The presence of functional groups resulted in chitosan has a high chemical reactivity. Functional groups present in chitosan allow for chemical modifications including a diverse reactions with crosslinking agent for, the excess can be used as ingredients allow bioplastics, ie plastics that can be degraded and does not pollute the environment (Putu, 2007).

Polyvinyl alcohol is a substance that is not tasteless, odorless, can be broken down by natural and biocompatible. It can be dissolved in water, polyvinyl alcohol also soluble in ethanol. However, these substances do not dissolve in organic solvents.

Electrolyte membranes with a mixture of PVA exhibit mechanical properties and thermal, the polymer membrane has good strength and easily modified, polymer electrolyte membrane is suitable for alkaline batteries and other electrochemical systems. as well as the type of alkaline solid polymer electrolyte membranes modified by using PVA was able to produce a high ion conductivity (Dewi, 2011).

Calcium carbonate (CaCO₃) is the major mineral-forming limestone, with its constituent chemical elements consisting of calcium (Ca) and carbonate (CO₃) and has a hexagonal crystal system rhombohedral cleavage. The properties of calcium carbonate is having a density or bulk density is 2.71; hardness scale 3 (Mohs scale), fine-grained to coarse; may form a stalactite; module tubleros; koraloidal; pisolitik and filler particles having a grain size of about 1.5-40. Calcium carbonate can be formed through chemical reactions, and its constituent elements is none other than

calcium (Ca), carbon (C) and oxygen (O). structure of the hexagonal-shaped calcium carbonate crystals (Junaidi, 2010).

Physical properties of CaCO₃

1. Molecular weight :100.09 g/mol
2. Density :2.8 gr/cm³
3. Melting point :825°C
4. Shaped crystals or powder.
5. Colorless or white.
6. Odorless and tasteless.

This study aims to determine the electrical properties of the membrane fuel cell made of chitosan-PVA with the addition of vary CaCO₃ concentration. Measurement of electrical properties includes proton conductivity, impedance and permittivity. The research was conducted in February 2013, in laboratory of Chemical Physics and Material Physics UIN Maliki Malang. Analyse use instrument was conducted in the laboratory Materials Physics and Instrumentation of Brawijaya University.

MATERIALS AND METHODS

The research was conducted by extracting chitosan from crab shells. Extraction chitosan of crab shell includes three stages. Deproteinization to eliminate the protein content, demineralization to eliminate the mineral content in the shell then obtained chitin. Deacetylation is to remove the acetyl group (-COCH₃) (Aryanto, 2002). 7 g of chitosan dissolved in 2% acetic acid 1:10 w/v, the solution was stirred for 6 hours. Polyvinyl alcohol dissolved in hot water with a ratio of 1:10 w/v and stirred until homogeneous. Chitosan-acetic acid added into the solution of PVA 1:4 wt% and stirred until homogeneous. 2.3 g of chitosan-PVA dissolved in 40 ml of 10% acetic acid solution. Added CaCO₃ with ratio 0, 0.1, 0.2, 0.3 and 0.4 g of chitosan-PVA. The suspension was heated at 70°C and stirred with a speed for 600 rpm. Mixture is poured into molds with a diameter of 10 cm and stored

at room temperature for 24 hours, then dried at a temperature of 60°C for 2 days. Formation of crosslinking is performed by immersing membrane in 0.8 ml Glutaraldehyde (C₅H₈O₂) solution 3%, were mixed with 30 ml of 6% H₂SO₄ for 2 days. Finally, the membrane was washed using distilled water and dried at room temperature for 24 hours (Rermux et al., 2010).

Data obtained from LCR Meter 816 made in America shows the electrical properties of the membranes covering the resistance (R) and capacitance (C), then determined the value of proton conductivity, permittivity and impedance data obtained by inserting equation.

RESULTS

A. Characterization of Electrical Properties of Membranes

Tests performed using the electrical properties of LCR Meter 816. The applied voltage is 1 volt.

1. Permittivity

Measurements permittivity real and imaginary using the equation (Hastuti, 2004)

$$\text{Permittivity real: } \epsilon_r' = \frac{\epsilon_r''}{\sqrt{1+D^2}} \dots\dots\dots (1)$$

$$\text{Permittivity img: } \epsilon_r'' = \epsilon_r' \cdot D \dots\dots\dots (2)$$

Where

$$D \text{ is the dissipation factor: } D = \frac{1}{2\pi fCR} \dots (3)$$

From the data obtained later graphed the relationship between permittivity with frequency.

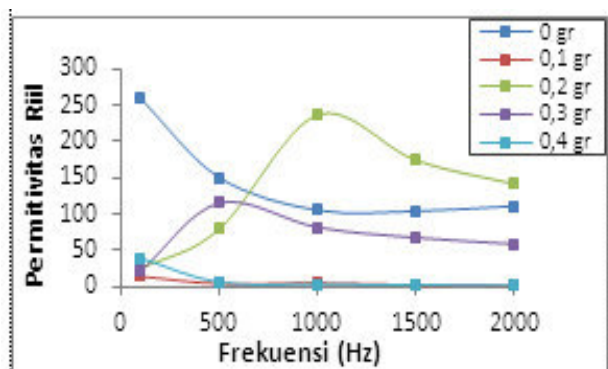


Figure 1. Relationship with the frequency of the variation of the real permittivity addition CaCCO₃

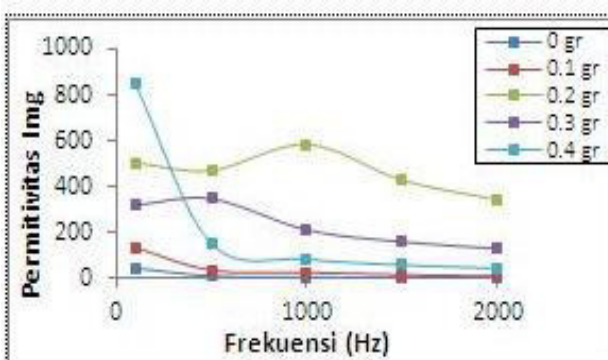


Figure 2. Relationship with the frequency of the variation of the imaginary permittivity addition CaCCO₃

2. Impedance

Impedance measurements using the equation:

Impedance riil

$$Z_{re} = R / \sqrt{1 + (2\pi fCR)^2} \dots\dots\dots (4)$$

Impedance imaginer

$$Z_{img} = 2\pi fCR^2 / \sqrt{1 + (2\pi fCR)^2} \dots\dots\dots (5)$$

By entering the data obtained into the equation then obtained graphs 3 and 4.

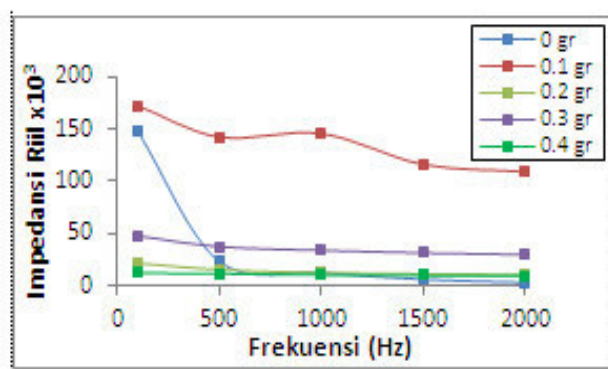


Figure 3. Relationship between frequency and impedance real of the variation concentration of CaCO₃

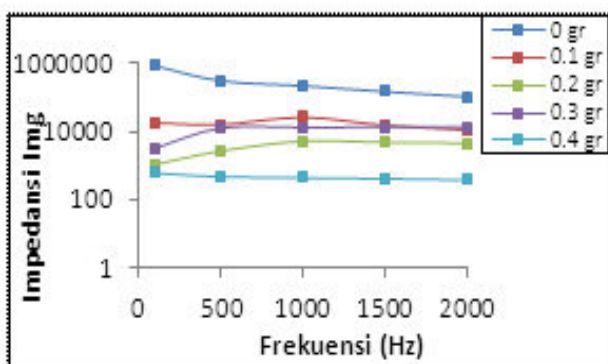


Figure 4. Relationship between frequency and impedance imaginary of the variation of concentration of CaCO₃

3. Proton conductivity

Proton conductivity measurements using equation (Liong, 2009):

$$\sigma = \frac{L}{AR} \dots\dots\dots (6)$$

Where σ = conductivity
 L = thickness of the membrane
 A = area of membrane
 R = resistance

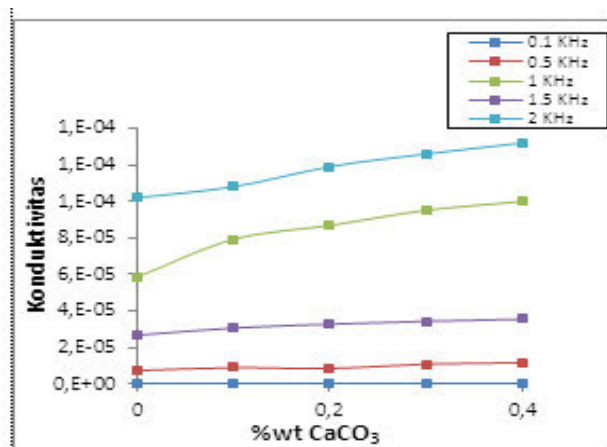


Figure 5. Relationship with the proton conductivity of CaCO₃ concentration on the frequency variation

DISCUSSION

The formation of peaks on the **Fig. 1** and **2** shows the polarization of the membrane. Polarization of the space charge polarization (Space Charge) that occurred at a frequency of 100 and 500 Hz and dipolar polarization that occurs at a frequency of 1000 Hz. Permittivity is strongly influenced by the amount of charge contained therein. The more the charge contained in the material, the higher the chances of polarization. The increase in capacitance caused by the weakening of the electric field between two capacitor plates.

Fig. 3 and **4** shows relation frequency with impedance. Impedance are influenced by the resistance, reactance and frequency. The average of value obtained occur reduction with increasing frequency. This suggests that at low frequencies the value of capacitive reactance (X_c) is greater than the inductive reactance (X_L). That is causing of increase impedance and maximum current is decrease. Testing of samples by using a variation of the impedance values obtained frequency is inversely to frequency, so that the resistivity of the material

properties of the small and large payload capabilities store. This means that the material has good conductivity.

Proton conductivity is intimately parameter of membrane and show by **Fig. 5** This is parameter also affected by the strength of the acid, the chemical structure, morphology of the membrane and temperature. The events of transfer proton caused leap the hydroxide ion from group of amine protonation to another group. This event is known as Grotthuss (jump mechanism) (Wilkinson *et al.*, 2010). From the data obtained by the addition of CaCO₃ variations affect the value of proton conductivity, which indicates that the addition of CaCO₃ concentration resulted in the conductive material of the proton. Therefore CaCO₃ has a high molecular weight and bringing the particles dispersed. Calcium carbonate particles as anions in the membrane and interacts with the proton.

Effect glutaraldehyde compound of membrane was made the formation of crosslinking to the membrane until proton has opportunity to jump greater. Crosslinking formed in the presence of glutaraldehyde is atom C in a mixed chitosan-PVA bonded with O atoms in the compound glutaraldehyde.

CONCLUSION

The characterization of the electrical properties of the membrane fuel cell the smaller the impedance values obtained with increasing frequency. Contained the smallest impedance value at frequency 2 KHz with a concentration of 0.4 g CaCO₃. Permittivity of the membrane showed a polarization space charge and dipolar polarization since the peak formed at a frequency of 100 Hz to 1000 Hz. Thus obtained membrane including a dielectric material. Proton conductivity greater with the addition of various concentration of CaCO₃. The maximum score is $1.32 \times 10^{-4} \text{ S/cm}^{-1}$ on the membrane with a concentration of 0.4 g CaCO₃.

ACKNOWLEDGMENT

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Natural Dyestuff Production

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ABSTRACT

Textile especially Batik manufacturer wishes not yet due to the availability of natural dyes are still rare, if it still takes time for long to get it because the distance between the dye and batik producers that far apart / out of town / outside of the island, as well as the use is not ready (instant) , still require considerable time. Manufacture of dyes performed using 5 (five) materials that are widely used by manufacturers of batik namely: Jolawe, Jambal, Tingi, Tegeran and indigo to dye results in the form of liquid, powder and paste. The Method are :

1. Cut material, extracted, concentrated to 50%, filtered and then the filtrate was taken (formed liquid dye)
2. filtrate + 30% dextrin was heated with tray dryer at 50 degrees for 4 hours (dye formed gel / paste)
3. Filtrate + 30% dextrin was heated in an oven at 100 degrees for 5 hours (formed dye powder). Number of substances produced in the colors of each material is measured. Quality dyes in fastness tests. From the results of experiments conducted in this study it can be concluded that the samples in the form of liquid , powder and pastes generally produce a positive value means that the dominant red and positive b values appear yellow. Wavelength of the test results obtained by color as follows: Jambal is blue, Tegeran is violet, jolawe is yellow, Tingi is blue and indigo is blue of the test results with the test color fastness to dry rubbing fastness 5 shows the material is as high as 4-5. Natural dye products will be sold to satisfy the demand of textile producers around , The East Java, Indonesian and the world

Key word : Natural, Dyestuff, Production

INTRODUCTION

Textile especially Batik companies in Indonesia are looking forward to the availability of natural dyes to get the color of soft colors and a variety of colors with high color fastness, low prices are also easily obtained. (Lemmens. et. at. 2010)

Textile especially Batik manufacturer wishes not yet due to the availability of natural dyes are still rare, if it still takes time for long to get it because the distance between the dye and batik producers that far apart / out of town / outside of the island, as well as the use is not ready (instant) , still require considerable time.

Problem Formulation

How to make natural dyes for textiles and batik in accordance with the needs of consumers

Literature Review

There are 2 groups of the dyes used in the Batik industry and textile are natural and synthetic dyes, (Manuntun Manurung ,2012). Most natural dyes derived from plants growing among others of bark, logs, roots, leaves and seeds while the synthetic dye derived from the double bond that many polymers derived from benzene (Hasanudin, dkk. 2001)

The emergence process color :

Colors can be seen when a substance absorbs visible light at a wavelength of 400-750 nm and accepted by the retina of the eye. Color seen by the retina is not a color that is absorbed but reflected the complementary color. The visible light spectrum and complementary colors can be seen in Table 1.

An organic compound with a conjugated double bond system can absorb certain

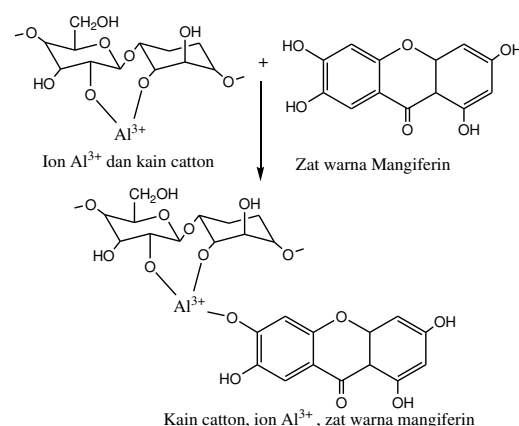
wavelengths of color due to the electron transition of $\pi \pi^*$ and π^* or n.

Table 1. The visible light spectrum and complementary colors

Wavelength (nm)	Absorbed Color	Complementary Color
400-435	Yellow	Violet-green
435-480	Blue	Yellow
480-490	Green-Blue	Orange
490-500	Blue-green	Red
500-560	Green	Purple
560-580	Yellow-green	green Violet
580-595	Yellow	Blue
595-610	Orange	Green-Blue
610-750	Red	Blue Green

Ties dye and fabric :

1. hydrogen bonds
2. ties elektrovalen
3. ties vanderwals
4. covalent bond



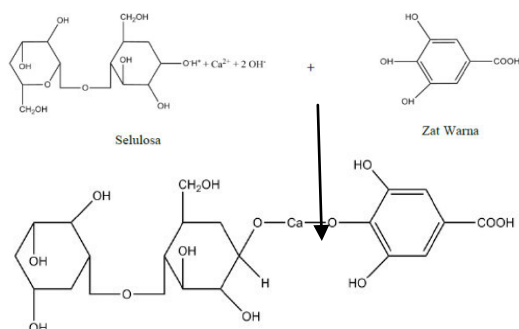


Figure 1. The Complect Of Mordant Reaction In Fiber (Ester K.S dan Adi K , 2008)

Research Objectives

1. Making natural dyes in the form of liquid, powder and paste
2. Contribute their knowledge in natural pembuatan zat warna liquid , powder and paste
3. Increase company profit
4. Open up new business opportunities

Benefit Research

1. Improve the quality of batik in particular in terms of colorin
2. Provide efficient and effective results
3. Provide an opportunity to export natural dyestuff

Implementation Of The Concept

Materials steeper bark TINGI, wood TEGERAN, JAMBAL Bark, bark and leaves INDIGO and JOLawe seeds, cuted, extracted subsequently become LIQUID, GEL / PASTE and POWDER, and then conducted color lightness test and direction of color with spectro fotometers, dyeing and then color fastness test with gray scale, staining scale and croct meters, ([http://.Suaramerdeka.com/harian,htm](http://Suaramerdeka.com/harian,htm), diakses, 22 maret 2007

Natural dye manufacture:

1. Cut material, extracted, concentrated to 50%, filtered and then the filtrate was taken (formed liquid dye)
2. filtrate + 30% dextrin was heated with tray dryer at 50 degrees for 4 hours (dye formed gel / paste)
3. Filtrate + 30% dextrin was heated in an oven at 100 degrees for 5 hours (formed dye powder)

Dyeing (coloring)

(<http://.www.Indonesia.com/bpost.htm>, diakses, 15 maret 2007) :

1. Preparation fabric:
 - with mordant:
 - For cotton fabrics:
 - soak the material with alum and soda ash
 - For silk fabrics:
 - soak the material with alum alone
 - 2. Fixaxi process (lock color), with an assortment of fixer, among others, lotus (FeSO₄), lime (CaCO₃), alum (K₂SO₄.Al₂ (SO₄) 3.24H₂O), lime, etc.
 - 3. The process of dyeing with Natural dye
 - Once the material has ready mordant and fixed solution then the dyeing of textile materials can be done in the following way :
 - a. Prepare a solution of natural dye extraction process results in spot dyeing.
 - b. Enter the textile material which has mordanted to a solution of natural dyes and dyeing process for 15-30 minutes.
 - c. Put the ingredients into the fixer solution , can be selected one among lotus, alum or lime. Material processed in the fixer solution for 10 minutes.

RESULT

From the results of experiments conducted in this study it can be concluded that the samples in the form of liquid , powder and pastes generally produce a positive value means that the dominant red and positive b values appear yellow.

Wavelength of the test results obtained by color as follows: Jambal is blue, Tegeran is violet, jolawe is yellow, Tingi is blue and indigo is blue of the test results with the test color fastness to dry rubbing fastness 5 shows the material is as high as 4-5. Natural dye products will be sold to satisfy the demand of textile producers around , The East Java, Indonesian and the world

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IDENTIFICATION OF SELECTION CRITERIA FOR GREEN AUTOMOTIVE COMPONENT INDUSTRY

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ABSTRACT

Indonesia's automotive component industry has the potential for growth that is in harmony with the automotive industry in Indonesia. In addition to the potential positive impact for the state and society, also a negative impact on environmental issues, especially environmental pollution caused by industrial waste and utilization of natural resources is not efficient. Green industry today has become a requirement of doing business. It is therefore very important for the automotive component industry to develop green industry to market expansion as well as its survival. In order to support the readiness component industry towards green industry criteria need to be formulated. The purpose of this study is to identify what criteria are considered for selection of green automotive component industry. The Fuzzy Delphi Method was used in this study. Initial criteria identification criteria are considered for selection of green automotive component came from integrating assessment model of green industry awards with performance ratings company valuation models in environmental managements. Results showed there are 22 sub criteria that grouped by 11 criteria and three aspects (factors).

Keywords

Automotive component industry, fuzzy Delphi method, green industry,

INTRODUCTION

The purpose of the National Long-Term Industrial Development in Indonesia is to build the industry with the concept of sustainable development, which is based on three inseparable aspects namely economic development, social development and the environment (Perpres. 28 of 2008). Sustainable development is basically an aspect of human development in inclusive, connected, equitable, prudence, and secure (Gladwin *et al*, 1995). To become sustainable, we must therefore decouple our economic well-being from environmentally

negative impacts and resource consumption (Greenovate Europe,2012).

The concept of sustainable development can be achieved through the role of the various parties, one of which is industrial. Indonesia is one of the countries in Asia are experiencing the growth of the automotive industry significantly. The automotive industry accounted for 4.937% of the gross domestic product which is the biggest contributor to the category manufacturing industry reached 23.84% (Badan Pusat Statistik, 2012). Therefore, the automotive industry is one of the three

industries which can encourage the growth rate of the national industry and economy of Indonesia.

Indonesia's automotive component industry has the potential for growth that is in harmony with the automotive industry in Indonesia. In Indonesia there are 200 companies engaged in the automotive components industry, which is 55% of which is a joint venture (joint venture) with a high level of technological dependence (Media Data (2010) in Dewayana *et al.* (2011)).

Although component automotive industry has brought different beneficiaries to human life, it is being pointed out as one of the major cause of global air pollution which resulted in climate change, smog, green house gases (GHGs), and human diseases by many reasons (Shatouri, 2012). In addition to the potential positive impact for the state and society, also a negative impact on environmental issues, especially environmental pollution caused by industrial waste and utilization of natural resources is not efficient.

With increasing global warming, green is becoming important strategy in the manufacturing sector, so sustainability in green manufacturing is a competitive need (Srinivasan, 2011). Green industry today has become a requirement of doing business. It is therefore very important for the automotive component industry to develop green industry to market expansion as well as its survival.

Green industry is an industry that prioritizes efforts in the production process efficiency and effectiveness of resource use in a sustainable manner so as to harmonize industrial development with preservation of the environment and can benefit society member (Kementerian Perindustrian, 2012). Application of Green industry (Kementerian

Perindustrian, 2013) through the concept of cleaner production (cleaner production).

Kristanto (2013) states that the waste is a logical consequence of every process that occurs in an industry (the factory). Furthermore, Kristanto (2013) also stated that the conventional strategy in waste management approach that is based on a form of waste management (end-of-pipe treatment) were considered less effective. Therefore we need an integrated approach as a preventive environmental management strategy, integrated, and applied continuously at all stages of the production process. The approach is known by the term Cleaner production.

Cleaner production aimed at preventing and minimizing the formation of waste or environmental pollutants as well as making efforts to improve the efficient use of raw materials, auxiliary materials, and energy throughout the stages of the production process. Cleaner production through the application of the 4Rs, ie Reduce (waste reduction at the source), Reuse (reuse of waste), and Recycle (recycling of waste), and Recovery (separation of a material or energy from a waste).

To encourage the growth of Green Industry, Indonesia Ministry of Industry presented awards to national industrial companies that have implemented the pattern of resource saving and use of raw materials and energy that are environmentally friendly and renewable. Green Industry Award (PIH) has been going on for four years. Assessment of green industry awards are based on the following

1. Production process, including raw materials and auxiliary materials, energy, water, technology, process, product, human resources, and work environment;
2. Company's management, including production efficiency program, Community

Development / Corporate Social Responsibility, awards ever received, and system management;

3. Industrial Environmental Management, include compliance with environmental quality standards, waste management facilities and emissions, and environmental management performance.

. Ranking Criteria used refers to the Minister of Environment (2010) Concerning Performance Rating Program in Environmental Management, namely: green ratings for businesses and or activities that have been done over environmental management required by regulation (beyond compliance) through the implementation of environmental management systems, efficient resource use through the efforts of the 4Rs (Reduce, Reuse, Recycle and Recovery), and the efforts of social responsibility (CSR / ComDev) well. According to the Ministry of Environment (2012) PROPER Assessment criteria to differentiate into two, namely:

1. Adherence to the criteria used for ranking the blue, red, and black. Compliance criteria is basically enterprise valuation observance of environmental regulations. Regulations are used as the basis of assessment is the rule: Implementation Document Environmental Management, Air Pollution Control, Air Pollution Control, Waste Management B3, Marine Pollution Control, Environmental Damage Criteria;

2. More aspects of the assessment criteria required (beyond compliance) for ranking the green and gold. Aspects assessed are: environmental management systems, energy efficiency, emission reduction, and the use of B3 waste reduction, application of R 3 non B3 solid waste, water conservation and water pollution load reduction, protection of biodiversity, the implementation of community empowerment.

The automotive industry is considering various ways to reduce operational costs while also mitigating its carbon, water, and energy footprint. The industry is already doing quite a bit of innovation to "green" the vehicle itself, which may entail the use of alternative propulsion technology to lower emissions or lightweight composites to improve fuel economy. However, Original Equipment Markets and suppliers are beginning to realize that being environmentally conscious also means being financially savvy, which has spurred a shift towards leaner, greener manufacturing (www.pwc.com/cleantech, 2013).

The raw material used by the automotive component industry in the form of a mixture of steel and steel (with different compositions), aluminum, silver, copper, materials for molds, rubber processing and rubber, foam, and paper for the manufacture of filters. According SENADA (2007) diversity in the quality of materials is mainly related to the allotment to original equipment manufacturers (OEM) or sold as a product without a brand .

Focus on general automotive component manufacturer that produces one type of component in accordance with its technical capabilities (Media Data, 2010 in Dewayana *et al* .,2011). There are two categories based manufacturer of automotive components production base (Media Data, 2010 in Dewayana *et al*, 2011) , namely the manufacturer with a production -based process (have the technology and machinery to carry out the production process in producing the product), and manufacturer with production based on the product (have the technology and machines to make a product) .

In order to support the readiness component industry towards green industry criteria need to be formulated. The purpose

of this study is to identify what criteria are considered for selection of green automotive component industry.

METHODS

Fuzzy delphi method (FDM) was used to establish criteria for evaluating green automotive component industry. Fuzzy theory can solve the fuzziness of common understanding of experts (Hsu *et. al*, 2013; Ross, 2004). The FDM that used in this research consists of five main steps :

1. Extracting initial criteria for evaluating green automotive component industry based on Green Industry Award (Ministry of Industry, 2012), The Company’s Environmental Rating Program (Ministry of Environment), criteria for green suppliers proposed bu Lin and Juang (2008) and criteria for supporting Low Cost and Green Car (LCGC) government program. Totally there are 39 factors or criteria.
2. Assessment of the correlation of each initial criteria with the concept of green industry and supporting LCGC program using linguistic variables in questionnaires and its fuzzy number using triangular fuzzy number (**Table 1**).

Table 1. Linguistic variable and triangular fuzzy number (TFN)

Linguistic variable for relationship	TFN		
	a	b	c
No	0	0	2.5
Weak	0	2.5	5
Medium	2.5	5	7.5
Strong	5	7.5	10
Very strong	7.5	10	10

3. Calculating score for two experts using the formula from Hsu et al (2013). For every sub criteria for two experts, we compute fuzzy weigthing $w_j = (a_j, b_j, c_j)$ where

$$a_j = \text{Min} \{a_{ij}\}, b_j = \frac{1}{n} \sum_{i=1}^n b_{ij}, c_j = \text{max}\{c_{ij}\}$$

$$i = 1, 2, \dots, n \text{ (n experts),}$$

$$j = 1, 2, \dots, m \text{ (m sub criteria)}$$

4. Defuzzification using center of gravity method.

$$S_j = \frac{a_j + b_j + c_j}{3}, j = 1, 2, \dots, m$$

5. Screening. Sub criteria must has defuzzification value greater than 6 based on the common concensus.

RESULTS AND DISCUSSION

After screening using FDM, 17 sub criteria are deleted so there are on only 22 sub criteria with 11 criteria (**Table 2**).

Table 2. Evaluation criteria and sub criteria after FDM screening

Criteria	Sub Criteria	Defuzzification
Input material	Type of input material	7.5
	Rasio of input material to product	7.5
	Efficiency of material input usage	6.25
	Sertification of input material	7.5
Energy	Energy efficiency	7.5
	Renewable energy usage	6.25
Water	Water efficiency	7.5
Process technology	Implementation of Reduce, Reuse, Recycle (3R)	7.5
	Separation of waste water	6.25
	Improvement of technology, machine and equipment	6.25
Product	Environmentally friendly product innovation	7.5
	Produce component that support LCGC program	7.5
	Environmentally friendly product package	7.5
Work environment	Monitor and evaluate performance of environmet, occupational health and safety	9.17
CO2 emission reduction program	CO2 emission reduction	9.17
Environment al standard compliance	Waste water	7.5
	Gas and dust waste	7.5
Waste treatment infrastructure	Operasional of waste infrastructure	7.5
	B3 waste management	7.5
Sertification	Product	7.5
	Environmetal Management System	7.5
Award	Environmental management and production award	7.5

Identification criteria results indicate that there are eleven criteria for selection of green automotive component industry (Table 2). According to Green Industry Award, those criteria related to three aspects, namely the production process, the company's management, and environmental management. Production process aspect has criteria such as material input, energy, water, process technology, product and work environment. Environmental management aspect has criteria such as CO2 emission

program, environmental standard compliance, and waster treatment infrastructure. Company's management has criteria such as sertification and award.

CONCLUSION

This research reduced the selection criteria for green industry compared to initial criteria from Green Industry Award. There was also new sub criteria, namely produce component that support LCGC program. This research also confirmed three aspects from Green Industry Award, namely the production process, the company's management, and environmental management. Production process aspect has criteria such as material input, energy, water, process technology, product and work environment. Environmental management aspect has criteria such as CO2 emission program, environmental standard compliance, and waster treatment infrastructure. Company's management has criteria such as sertification and award.

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INFLUENCE OF BINDER TYPES AND LEVELS ON CALORIFIC VALUE OF "CHAR JERBAS" BRIQUETTES

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ABSTRACT

Bagasse and hay are abundant agricultural biomass wastes. Bagasse and hay biomass wastes are converted into briquettes which is used as an alternative energy. The research was conducted in May-June 2013. The design is factorial RAL with 3 times of replication. The first factor is types of binder (molasses, tapioca starch, extract of hibiscus leaf) and the second factor is levels of binder (50%, 75%, 100%). The findings show that the binder types suggested significant influence on the calorific value of briquettes, while the binder levels does not show significant influence and neither do the interaction of types and levels of binder.

Keywords

binder types, binder levels, calorific value, "char jerbas"

INTRODUCTION

Indonesia is a country rich in natural resources, which has abundant natural yields. One of the abundant natural yield is sugar cane (*Saccharum officinarum*), paddy (*Oryza sativa*), these plants are capable for living in many regions of Indonesia, particularly Java and Sumatera, producing rice which is the staple food for Indonesian people. Overflowing rice unwittingly will produce agricultural waste of rice straw hay.

Utilization of sugar cane dregs waste (*bagasse*) and rice straw hays biomass which has the ability to become alternative energy, will be able to answer problems that arise nowadays where the availability of energy sources are depleting an expensive fossil and mineral fuels in Indonesia. Usage was done through converting it, by *pirolisis* process, into charcoal briquettes which has higher calorific value than biomass and other energy sources (Aq 2010: 2). Charcoal briquettes is an alternative fuel which is similar to charcoal and can be used as brand new fuel. Before formed into briquettes, sugar cane dregs waste (*bagasse*) and hays were converted into charcoal (*char*) through a process of carbonization. Charcoals that were produced from both materials will be formed into briquettes. Charcoals were formed into briquettes to get the density and high calorific value, so it needs some adhesive (*binder*). Adhesive (*binder*) are highly variable and have thick and sticky characteristics.

Molase is a liquid-shaped, dark brown colored by-product of sugar production, and have condensed, thick, and sticky characteristics which can be used as adhesive (*binder*). Tapioca flour is a product of cassava and yam extraction, especially the ones with characteristics like *molase*, and qualified as good adhesive when it is heated.

While waru leaves extracts is a part of waru plant (*Hibiscus tiliaceus*) which contains *saponin* and has density and adhesiveness when extracted so that the extract will be able to be adhesive or glue. The manufacture of briquettes by using various types of *binder* and different variations of *binder* levels can be used to find out heat value and briquettes quality so briquettes will have some potential to be developed.

MATERIALS AND METHODS

Tools that were used during the research are scissors, muffle, spices grinder tool, sieve screen, digital scales, stove, *teflon*, blender, briquettes pressing tool, oven, carbonization barrel, bomb calorimeter, thermometer, chamber, wire, and thread.

Materials that were used sugar cane dregs (*bagasse*), rice straw (*Oryza sativa*) hays, drops of sugar cane (*molase*), tapioca flour, waru leaves extract (*Hibiscus tiliaceus*), oxygen, and *aquadest*.

The design that was used in this research is factorial *Rancangan Acak Lengkap* (RAL) with 3 times of replication. This research used 3 x 3 factorial, which means that there were 2 experiment factors, namely P_1, P_2, P_3 and K_1, K_2, K_3 , treatment arrangement obtained 9 combinations of treatment. First experiment factor was treatment with different types of binder, which are *molase*, tapioca flour, and waru leaves extract (*Hibiscus tiliaceus*). Second experiment factor was treatment of different binder levels that consist of 50%, 75%, and 100%.

RESULTS

Data that was successfully collected were calorific value of briquettes which was used to find out the effect of type and level of binder towards calorific value of “*char jerbas*” briquettes. Data of calorific value is stated in calorie/gram (cal/gram). Data of briquette test

result to find out calorific value is stated in the following table.

Table 1. Data of Briquette Calorific Value Test Result Data (cal/gram).

Binder Types and Levels	Calorific Value (Ulangan ke-)			Average
	1	2	3	
Molase 50% (P ₁ K ₁)	3577,649	3595,193	3690,431	3621,091
Molase 75% (P ₁ K ₂)	3405,629	3591,478	3671,500	3556,202
Molase 100% (P ₁ K ₃)	3397,590	3297,103	3445,838	3380,177
Tepung Tapioka 50% (P ₂ K ₁)	4352,365	4472,556	4472,330	4432,417
Tepung Tapioka 75% (P ₂ K ₂)	4194,181	4201,270	4369,438	4254,963
Tepung Tapioka 100% (P ₂ K ₃)	4532,273	4234,597	4262,570	4343,147
Daun Waru 50% (P ₃ K ₁)	4453,304	4277,153	4482,033	4404,163
Daun Waru 75% (P ₃ K ₂)	4343,656	4376,674	4427,011	4382,447
Daun Waru 100% (P ₃ K ₃)	4416,764	4514,199	3724,634	4218,532

Based on the **Table 1**, it was known that briquette with highest calorific value is briquettes using tapioca flour binder with level of 50% from char total mass which has calorific value of 4432,417 cal/gram, in addition briquettes using waru leaves extract binder (*Hibiscus tiliaceus*) with level of 50% from char mass which has calorific value of

4404,163cal/gram. Meanwhile briquette with lowest calorific value was briquettes using molase binder with level of 100% from total char mass which has calorific value of 3380,177 cal/gram.

The result of *char “jerbas”* briquettes research test with variety types of binder that were given then variation of calorific value acquired and can be seen in the following picture.

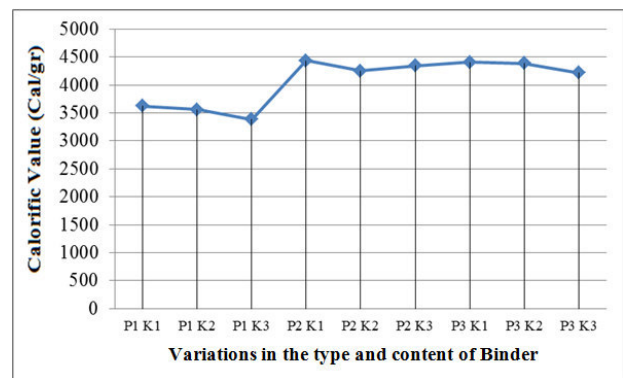


Figure 1. Char “Jerbas” Briquette Calorific Value Graphics

Based on **Fig. 1**, it can be seen that variation of tapioca flour binder with level of 50% (P₂K₁) has the highest calorific value compared to other variations, so it is possible that addition of tapioca flour binder changed calorific value of briquettes.

DISCUSSION

The content of carbon that exists in tapioca flour increased calorific value in briquettes because C atom is a raw material of heating. Ismayana Andes *et all* (2011) stated that adhesive has trait which increase calorific value because it consists element of C (carbon). Budiarto Arief *et all* (2012) mentioned that carbon level affects calorific value, which much higher carbon level, calorific value will be higher, and tapioca flour has carbon value of 84,7% (a total of carbohydrate, protein, and fat).

Uemura's statement is contrast with the result of research that has been done which stated that addition of tapioca flour binder with higher concentration of 75% and 100% has lower calorific value, compared to tapioca flour with concentration of 50%. In this matters, it was related with carbon (C) level and water level, where despite the higher carbon (C) level, it was also comparable with higher water level. Carbon (C) which was supposed to increase calorific value thus lowering calorific value because carbon (C) was used for vaporizing the high water level in the briquettes, so that carbon (C) that was supposed to increase calorific value thus lowering calorific value because carbon (C) was used for vaporizing high water level in the briquettes. Chemically, reactions between carbon (C) atom and H₂O will results evaporation in the form of CO₂ and H₂.

Binder from molase also has carbon content because it consist sugar content with level of 50-60%, amino acid, and minerals. But even though it consisted carbon content, in the research it has lower calorific value compared to other variations. When being dried with the same time and temperature, briquette with tapioca flour dried faster while molase binder still contained quite high humidity because molase has more water and sugar content with level of 50-60%, made it difficult to evaporate, and in the process of briquettes heating, tapioca flour binder has higher calorific value compared to molase binder. Riseanggara in the (Ismayana *et al*, 2011) argued that briquette with higher water level will decrease the quality of briquette because of microbes influence and caused a lot of smoke when heating. Decreasing quality of briquettes because of microbes influence was caused by water level that is still remained in the briquettes, making briquettes more humid and allowed microbes that come from air to be capable of living, have a metabolism, and also turn the briquettes into microbe's life media. Living capable of microbes in the humidity of

briquette will cause fungi on the briquettes and lowering the quality, especially on calorific value of briquettes. Calorific value of briquettes on waru leaves extract binder has almost the same value with calorific value on tapioca flour binder. This is because in the drying process, water level in waru leaves extract evaporated easily so that in the heating process the water level was low and made the calorific value of briquettes higher. Chemical content in waru leaves consist element of carbon (C) and also able to increase calorific value so it has high calorific value.

Variable of binder levels on this research analysis test affect abstractly because the more increased binder levels that were given, the higher water level in the briquettes so it affected abstractly because high water level will decrease calorific value of briquettes. Ismayana *et al*, 2011 mentioned that the lower water level, the higher calorific value and its heating capacity, and also the higher water level, the lower calorific value, because much absorbed energy are used to evaporate the water. Ismayana *et al*, 2011 argued that the more concentration of adhesive is added, the water level will also increase and lowering the calorific value.

CONCLUSION

Based on the result of data analysis, it shows that binder levels does not show significant influence on the calorific value of *char "jerbas"* and neither do the interaction of types and levels of binder, because due to the more increased binder levels, water level will increase and decrease calorific value. While binder types affected calorific value of *char "jerbas"*, which binder type with highest calorific value was tapioca flour binder with level of 50% from the char total mass.

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NOTULEN FORM

Room : Architecture Meeting Room I, 4th Floor
 Time : 12.30 - 13.45
 Group : Green Architecture (GA)

[Handwritten Signature]
 Agung Sedyo

- presenter utama radior mempresentasikan paper-nya. fiau presenter ferdini dari 2 orang yaitu gustan Adeputra Yusran & Agung Dedy Patranoto. dan hasil penelitian dapat dikumpulkan dengan aplikasi skala 1:1 sehingga spt dirasakan manfaat.
- presenter utama pada pameran/wurutan kedua. radior untuk presentasi bernama Yudi Widodo. dengan hasil penelitian ketahanan pangan akibat pengaruh konsumsi pangan.
- presenter utama pada wurutan ketiga. radior untuk presentasi bernama Alvin Gusni. fiau dengan hasil penelitian. bahwa ada peran penting dalam may fertilitas air jair pada bangunan.

NOTULEN FORM

Room : Informatic Meeting Room, 3rd Floor
Time : 12.30 – 13.45
Group : Environment and Biodiversity (ENVB) II

Presentasi o/ Noverita

1. Menentukan jamur beracun dg tidak → dimasak air tdk berubah, dibelak warna tdk berubah.
2. Saran y/ peneliti ⇒ pembudidaya jamur clitete dg kandungan gizi, racun yg d'andung.

Presentasi o/ Bagus Setiawan.

1. Lautace → masuk suku kayu manis.
keistimewaan : penyerapan karbon yg banyak.

NOTULEN FORM

Room : Informatic Meeting Room, 3rd Floor
Time : 12.30 – 13.45
Group : Environment and Biodiversity (ENVB) II

Presentasi I oleh Kuntoro Boga Andri, sampai pasar

1. Apakah pengaruh proses produksi berpengaruh pd harga economic?

Ta, Pengaruh sekali.

2. → Jalur pemasaran berpengaruh pd produksi lokal menurun & masuknya barang import.

→ Pengaruh musim membuat produksi lokal turun. Shg mengakibatkan impor u/ memenuhi kebutuhan.

Presentasi II oleh Kuntoro Boga Andri.

1. Dusun Tiron di Kediri → dipilih frna jenis ^{species} ~~varietas~~ yg bermacam². bkan general production.

2. Apakah ada keinginan u/ perluasan keragaman diseluruh Ind? ada dibuat project kerjasama u/ perluasan tanam dg species berbeda.

1.

NOTULEN FORM

Room : Chemistry Meeting Room I, 2nd Floor

Time : 12.30 – 13.45

Group : Green Chemistry (GC)

14.20. Presentasi : A.G. Fauz

Pertanyaan yg para hadirin: ~~Erasmus~~
 - Ujt alkaloid paice apa ?

- Cateola adalah tumbuhan tingkat rendah → alkaloid ↓
- efektifitas pada ketiga uji apakah saling berkorelasi?

∴ data lengkap

*

g

14.25. Presentasi : Widiyati (Eus)

Quest- 1 : Apakah diindikasikan
 → Sdk lama dikeh (laborasi?)

→ byu kl enkup menua paji.

answer : → bedanya atau (at) WCL.

an 2 : abay pd ptt w pdh ptt mpt 7

an → manula y/ industri.

NOTULEN FORM

Room : Chemistry Meeting Room I, 2nd Floor
 Time : 12.30 – 13.45
 Group : Green Chemistry (GC)

13.30: Presenter 1 : Nely Kurniasih, M.Sc.
 Question 1 : Any Nawa ?
 → tingkat keberkhasan nukleus ?
 → kemampuan hasil lain
 answer : → Identifikasi 2 HPLC
 → rendemen 90%
 Question 2 : sifatnya zat
 → anal bisa analisis
 → layer 2 nukleus
 answer 2 : → analisis keserasian (Ged)
 → diteliti satu 2.

13.45. Presenter 2 : Sri Endang Rahayu
 Question 1 : Nur Fobri Adhary
 → layer la deep penelitian.
 answer : → ada tabung penelitian inkubasi

14.05. Presenter 3 : Poikhatun Nektira
 Question 1 : Omg
 → ada penulisan dan maknanya
 → analisisnya ⇒ persyaratan.
 answer : → empiris dan menggunakan 2 analisis

NOTULEN FORM

Room : Physics Discussion Room , 2nd Floor
Time : 13.30 – 14.30
Group : Green Physics and Engineering (GPE)

13.35 - 13.47 Eko Prasetyo Kuncoro
Penambahan absorban

13.47 - 14.00 Nurul Rohah Hidayah
Ukuran Briket yang digunakan dalam 1 kg: pemasakan
air
Media Menormalisasikan briket for (pda
Masyarakat

14.00 - 14.12 Amilatul Faizah
Proses Pembuatan Kratik
Manfaat dan Penelitian

14.12 - 14.24 Peni Ismawati
Mengapa menggunakan Newton gregori

14.24 - 14.36 A.M. Mytahul Hus.

NOTULEN FORM

Room : Chemistry Meeting Room2, 2nd floor

Time : 12.30 – 13.45

Group : Biotechnology I

③ Title : Potention of Black Tea Extract
as Dye Sensitizer on A DSSC

Presenter : Rachmawati N.

Questions :

- Dye apa yg sudah dicoba?
Cumi x & black tea

NOTULEN FORM

Room : Chemistry Meeting Room2, 2nd floor
Time : 12.30 – 13.45
Group : Biotechnology I

① Title : Isolation of Cellulolytic mold from Soil
of Teak Forest in Kresch - Madian

Presenter : M. Waskito Ardhi

Questions :

- Pengambilan sampel ? acak ± 5 cm
(Fundamental research)

② Title : Study on the composition of dietary
fiber on the parts of the thallus
brown algae

Presenter : Kartini Zailanie

Questions :

Varietas seaweed yg unggul ? byk varietas
Ada yg dibudidayakan & #

NOTULEN FORM

Room : Mathematic Classroom, 3rd Floor
Time : 12.30 – 13.45
Group : Environment and Biodiversity (ENVB) I

- Presentasi 1. Rini Fitri . Aspek sosial budaya tanaman di Krueg Simpo Aceh.
- Presentasi 2. Marheny Lukitawari
Komunitas Plankton Di Bening Reservoir
- Presentasi 3. Soeyono , Quality of Trees Diversity Growing Around water Springs At Southern Part of Patuman
- Presentasi 4. Nia Yudha Junji. Macroinvertebrate Diversity at Bengkung An Sukarni
- Presentasi 5. Mr. Abdulkadir Pahardianto
Activation Communitres on Sustainable River
- Presentasi 6. Anis Arhyanji Processing Heavy Metal In River Water Trough Coagulant
- Flocculation Process Using Moringa seeds

NOTULEN FORM

Room : Mathematic Meeting Room, 3rd floor

Time : 12.30 – 13.45

Group : Biotechnology II

1. Presentasi I : Solihin : Lancar (13.30 - 14.00).
2. Presentasi II : Dr Harri Nurcahya (14.00 - 14.30)
lancar.
3. Presentasi III : Prof Dr Emawati Anaya (14.30 - 15.15)
- Ekstrak zerumbon & anti kanker.
4. Presentasi IV : Dr Irena Suyatna Jalib : (15.15 - 15.45)
(Dra Suprihatin , M.Si)



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NOTULEN FORM

Room : Architecture Meeting Room I, 4th Floor

Time : 12.30 – 13.45

Group : Green Architecture (GA)

- presenter utama pada pendaklah
ke grupat bermaksud. Diantara.
Davina M. Hadin untuk
menyajikan hasil penelitiannya.