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Planta Tropika focuses related to various themes, topics and aspects including (but not limited) to the following topics Agro-Biotechnology, Plant Breeding, Agriculture Waste Management, Plant Protection, Soil Science, Post Harvest Science and Technology, Horticulture. Planta Tropika published two times a year (February and August) by Universitas Muhammadiyah Yogyakarta in collaboration with Indonesian Association of Agrotechnology / Agroecotechnology (PAGI). The subscriptions for one year : IDR 350.000.

Editorial Address

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Editorial

Journal of Planta Tropika ISSN 0216-499X published by Study Program of Agrotechnology, Faculty of Agriculture, Universitas Muhammadiyah Yogyakarta, is journal presenting scientific articles of agricultural science (Journal of Agro Science). With full sense of gratitude to the Almighty Allah, Volume 7 Number 2 for the year of 2019 has been published.

In this edition, Journal of Planta Tropika presents seven research articles in the field of Agro sciences comprising post harvest physiology, crop cultivation system, weeds management, tissue culture, land management, and climate. The scientific articles discuss about:

(1) Oil Palm (*Elaeis guineensis*) Responses to Indigenous Mycorrhizae and Cow Manure in Ultisol, (2) Susceptibility of Sorghum Cultivars to *Sitophilus oryzae* L. (Coleoptera: Curculionidae) During Storage, (3) Acceleration of *Echinacea purpurea* (L.) Moench Shoot Growth by Benzyl Adenine and Indole Butyric Acid Addition, (4) The Diversity of Rot Fungi from Cocoa Plantation and Its Ability to Grow on Carbon Source Media, (5) Physical Characteristics of Active Packaging Based on Methyl Cellulose with The Addition of Glutaraldehyde and Klutuk Banana (*Musa balbisiana* Colla) Leaf Extract, (6) Parasitization and Identification of The Red Guava Fruit Fly Parasitoids in The Deli Serdang District, (7) Methane Emissions and Rice Yield in Rainfed Bed System (*Surjan*) as Affected by Manure and Zeolite Treatment, and (8) The Effect of Light Color Variation in Simple Light Traps on the Number of Fruit Flies (*Bactrocera* sp.).

The editors would like to thank the authors, reviewers, executive editors, leaders and LP3M UMY for their participation and cooperation. Our hope, this journal can be useful for readers or be a reference for other researchers and useful for the advancement of the agriculture.

Editors

GUIDE FOR AUTHORS

TYPE OF PAPERS

PLANTA TROPIKA receives manuscripts in the form of research papers in Bahasa Indonesia or English. The manuscript submitted is a research paper that has never been published in a journal or other publication.

SUBMISSION

The submission of the manuscript is done through our journal website http://journal.umy.ac.id/index.php/pt/index. If you need information regarding the process and procedure for sending the manuscript, you can send it via email at plantatropika@umy.ac.id. Editor's address: Program Studi Agroteknologi, Fakultas Pertanian, Universitas Muhammadiyah Yogyakarta, Jl. Ring Road Selatan, Tamantirto, Kasihan, Bantul, Telp (0274) 387646 psw 224, ISSN: 2528-7079.

ARTICLE STRUCTURE

The submitted manuscripts should consist of 15-20 pages of A4 size paper with 12-point Times New Roman fonts, 1.5 spacing with left-right margin and top-bottom of the paper is 2.5 cm each. All manuscript pages including images, tables and references should be page-numbered. Each table or picture should be numbered and titled.

The systematic of the manuscript writing is as follows:

TITLE : The title should be brief and informative and written bold. Only the first letter of the words is written in uppercase. Maximum length should be 14 words.

AUTHOR NAMES : The author names should be written in lowercase letters (only the first letter of the words is written in uppercase) and should be written from the first author and followed by the others along with the marker of each author's affiliation.

AUTHOR AFFILIATIONS : The author affiliation should be written in lowercase letters (only the first letter of the words is written in uppercase) and it is written according to the order of the number marker of each author's affiliation.

EMAIL : Please list one of authors' email address used for paper's correspondence.

ABSTRAK : Abstrak is written in Bahasa Indonesia using single space in a paragraph with maximum length of 200 words. It should contain background, objective, method, results, and conclusion followed by keywords containing maximum of 5 words.

ABSTRACT : Abstract is written in English using single space in a paragraph with maximum length of 200 words. It should contain background, objective, method, results, and conclusion followed by keywords containing maximum of 5 words.

INTRODUCTION : Introduction contains background, hypothesis or problem outline, and the objective of the research.

MATERIALS AND METHOD : Explaining in detail about materials and method used in the research as well as the data collection and analysis.

RESULT AND DISCUSSION : The results of the research should be clear. State the results collected according to analyzed data. Discussion should include the significance of the results.

CONCLUSION : Authors are expected to give brief conclusion and to answer the objective of the research.

ACKNOWLEDGEMENT : If necessary.

REFERENCES : Single space, according to the authors' guide of Planta Tropika.

EXAMPLES ON HOW TO WRITE REFERENCES

References are written in alphabetical order according to the rules below:

REFERENCE TO A BOOK

Gardner, F.P., R.B. Pearce, and R.L. Mitchell. 1991. Fisiologi Tanaman Budidaya (Translated by Herawati Susilo). UI Press. Jakarta.

REFERENCE TO A JOURNAL PUBLICATION

Parwata, I.G.M.A., D. Indradewa, P.Yudono dan B.Dj. Kertonegoro. 2010. Pengelompokan genotipe jarak pagar berdasarkan ketahanannya terhadap kekeringan pada fase pembibitan di lahan pasir pantai. J. Agron. Indonesia 38:156-162.

REFERENCE TO A THESIS/DISSERTATION

Churiah. 2006. Protein bioaktif dari bagian tanaman dan akar transgenic Cucurbitaceae serta aktivitas antiproliferasi galur sel kanker *in vitro*. Disertasi. Sekolah Pascasarjana. Institut Pertanian Bogor. Bogor.

REFERENCE TO AN ARTICLE IN PROCEEDING

Widaryanto dan Damanhuri. 1990. Pengaruh cara pengendalian gulma dan pemberian mulsa jerami terhadap pertumbuhan dan produksi bawang putih (Allium sativum L.). Prosiding Konferensi Nasional X HIGI hal. 376-384.

FIGURE FORMATTING

Title should be given **below each figure**. Additional information (notes) should be written in lowercase letters except the first letter in each sentence. All figures need to be numbered respectively. Figures should be placed close to explanation/ discussion about the figure.

Examples :

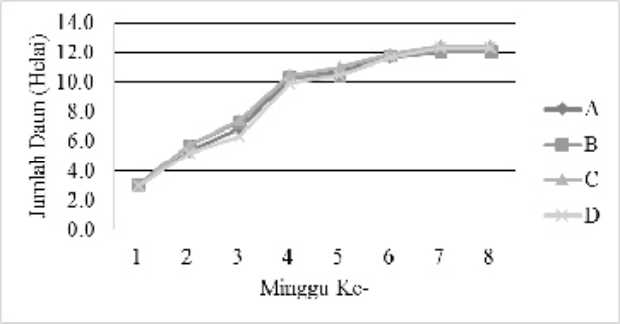

Figure 1. Number of leaves of corn plant

Notes:
A = 250 kg KCl/ha + 0 kg KJP/ha
B = 125 kg KCl/ha + 273,89 kg KJP/ha
C = 62,5 kg KCl/ha + 410,84 kg KJP/ha
D = 0 kg KCl/ha + 547,79 kg KJP/ha

Fig. 1., Fig. 2., and so on. The title of the figure is written with lowercase letters (use uppercase letter at the beginning of the title only) and without full stop (.). Additional information (notes) is placed below the figure.

TABLE FORMATTING

The title of the table should be written **above the table** started from the left (left alignment). Additional information related to the table (notes) is placed below the table. The information is written in uppercase letters at the beginning only as well as the titles inside the table. Table is placed close to the discussion of the table.

Examples :

Table 1. Fruit compost analysis

Variable	Jatropha before composted	Jatropha after composted	SNI (National standard) for compost	Category
Water content	22,49 %	45,79 %	≤ 50 %	Qualified
pH	7,05	8,02	4-8	Qualified
C-Organic content	10,01	5,11	9,8-32 %	Not qualified
Organic matter	17,42 %	8,81 %	27-58	Not qualified
N-Total	0,97 %	2,69 %	< 6 %	Qualified
C/N Ratio	10,44	1.90	≤ 20	Qualified
Potassium	-	9,06 %	< 6 %**	Qualified

Notes: **) Certain materials originated from natural organic matters are allowed to contain P_2O_5 dan K_2O level > 6% (proved with the results of laboratory analysis).

Oil Palm (*Elaeis guineensis*) Responses to Indigenous Mycorrhizae and Cow Manure in Ultisol

DOI: 10.18196/pt.2019.099.103-109

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ABSTRACT

The experiment was conducted to examine the effects of indigenous mycorrhizae inoculation and cow manure doses on the growth and yield of oil palm (Elaeis guineensis) at production stages I. It was conducted at farmer plantation in Semabu Village Tebo Regency located at -1.473543, 102484062. This research was arranged in a randomized block design consisting of two factors. The first factor is inoculation of mycorrhizae isolates comprising two levels, i.e. without and with inoculation, and the second factor is the dose of cow manure comprising five levels, i.e. without cow manure, 25%, 50%, 75%, and 100% of the recommended dose of cow manure at Production Stages I (30 kg plant-1). The variables observed were plants girth, leaf midrib, number of bunches per plant, weight per bunch, weight of fresh fruit bunches per plant, and root infection. The results showed that there was interaction effect between inoculation of mycorrhizae and cow manure doses. The inoculation of mycorrhizae and cow manure at a dose of 50% of the recommended dose were able to increase oil palm growth and yield.

Keywords: Cow manure, Inoculated, Mycorrhizae, Oil palm, Ultisol

ABSTRAK

Tujuan dari penelitian ini adalah untuk mempelajari pengaruh inokulasi mikoriza indigenus dan pupuk kandang kotoran sapi terhadap pertumbuhan dan hasil kelapa sawit (Elaeis guineensis) pada Tanaman Menghasilkan tahun pertama (TM I). Penelitian ini dilakukan di kebun petani di Desa Semabu Kabupaten Tebo (lokasi -1.473543, 102484062). Percobaan ini menggunakan Rancangan Acak Kelompok dengan 2 faktor. Faktor pertama adalah isolat mikoriza indigenus yang terdiri dari dua level yaitu tanpa inokulasi dan inokulasi mikoriza, and faktor kedua adalah dosis pupuk kandang kotoran sapi yang terdiri dari lima level yaitu tanpa pupuk kandang kotoran sapi, 7.5, 50, 75, and 100 % dosis rekomendasi pupuk kandang kototan sapi untuk TM I (30 Kg tanaman^{-1}). Variabel yang diamati yaitu lingkar batang, jumlah pelepah, jumlah tandan per tanaman, bobot buah per tandan, bobot tandan buah segar per tanaman dan infeksi akar Hasil penelitian menunjukkan bahwa terjadi interaksi antara inokulasi mikoriza indigenus dan dosis pupuk kandang kotoran sapi. Inokulasi mikoriza indigenus dan pupuk kompos kotoran sapi 50% dari dosis yang direkomendasikan mampu meningkatkan pertumbuhan dan hasil kelapa sawit.

Kata Kunci: Pupuk kendang, Inokulasi, Mikoriza, Kelapa sawit, Ultisol

INTRODUCTION

The global demand for oil palm is continuously increasing due to the population increase and oil palm diversification by the producer. Hence, oil palm production has good prospects to be developed in Indonesia. The production of oil palm production could be increased through several ways such as area expansion, intensification and rehabilitation programs. In Jambi Province, the main problem in expanding oil palm plantation is the limited availability of fertile land. Mostly, the land in Jambi is composed of infertile soil Ultisol. Ultisol is known as marginal soil that has low fertility, shallow solum, low water holding capacity, vulnerability to erosion, low pH, and high Al levels. One of the efforts to overcome the problem is the application of indigenous mycorrhizae fungi that could help plants uptake nutrients. Furthermore, the application could be accompanied with the addition of organic material from cow manure to optimize the function of mycorrhizae in plant.

The application of mycorrhizae in plants has been widely proven to be able to improve nutrient and water absorption and to promote plant growth. Nutrients uptake, especially P, is improved in plants infected by mycorrhizae. As it is known, P is largely required by plants despite its limited availability in soils nutrient. A research by Kartika (2012) showed the water use of oil palm seedlings inoculated with

mycorrhizae was more efficient compared to the ones without mycorrhizae. The effect of mycorrhizae on ultisol will be more optimal if combined with organic material. According to Tampubolon and Hendriansyah (2011), organic material from cow manure can improve physical, chemical, and biological properties of the soil. For those reasons, the purpose of this research was to determine the oil palm responses to the mycorrhizae inoculation and cow manure doses.

MATERIALS AND METHODS

This experiment was conducted in land with ultisol soil in Semabu village, Tebo Regency located at -1.473543, 102484062. The experiment was arranged in Randomized Complete Block Design, consisting of two factors and four replications. The first factor is mycorrhizae inoculation (without and with inoculation of indigenous mycorrhizal *Glomus sp-16*). Indigenous mycorrhizal Glomus sp-16 is an indigenous mycorrhizal isolate from marginal land in Tebo Regency, cultured at Teaching and Research Farm, Faculty of Agriculture, Jambi University (Kartika, et al., 2010). The second factor is the dose of organic fertilizer (without cow manure (0 %) and with cow manure at 5, 50, 75, and 100 % of the recommended dose (30 kg plant^{-1})). Cow manure was fermented four weeks until composted perfectly, and it was characterized from the texture, which is crumbly, dry, cold and odorless. The nutrient contents of cow manure were Carbon 28.11 %, Nitrogen 1.98%, C/N ratio 14, P_2O_5 805 ppm, and K_20 468 ppm.

Oil palm cv. Tenera at the age of 12 months was used in this study. In the field, the plants were arranged with spacing of 9× 9 m and planting holes of 30 x 30 x 30 cm. Three months before transplanted to the field, the plants were inoculated with mycorrhizae according to the treatments. Meanwhile, the cow manure treatment was applied to the field seven days before planting time. Two plants from each plot were randomly selected and tagged for observation. The variables observed included plants girth, number of leaf midribs, number of bunches per plant, weight of fresh fruit bunches per plant and root infection. Plants girth was measured at a height of 5 cm from the base of the stem, leaf midrib number was calculated by counting all the midribs which opened perfectly and fresh, bunch number per plant was calculated by counting all bunches formed in one plant, fruit weight per bunch was weight of all fruits in one bunch, fresh fruit bunches weight was measured all fresh fruit bunches (FFB) per plant at harvest, and root infection was carried out on the roots of sample plants based on root staining techniques according to the method of Kormanik and McGraw (1982). The collected data were subjected to standard statistical analysis by Steel and Torrie (1980), followed by DMRT at α =5%.

RESULTS AND DISCUSSION

Plants Girth

The results showed that there was interaction effect between mycorrhizae inoculation and cow manure doses on the plants girth. The highest plants girth was observed in the plants treated with mycorrhiza inoculation and cow manure at a dose of 50 % of recommended dose (Table 1).

Number of Leaf Midrib

The results showed that there were interaction effects between mycorrhizae inoculation and cow manure doses on the number of leaf midrib. The plants treated with mycorrhizae inoculation and cow manure doses of 75% had significantly higher number of leaf midrib compared to the plants treated without mycorrhizae inoculation and without cow manure (0%) (Table 2).

Table 1. Plants Girth as Affected by Mycorrhizae Inoculation and Cow Manure Doses

Mycorrhizae	Cow manure (percentage of recommended doses fertilizer for oil palm)					Mean
	0	25	50	75	100	
Without inoculation	64 b A	67.50 ab A	68.33 ab B	68.00 ab B	72.83 a B	68.13 B
With inoculation	67.83 c A	73.67 bc A	80.83 a A	79.00 ab A	79.67 ab A	76.20 A
Mean	65.92 b	70.58 ab	74.58 a	73.50 a	76.25 a	

Remarks: Means followed by the same uppercase letter in the same column and by the same lowercase letter in the same row are not significantly different according to DMRT at 5%.
Recommended dose of organic fertilizer (cow manure): 30 kg plant^{-1}

Table 2. The Number of Leaf Midrib as Affected by Mycorrhizae Inoculation and Cow Manure Doses

Mycorrhizae	Cow manure (percentage of recommended doses fertilizer for oil palm)					Mean
	0	25	50	75	100	
Without inoculation	36.67 b B	42.00 a A	42.17 a A	43.00 a A	43.33 a A	41.43 A
With inoculation	42.50 a A	41.00 a A	43.50 a A	44.00 a A	43.83 a A	42.97 A
Mean	39.58 a	41.50 a	42.83 a	43.50 a	43.58 a	

Remarks: Means followed by the same uppercase letter in the same column and by the same lowercase letter in the same row are not significantly different according to DMRT at 5%.
Recommended dose of organic fertilizer (cow manure): 30 kg plant^{-1}

Number of Bunches per Plant

The results showed that there were interaction effects between mycorrhizae inoculation and cow manure doses on the number of bunches per plant. The highest number was obtained int the plants treated with mycorrhizae inoculation and cow manure doses of 50% of recommended dose (Table 3).

Fruit Weight per Bunch

The results showed that there were no interaction effects between mycorrhizae inoculation and cow manure doses on fruit weight per bunch. However, there was a tendency that the highest fruit weight per bunch was achieved in the plants treated with mycorrhizae inoculation and cow manure doses of 50% of recommended dose.

Fresh Fruit Bunches Weight per Plant

The results showed that there were interaction effects between mycorrhizae inoculation and cow manure doses on the weight of fresh fruit bunches per plant. The highest value was obtained in the plants treated with mycorrhizae inoculation and cow manure doses of 50% of recommended doses (Tabel.5)

Root Infection

Data in Table 6 showed that the plants inoculated with mycorrhizae had high percentage of root infections. Meanwhile, slight root infection was also observed in the plants that were not inoculated with mycorrhizae. This result showed that even though the plants were not inoculated with mycorrhizae, however, there were local mycorrhizae (natural mycorrhizae) in the field that could infect the roots. High root infection will help plants absorb nutrients, especially phosphate.

The highest values of plants girth, number of bunches, fruit weight per bunches and fresh fruit bunches weight per plant were obtained in the plants treated with with mycorrhizae inoculation and cow manure at a dose of 50% of the recommended dose (Table 1, Table 3, Table 4 and Table 5). It means that the application of cow manure at a dose of 50% of the recommended dose and

Table 3. The Number of Bunches Per Plant as Affected by Mycorrhizae Inoculation and Cow Manure Doses

Mycorrhizae	Cow manure (percentage of recommended doses fertilizer for oil palm)					Mean
	0	25	50	75	100	
Without inoculation	19.22 b A	15.33 c B	15.17 c B	22.28 a A	21.89 ab A	18.78 B
With inoculation	14.72 d B	19.17 c A	24.44 a A	23.89 ab A	21.28 bc A	20.70 A
Mean	16.97 d	17.25 cd	19.81 bc	23.08 a	21.58 ab	

Remarks: Means followed by the same uppercase letter in the same column and by the same lowercase letter in the same row are not significantly different according to DMRT at 5%.
Recommended dose of organic fertilizer (cow manure): 30 kg plant^{-1}

Table 4. The Weight of Fruit Per Bunch as Affected by Mycorrhizae Inoculation and Cow Manure Doses

Mycorrhizae	Cow manure (percentage of recommended doses fertilizer for oil palm)					Mean
	0	25	50	75	100	
Without inoculation	3.86 a A	3.92 a A	3.90 a A	3.93 a A	4.03 a A	3.93 A
With inoculation	3.94 a A	3.93 a A	4.20 a A	4.17 a A	4.16 a A	4.08 A
Mean	3.90 a	3.92 a	4.05 a	4.05 a	4.09 a	

Remarks: Means followed by the same uppercase letter in the same column and by the same lowercase letter in the same row are not significantly different according to DMRT at 5%.
Recommended dose of organic fertilizer (cow manure): 30 kg plant^{-1}

Table 5. The Weight of Fresh Fruit Bunches Per Plant as Affected by Mycorrhizae Inoculation and Cow Manure Doses

Mycorrhizae	Cow manure (percentage of recommended doses fertilizer for oil palm)					Mean
	0	25	50	75	100	
Without inoculation	27.83 c A	27.67 c B	32.17 b B	35.83 ab B	46.60 a B	34.02 B
With inoculation	28.33 c A	34.50 b A	53.17 a A	52.00 a A	50.60 a A	43.72 A
Mean	28.083 c	31.08 c	42.67 b	43.92 ab	48.60 a	

Remarks: Means followed by the same uppercase letter in the same column and by the same lowercase letter in the same row are not significantly different according to DMRT at 5%.
Recommended dose of organic fertilizer (cow manure): 30 kg plant^{-1}

mycorrhizae inoculation can improve the growth and yield of oil palm. Therefore, mycorrhizae can save the use of cow manure by 50% of the recommended dose. Based on those results, it is proven that arbuscular mycorrhiza fungi (AMF) can support the growth and yield of oil palm as well as increase the ability of plants to absorb more nutrients from soil solution. Mycorrhizae hyphae can facilitate the plant to absorb water and nutrients. Mycorrhizal colonization (Table 6) increases the absorption of water and nutrients. Several researches (Same, 2011; Ortas and Akpinar, 2011; Bhattacharjee and Sharma, 2012; Ortas, 2012; Zhang et al., 2012; Kathlee and Treseder, 2013; Watts-Williams Stephanie, et al., 2014; Lu et al., 2015; Binu et al., 2015; İncesu et al., 2015; Liu et al., 2015) also reported that AMF (Arbuscular Mycorrhizae Fungi) had the ability to promote plants growth and yield by enhancing absorption of macro nutrients, especially phosphate and some micro elements.

AMF is an alternative technology to support growth and to improve productivity and quality of crops primarily grown on ultisols. AMF can

Table 6. The Percentage of Oil Palm Root Infection as Affected by Mycorrhizae Inoculation and Cow Manure Doses

No.	Treatments	Percentage of oil palm root infection		
		Months after transplanting (MAT)		
		4	15	28
1.	M_0P_0	1.33	1.33	1.33
2.	M_0P_1	0.67	1.33	1.33
3.	M_0P_2	1.67	1.67	1.67
4.	M_0P_3	1.33	1.67	1.67
5.	M_0P_4	2.33	2.33	2.33
6.	M_1P_0	97.33	100.0	100.0
7.	M_1P_1	98.33	100.0	100.0
8.	M_1P_2	100.0	100.0	100.0
9.	M_1P_3	100.0	100.0	100.0
10.	M_1P_4	100.0	100.0	100.0

Remarks: M: Mycorrhizae inoculation
M_0 (without mycorrhizae inoculation). M_1 (with mycorrhizae inoculation)
P: The percentage of cow manure dose from recommended doses of fertilizer for oil palm.
P_0 (without cow manure), P_1 (25% = 7.25 kg plant^{-1}), P_2 (50% = 15 kg plant^{-1}), P_3 (75% = 22.5 kg plant^{-1}), P_4 (100% = 30 kg plant^{-1})
Recommended dose of organic fertilizer (cow manure): 30 kg plant^{-1}

colonize root system by generating direct benefits for the host plants. They can improve nutrient uptake, especially phosphate (Same, 2011; Ortas and Akpinar, 2011; Bhattacharjee and Sharma, 2012; Ortas, 2012; Kathlee and Treseder, 2013; Watts-Williams Stephanie et al., 2014), improve plant resistance to abiotic stresses (Zhu et al., 2012) and heavy metal stress (Krishnamoorthy et al., 2015), and protect palm oil from stem rot disease (Simanjuntak et al., 2013).

Organic fertilizer from cow manure combined with mycorrhizae inoculation can increase plant growth, yield and levels of N, P, K and Mg in the leaves of oil palm grown on ultisol. This is due to the organic fertilizer (cow manure) that can improve the physical, chemical and biological properties of the soil (Tampubolon and Hendriansya, 2011) and Gomes et al. (2014). The roots of plants inoculated with mycorrhizae are protected from pathogen attack. The plants are physically protected by the hyphae, which produce hormones and growth regulators for plants (Hahn et al., 1999)

The application of mycorrhizae to the soil can improve soil structure (Leifheit et al., 2014) and trigger growth regulator substances (auxin, cytokinin, and gibberellin). Plant growth regulators are needed in the process of cells division, which stimulate growth and prevent or slow the aging process thereby increasing root function to absorb nutrients and water (Auge, 2001).

The structure of mycorrhizae on the plant roots can increase the area for metabolic exchange between the host plant and mycorrhizae. AMF can also absorb organic phosphate and convert it into inorganic P that can be absorbed by plants with the help of acid phosphatase enzyme. Accordingly, acid phosphatase enzyme produced by fungal hyphae is currently active, hence developing and enhancing the activity of phosphatases on the root surface. Mycorrhizae fungi release inorganic phosphate from organic phosphates in the area near the surface of the cell so that it can be absorbed through the mechanism of nutrient uptake (Gunawan, 1983). Phosphorus is one of the macro nutrients that are important in plant growth and development. The element serves as a constituent metabolite in complex compounds, as activator, and as enzyme cofactors or unifying fibers. It also plays a role in

physiological processes as structural component of several important compounds and in molecular energy transfer of ADP and ATP (Gardner et al., 1991; Marschner 1997).

ATP compounds is an important compound for metabolite reactions, which is the reaction of the biosynthetic formation of compounds essential for the maintenance and growth of cells, including proteins and nucleic acids. In addition, ATP is required for the synthesis of food reserves, such as lipids and polysaccharides. It is also needed in the process of active transport and flow of protoplasm. Phosphorus is an element that is critical for plant growth, where the P deficiency leads to inability of plants to absorb other elements. As an important element in the formation of energy for plant growth, sufficient P will improve plant growth. If the energy is available in sufficient quantity, all the metabolic processes can occur properly. The effects of AMF on plant growth were caused by the increase in absorption of nutrients by the larger surface area of absorption or the ability to mobilize sources of nutrients that are not easily available. AMF have a very important function for plant growth, primarily due to increased absorption of P (Prawiranata et al., 1992).

CONCLUSIONS

The combination of indigenous mycorrhizae and cow manure could improve oil palm growth and yield. Oil palm treated with mycorrhizae inoculation and cow manure doses of 50% of recommended dose fertilizer could increase the growth, yield component, and yield of oil palm.

ACKNOWLEDGEMENTS

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Susceptibility of Sorghum Cultivars to *Sitophilus oryzae* L. (Coleoptera: Curculionidae) During Storage

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ABSTRACT

Sitophilus oryzae L. is a primary pest that causes damage to stored sorghum. The aim of this study was to evaluate the susceptibility of some sorghum cultivars to S. oryzae infestations and the damage resulted during storage period. The research was carried out at Plant Pest and Disease Laboratory, Department Agroecotechnology, Faculty of Agriculture, Malikussaleh University from February to June 2017. Nine cultivars of sorghum were screened for their susceptibility to S. oryzae attacks and the damage resulted. The Dobie sesceptibility indexwas used to classify the susceptibility of sorghum cultivars. Susceptibility experiment of several sorghum cultivars to S. oryzae was done by no choice assay. The results exhibited that sorghum cv. Suri 3, Suri 4, Kawali, and Numbu was categorized as moderate. Cv. Samurai 1 was included in moderate to susceptible, and cv. Super 1, Super 2, Samurai 2, and Pahat were categorized as susceptible to S. oryzae. The susceptibility of sorghum cultivars was determined by high number of F1 progeny, the high percentage of seed weight loss, damaged seeds, low median development time and low width of sorghum seeds.

Keywords: Sorghum cultivar, Susceptibility, Storage period, Sitophilus oryzae

ABSTRAK

Sitophilus oryzae L. merupakan hama primer yang menyebabkan kerusakan sorgum di penyimpanan. Penelitian bertujuan mengevaluasi kerentanan dan kerusakan beberapa varietas sorgum terhadap infestasi *S. oryzae* selama di penyimpanan. Penelitian telah dilakukan di Laboratorium Hama dan Penyakit Tanaman, Program Studi Agroekoteknologi, Fakultas Pertanian, Universitas Malikussaleh dari bulan Februari–Juni 2017. Sembilan varietas sorgum dinvestigasi tingkat kerentanan dan kerusakan terhadap serangan *S. oryzae*. Indeks kerentanan Dobie digunakan untuk mengelompokkan derajat kerentanan varietas sorgum terhadap *S. oryzae*. Pengujian kerentanan beberapa varietas sorgum terhadap *S. oryzae* dilakukan tanpa uji pilihan. Hasil penelitian menunjukkan bahwa sorgum dari Varietas Suri 3, Suri 4, Kawali, dan Numbu tergolong moderat, sedangkan Varietas Samurai 1 tergolong moderat sampai rentan, dan Varietas Super 1, Super 2, Samurai 2, dan Pahat tergolong rentan terhadap *S. oryzae* selama penyimpanan sorgum. Kerentanan varietas sorgum ditentukan oleh jumlah F1 yang banyak, persentase kehilangan bobot biji dan persentase biji berlubang yang tinggi serta median waktu perkembangan dan lebar biji sorgum yang rendah.

Kata Kunci: Kultivar sorgum, Kerentanan, Periode penyimpanan, *Sitophilus oryzae*

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench.) is a major food cereal for millions of people in the world. It is considered as an alternative source of carbohydrates, and it has the potential as a rice supplementary food in Indonesia. Meanwhile, sorghum is mainly consumed in Africa and South Asia (Subagio & Aqil, 2014; Griebel et al., 2019). Sorghum has great potential to be cultivated in Indonesia because it is relatively drought tolerant, and it has a high nutrient content compared to rice. Sorghum storage is a part of postharvest activities, which is done after threshing and exfoliation (Subagio & Aqil, 2014). Generally, sorghum is stored as seeds or panicles. This is done to maintain the quantity and quality of sorghum from several factors that affect the commodity, such as the presence of stored-product pests and the increase in water content which triggers the appearance of fungi (Firmansyah et al., 2013). The main problem in developing sorghum is that sorghum is easily damaged during the storage period (Sirappa, 2003). The most common postharvest damage during the storage period is caused by the attack of stored-product pests. Stored-product pests that cause damage to stored sorghum are *Sitophilus spp.*, *Corcyra cephalonica*, *Sitotroga cerealella*, *Plodia*

interpunctella, Rhyzoperta dominica and *Ephis cautella* (Firmansyah et al., 2013; Tenrirawe et al., 2013).

Sitophilus oryzae L. is one of important pests attacking agricultural commodities such as cereals, and it is commonly found in Asian countries (Zunjare et al., 2016). This pest is classified as major and polyphagous pest, which causes intense damage to stored sorghum (Ladang et al., 2008; Bhanderi et al., 2015). Weight loss of sorghum during the storage period is caused by feeding activities of both larvae and adults (Prasad et al., 2015). The adults and larvae attack from the inside of sorghum seeds, causing economic losses (quantity and quality damage) to sorghum during the storage period (Bhanderi et al., 2014). The infestation of these pests on sorghum also deteriorates seed germination and contaminates the seeds with exuvia, excretion accumulation, and fungal contamination during storage. Other qualitative losses are related to changes in the biochemical components of cereals such as the decrease in carbohydrate, starch, and protein content (Danjumma et al., 2009). Sorghum damage during storage could lower the value of sorghum (Reddy et al., 2002).

Damage to sorghum during the storage period caused by *S. oryzae* can be reduced by storing resistant sorghum. Bamaiyi et al. (2007) reported that there was a variability of each sorghum cultivar to the population and median development time of *S. oryzae*, susceptibility index, and percentage of damage and loss of yield weight. The results of the study by Pradeep et al. (2015) showed that there were 5 out of 20 sorghum cultivars that had a high level of resistance to *S. oryzae* with lower damage to sorghum seeds for 120 days of storage. Variations in sorghum damage caused by larvae and adults of *S. oryzae* are related to the differences in the characteristics of sorghum cultivars, thus affecting the susceptibility of sorghum (Pradeep & Jagginavar, 2015). To lower the damage during storage period, the use of resistant sorghum cultivars is highly recommended. In Indonesia, sorghum is still considered as unpopular food. However, this plant is promising for the economic growth and also prospective for grain cultivation due to its drought-resistance. Because of these reasons, it is necessary to conduct further evaluation, especially on its susceptibility to *S. oryzae* attack. Information on sorghum susceptibility is needed as a guideline to sorghum breeding program to support the development of sorghum. Hence, this study aimed to evaluate the susceptibility and damage of several sorghum cultivars to *S. oryzae* infestations during the storage period.

MATERIALS AND METHODS

Mass-rearing and infestation of *S. oryzae*

Insects were prepared following the method of Hendrival & Meutia (2016). A total of 40 adults were reared on 250 g of red rice and stored in maintenance jars for 4 weeks. After 4 weeks, the insect were removed from the jars. Then, the insects were re-incubated to red rice until the progeny appeared. Separation was carried out continuously every day until certain number of adults was obtained. A total of 10 *S. oryzae* adult pairs from stock rearing (aged 7 days) were placed in glass vials (diameter of 15 cm and height of 12 cm). Each glass vials contained sorghum (200 g) of various cultivars and they were maintained in laboratory at a temperature of 27 - 30 ^{o}C and RH of 70 - 75 %.

Characteristic of various sorghum cultivars

Nine sorghum cultivars were screened for their susceptibilities to *S. oryzae*. Cultivar Super 1, Super 2, Suri 3, Suri 4, Kawali, and Numbu were obtained from Cereals Research Institute, Maros, South Sulawesi. Cv. Samurai 1, Samurai 2, and Pahat were obtained from the National Nuclear Energy Agency of Indonesia (BATAN). The seed

dimension (length, width, and diameter) was measured from 20 seeds randomly observed. The seed length was measured between the two ends of whole seeds, while the seed width was measured between the back and abdomen of whole seeds. The digital calipers (mm) were used to measure the seed dimension (Table 1). The moisture content of sorghum seeds ranged from 10.55 - 10.88 %.

Determination of sorghum susceptibility

The susceptibility of sorghum was determined by Dobie susceptibility index (Dobie, 1974) which calculation is based on the appearance of F1 progeny and median development time of *S. oryzae*. The adults of *S. oryzae* were allowed to infest each of three glass vials containing 200 g sorghum seeds for ten days. After ten days, oviposition period of *S. oryzae* was discharged from each glass vials. The insects were counted 35 days post-infestation when the F1 progenies started emerging (the mean developmental period is 35 days). The emergent adults were counted daily and recorded. Sampling for adult emergence continued up to the 50th day when most F1 progenies had emerged (Bamaiyi et al., 2007). The median developmental period (days) is estimated as the time from the middle of the oviposition period to the emergence of 50 % of the F1 progeny. Median development time was observed daily since oviposition period (10 days after infestation) until 50 % progeny appeared. The susceptibility level of sorghum can be categorized as resistance (susceptibility index range of 0 - 3), moderate (range of 4 - 7), susceptible (range of 8 - 10), and very susceptible (> 11). The susceptibility index was calculated using the following formula.

$$\text{Susceptibility index} = 100 \times \frac{\left(\text{Log}_e \times \text{number of F1 progeny of } \textit{S. oryzae}\right)}{\text{Median development time of } \textit{S. oryzae}}$$

Determination of Damaged Seeds

The damaged seeds were measured by calculating the percentage of seed weight loss and damaged seeds in samples of 100 seeds which had been stored for 60 days. The damaged seeds were expressed as a proportion of the total number of seed samples from each glass vials. Sorghum seeds which were used in the research needed to be stirred so that the damaged and undamaged seeds mixed perfectly. The seed weight loss and damaged seeds were calculated using the following formula (Gwinner et al., 1996).

$$\text{Weight loss} = \frac{(W_u \times N_d) - (W_d \times N_u)}{W_u \times (N_d + N_u)} \times 100\%$$

$$\text{Damaged seed} = \frac{N_d}{N} \times 100\%$$

Where:

Wu = weight of undamaged seeds
Nu = number of undamaged seeds
Wd = weight of damaged seeds
Nd = number of damaged seeds
N = number of samples

Data Analysis

Data collected were analyzed using Analysis of Variance (ANOVA) with the tool of Statistical Analysis System (SAS) software. Pearson's coefficient correlation was obtained using the same statistical analysis.

RESULTS AND DISCUSSION

The number of F1 Progeny

Sorghum cultivars significantly affected the number of F1 progeny in 200 g of sorghum seeds ($F = 15.17^{**}$; df = 8; $P < 0.0001$). Sorghum cv. Samurai 2, Pahat, Super 1, and Super 2 significantly had a higher number of F1 progeny compared to cv. Samurai 1, Suri 3, Suri4, Kawali, and Numbu. The highest number of F1 progeny was found in cv. Samurai 2 (541 adults), however, not significantly

Table 1. Seed Dimension of Several Sorghum Cultivars

Sorghum Cultivars	Seed dimension		
	Length (mm)	Width (mm)	Diameter (mm)
Samurai 1	4.08	3.94	2.82
Samurai 2	3.98	3.90	2.84
Pahat	3.97	3.98	2.89
Super 1	4.14	3.90	2.78
Super 2	4.85	3.91	2.68
Suri 3	4.95	4.08	2.77
Suri 4	4.20	3.92	2.19
Kawali	4.18	4.14	3.00
Numbu	4.18	4.07	2.81

different from those found in cv. Pahat, Super 1, and Super 2. The lowest number of F1 progeny was found in cv. Kawali (153.67 adults) but was also not significantly different from those found in cv. Suri 3, Suri 4, and Numbu. Cv. Samurai 1 reached up to 384.33 adults. Sorghum seeds cv. Samurai 2, Pahat, Super 1, and Super 2 were preferred by *S. oryzae* compared to sorghum seeds cv. Samurai 1, Suri 3, Suri 4, Numbu, and Kawali (Table 2). The preference level of *S. oryzae* on sorghum was shown on the number of F1 progeny appeared. This preference level of *S. oryzae* on sorghum cultivars can also be described, consecutively, as follows Samurai 2 = Pahat = Super 1 = Super 2 > Samurai 1 > Numbu = Suri 3 = Suri 4 = Kawali.

The difference in the number of adults might be determined by nutrient content and physical properties of each sorghum cultivar. These differences indicated that variability existed between the sorghum cultivars evaluated, allowing the identification of resistant cultivars. The difference in sorghum cultivars determines the appearance of F1 progeny due to differences in physical characteristics between them (Bamaiyi et al., 2007). These physical characteristics (pericarp texture, skin hardness, temperature and moisture content of sorghum seeds) are a source of resistance against *S. oryzae* (Gerema et al., 2017). Khan & Halder (2012) also revealed that the type, skin hardness and size of rice influenced the oviposition, reproduction, and development of *S. oryzae*. This result is similar to the findings of Prasad et al. (2015) reporting that the size of sorghum seeds determined the size and number of *S. oryzae* progeny. The adults of this pest preferred sorghum with a bigger size, which is the best for laying their eggs compared to small sorghum ones. Sorghum cv. Samurai 1, Suri 3, Suri 4, Kawali, and Numbu exposed the characteristics preferred by *S. oryzae*.

Median Development Time

The results described in Table 2 show that different cultivars of sorghum significantly affected the median development time of *S. oryzae* during storage period (F = 13,47**; df = 8; P< 0,0001). The shortest median time development was shown by *S. oryzae* found in cv. Samurai 2 and Pahat (32.33 days), however, it was not significantly different from the median time development of the insects found in cv. Super 1 and Super 2. The longest median development time was observed in cv. Kawali (36.67 days) though there was no significant difference compared to those found in cv. Suri 3, Numbu, and Suri 4. Manwhile, the median development time observed in cv. Samurai 1 reached up to 34 days (Table 2).

The median development time from eggs to adults ranged from 32.33 to 36.67 days. This finding was slightly different from the research done by Bamaiyi et al. (2007) which found out that the median development time ranged from 32.97–42.97 days. The median time for development of *S. oryzae* in sorghum also has similarities to the development time of S. zeamais in the same stored product (Chuck-Hernández et al., 2013; Goftishu & Belete, 2014). Short median development time causes sorghum to be more susceptible to *S. oryzae*. According to Gerema et al. (2017), susceptible sor-

Table 2. Number of F_1 Progeny, Median Development Time, and Susceptibility Index of Sorghum Cultivars

Cultivars	Number of F1 progeny	Median development time (days)	Susceptibility index	Susceptibility category
Samurai 1	384.33 b	34 b	7.60 b	Moderate-susceptible
Samurai 2	541 a	32.33 c	8.45 a	Susceptible
Pahat	508.67 ab	32.33 c	8.37 a	Susceptible
Super 1	505 ab	32.67 bc	8.23 ab	Susceptible
Super 2	515.33 ab	33.67 bc	8.03 ab	Susceptible
Suri 3	192.33 c	36.33 a	6.25 c	Moderate
Suri 4	165.33 c	35.67 a	6.20 c	Moderate
Kawali	153.67 c	36.67 a	5.94 c	Moderate
Numbu	236.67 c	36 a	6.59 c	Moderate

Remarks: Means in the same column followed by the same letters do not differ significantly (P = 0.05) as determined by DMRT at 5%.

ghum resulted from shorter median development of *S. oryzae* infesting it, and Chuck-Hernández et al. (2013) also revealed the similar results on the sorghum susceptibility to infestation of S. zeamais. Interestingly, the median development time of *S. oryzae* also influenced the number of eggs laid by *S. oryzae*. The short median development time causes a greater number of eggs laid and more adults to appear (Prasad et al., 2015). From these results, it can be concluded that shorter median development attributed to a greater number of F1 progeny, while the insects with longer development time produced a lower number of F1 progeny (Bamaiyi et al., 2007).

Susceptibility Index of Sorghum Cultivars

Table 2 showed that there were significant effects of sorghum cultivars of sorghum on the susceptibility index (F = 20.22**; df = 8; P < 0.0001). The highest susceptibility index was demonstrated by cv. Samurai 2 and Pahat, reaching 8.45 and 8.37 though it was not significantly different from the susceptibility index of cv. Super 1 and Super 2. Meanwhile, the lowest index was observed in varieties Kawali, Suri 4, Suri 3 and Numbu. According to these findings, cv. Samurai 2, Pahat, Super 1, and Super 2 were categorized as susceptible varieties, cv. Samurai 1 was moderate-susceptible, and cv. Kawali, Suri 4 and Suri 3 were moderate to the attack of *S. oryzae*. The susceptibility of sorghum seeds was also influenced by the number of F1 progeny (r = 0.988**; P <0.01), width of sorghum seeds (r = -0.726*; P <0.05), and median development time (r = -0,978**; P <0.01) (Table 4). This result was in accordance with that of Bamaiyi et al. (2007) and Goftishu & Belete (2014) reporting that the susceptibility of sorghum cultivars was influenced by the number of F1 progeny and median development times of *S. oryzae* and S. zeamais. A large number of F1 progeny and short median development time led to high susceptibility index, causing the sorghum to be more susceptible to both *S. oryzae* and S. zeamais. The results of this study showed that susceptibility of sorghum to *S. oryzae*

Table 3. Percentage of Weight Loss and Damaged Seeds of Different Sorghum Cultivars

Cultivars	Percentage of weight loss	Percentage of damaged seeds
Samurai 1	3.24 de	12 abc
Samurai 2	8.82 a	19.67 a
Pahat	6.37 abc	16.33 ab
Super 1	5.92 abcd	15.67 ab
Super 2	6,57 ab	18.67 a
Suri 3	3.65 bcde	9.33 bc
Suri 4	2.95 e	8 c
Kawali	3.62 bcde	8.76 c
Numbu	3.80 cde	9.33 bc

Remarks: Means in the same column followed by the same letters do not differ significantly (P = 0.05) as determined by DMRT at 5%.

Table 4. Correlation Coefficient Between Seed Length, Seed Width, Seed Diameter, Number of F_1 Progeny, Median Development Time, Percentage of Weight Loss, Percentage of Damaged Seeds and Susceptibility Index of Sorghum Cultivars

Characteristics	Seed length	Seed width	Seed diameter	Number of F_1 progeny	Median development time	Percentage of weight loss	Percentage of damaged seeds	Susceptibility Index
Seed length	1							
Seed width	0.190	1						
Seed diameter	-0.153	0.440	1					
Number of F_1 progeny	-0.208	-0.712	0.258	1				
Median development time	0.381	0.767**	-0.158	-0.944**	1			
Percentage of weight loss	-0.161	-0.518	0.282	0.851**	-0.694*	1		
Percentage of damaged seeds	-0.087	-0.679*	0.228	0.967**	-0.848**	0.933**	1	
Susceptibility Index	-0.283	-0.726*	0.245	0.988**	-0.978**	0.780**	0.921**	1

Remarks: ** Significant at 1% level, * significant at 5% level

was also influenced by physical characteristic of sorghum, which is the seed width. The physical characteristic of sorghum seeds is an indicator of its susceptibility to *S. oryzae*. According to Siwale et al. (2009), the resistance of seeds to insect is influenced by the physical characteristic. Physical characteristics of cereals are attributed to their sensitivity to the attack of S. zeamais (Akpodiete et al., 2015; Throne & Eubanks, 2015; Rahardjo et al., 2017).

Determination of Sorghum Losses

There was significant difference in sorghum losses between cultivars (Table 3). Each cultivar demonstrated significantly different losses compared to others such as weight loss (F = 3.73**; df = 8; P <0.0097) and damaged seeds (F = 3.55*; df = 8; P <0.0122). Sorghum damage during storage occurred mostly in cv. Samurai 2, Super 2, Pahat, and Super 1, while the least damage occurred in cv. Kawali, Suri 4, and Numbu. The damage is related to the feeding activities of larvae and adults by causing symptoms such as cracked and perforated seeds as well as the production of frass. The frass production disables sorghum seeds to be processed into livestock feed, and also, it is inappropriate for human consumption. Sorghum damage leads to their susceptibility. Sorghum with high damage is immensely susceptible to *S. oryzae*. The results of the correlation analysis showed that there was a significant positive correlation between the percentage of weight loss (r = 0.780**; P <0.01) and the percentage of damaged seeds (r = 0.921**; P <0.01) and the susceptibility of sorghum. Correlation between these characters indicated that heavy damage enables sorghum to be highly susceptible. Sorghum damage during storage period was also influenced by the number of F1, which affected the percentage of weight loss (r = 0.851 **; P <0.01) and the percentage of damaged sorghum (r = 0.967 **; P <0.01) (Table 4). It is in accordance with the results of Gerema et al. (2017). They reported that the number of F1 progeny of *S. oryzae* influenced the damage of sorghum and caused weight loss, which was positively correlated with the susceptibility index. Sorghum cv. Suri 3, Suri 4, Kawali, and Numbu were moderately susceptible to *S. oryzae*. These cultivars could be recommended as they exposed an important role in minimizing sorghum losses during storage period in the tropics.

CONCLUSIONS

These nine sorghum cultivars can be categorized from moderate to susceptible to infestations of *S. oryzae*. Cv. Suri 3, Suri 4, Kawali, and Numbu were categorized as moderate, while cv. Samurai 1 was

categorized as moderate to susceptible. Meanwhile, cv. Super 1, Super 2, Samurai 2, and Pahat were susceptible. The susceptible sorghum seeds are not recommended to be stored for long periods as it deteriorates further due to the attack of *S. oryzae*.

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Acceleration of *Echinacea purpurea* (L.) Moench Shoot Growth by Benzyl Adenine and Indole Butyric Acid Addition

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ABSTRACT

Echinacea (Echinacea purpurea (L.) Moench) is a medicinal plant known to boost the immune system. Propagation is necessary to increase production. One of the methods of propagation in tissue culture. This research was conducted to understand the most suitable concentration of plant growth regulators. The treatment was given a combination of Benzyl Adenine (BA) and Indole Butyric Acid (IBA) with BA concentration of 1 ppm, 2 ppm, 3 ppm and 4 ppm while the IBA concentration used was 1 ppm and 2 ppm. The next step was subculture by using the combination among IBA 0 ppm, 0.5 ppm, 0.75 ppm and BAP 0 ppm, 0.5 ppm, 0.75 ppm of BAP. The result showed that the most shoots produced by the combination treatment of BA 2 ppm and IBA 1 ppm while the highest shoot and leaf number is best produced in treatment BA 1 ppm and IBA 2 ppm. The largest number of shoots was shown by treatment BA 2 ppm and IBA 1 ppm. This study can be concluded that BA 1 ppm and IBA 2 ppm, and BA 2 ppm and IBA 1 ppm gave the best treatment for shoot growth and control for root induction.

Keywords: Echinacea purpurea, Echinacea, Benzyl Adenine, Indole Butyric Acid, Callus

ABSTRAK

Ekinase (*Echinacea purpurea* (L.) Moench) merupakan tumbuhan obat yang dikenal berkhasiat meningkatkan kekebalan tubuh. Peningkatkan produksi Ekinase sangat penting dilakukan yaitu dengan perbanyakan, salah satunya dengan kultur jaringan. Tujuan dari penelitian ini adalah untuk mengetahui konsentrasi zat pengatur tumbuh (ZPT) yang paling sesuai untuk pertumbuhan Ekinase. Perlakuan ZPT yang diberikan berupa kombinasi Benzil Adenin (BA) dan Indole Butyric Acid (IBA) dengan konsentrasi BA 1 ppm, 2 ppm, 3 ppm dan 4 ppm sedangkan konsentrasi IBA yang digunakan yaitu 1 ppm dan 2 ppm. Selanjutnya dilakukan subkultur dengan menggunakan IBA dan BAP dengan kombinasi 0 ppm, 0,5 ppm, 0,75 ppm dan BAP 0 ppm, 0,5 ppm, 0,75 ppm. Hasil penelitian menunjukkan bahwa tunas paling banyak pada perlakuan kombinasi BA 2 ppm dan IBA 1 ppm sedangkan tinggi tunas dan jumlah daun paling baik dihasilkan perlakuan BA 1 ppm dan IBA 2 ppm serta jumlah tunas paling banyak ditunjukkan oleh perlakuan BA 2 ppm dan IBA 1 ppm. Penelitian ini dapat menyimpulkan bahwa perlakuan ZPT paling tepat untuk pertumbuhan tunas *E. purpurea* adalah BA 1 ppm dan IBA 2 ppm dan BA 2 ppm dan IBA 1 ppm. Sementara, pertumbuhan akar terbaik dihasilkan oleh kontrol.

Kata Kunci: Echinacea purpurea, Benzyl Adenin, Indole Butyric Acid, Kalus

INTRODUCTION

Echinacea (Echinacea purpurea (L.) Moench) is one of the medicinal plants widely used in the pharmaceutical industry. Echinacea is an introduced crop originating from America. This plant which is a member of the Asteraceae family. The plant is used extensively as a raw material by the pharmaceutical industry in Indonesia and is produced in the form of drugs, multivitamins, and energy drinks. The active ingredient in Echinacea consists of alkylamide, polyacetylene, caffeine acid esters, cichoric acid, polysaccharides and flavonoids such as kaempferol, quercetin, and isorhamnetin. Echinacea also contains several types of phenolic acids such as p-kumarat, p-hydroxybenzoate and p-protocatechuic (Kumar & Ramaiah, 2011; Manayi, et al., 2015). Almost all parts of Echinacea have medicinal properties, some of which are as immune enhancers, and to treat respiratory infections, urinary tract infections, colds, and arthritis (Alamgir and Uddin, 2010; Hudson, 2012). Recently, the research has been conducted on the possibility of Echinacea as an HIV therapy material. Several HIV patients choose to use Echinacea as the herbal remedy due to its immunostimulatory properties as hypothesized in various studies (Moltó et al., 2012).

The need of Echinacea plants is estimated will continue to increase each year. However, Echinacea in Indonesia is still imported. To obtain the medi-

cine raw materials, domestic production in Indonesia is necessary. Indonesian Minister of Health Regulation No. 88 of 2013 concerning the master plan for the development of pharmaceutical raw materials, it is stated that to produce raw materials for traditional medicines in order to meet the needs of domestic raw materials guaranteed in high quality, it is necessary to increase the development and production of traditional pharmaceutical raw materials in the country and reduce the number of imports (Menkes, 2013). Thus, Indonesia can be independent of pharmaceutical raw materials and not depend on other countries.

Echinacea plants can grow well at an altitude of 450-1,100 m asl with a soil pH of 5.5 - 7.5. Echinacea cultivation requires full sun with loose soil and enough water (Raharjo, 2005). Echinacea is widely cultivated using seeds. However, the use of seeds has several obstacles, such as seeds provide a variety of germination responses, seeds collected from various regions require special treatment for the maintenance, seeds availability depend on the season, and the resulting plants from seeds are not free from pathogens (Abbasi et al., 2007). According to Raharjo (2005) plant death is caused by a virus attack or root rot due to root fungus. Therefore, it is necessary to propagate through tissue culture, instead of using seeds so the explant growth is not season-dependent and is pathogen free.

This study uses a Plant Growth Regulator (PGR) that consists of Benzyl Adenine (BA) and Indole Butyric Acid (IBA). PGR is an organic compound instead of nutrient which in the low concentration can give effect on the plant growth and development (Hariadi et al. 2019). According to Niedz and Evens (2011) BA is one type of cytokinin hormone that plays a role in stem organogenesis. Whereas according to Sidhu (2010) cytokinins plays a role in cell division, shoot induction and cell proliferation. In this study the hormone is combined with IBA, which is a type of auxin hormone. According to Sidhu (2010) auxin plays a role in cell division, cell elongation and root differentiation. This study aims to determine the effect of BA and IBA on the growth of *E. purpurea* shoots and knowing the concentration of PGR which most influences the growth of the plant. BA is one type of cytokinin that has a strong and effective activity to stimulate the multiplication of shoots because it has a benzyl group while IBA at low concentrations can produce root growth (Lestari 2011). Research conducted by Mechanda, Baum, and Johnson (2003) produced shoot growth of 53.3 % while the research conducted by Yusnita et al. (2013) applied BA 5 ppm and IBA 2000 ppm with Sansivera (Sansevieria trifasciata) explants for 3 months resulting in shoots 3.5 stems in 3 months and 19,7 roots in 2 months. Based on these studies it can be seen that BA is a PGR that produces shoot growth of shoots growth better than other PGR types of cytokines such as kinetin. BA is important tested on Echinacea because other PGRs have not been able to produce shoots of Echinacea. In the study conducted by Sudrajad and Saryanto (2011) using Ekinase leaf as explant with single PGR consist of BA 1,2,3 and 4 mg/l produced callus growth without shoots. Therefore it is needed research of BA combination with auxin for shoot growth.

MATERIALS AND METHODS

The study was conducted at the Center for Research and Development of Medicinal Plants and Traditional Medicines in June 2017.

Experimental Design

This study applied a completely randomized design (CRD) with test parameters including the number of callus, number of shoots, number of leaves, and number of roots. In this study two stages of research were carried out, namely shoot growth

and root growth. The treatments used for shoot growth were a combination of 1 ppm, 2 ppm, 3 ppm, and 4 ppm BA with a combination of 1ppm and 2 ppm IBA for shoot growth media. The treatments for root growth were PGR combination of 0.25 ppm, 0.5 ppm, 0.75 ppm and 1 ppm BAP and 0.25 ppm, 0.5 ppm, 0.75 ppm and 1 ppm IBA. This study used 3 replications for each treatment and no single ZPT treatment was used because the study only observed the effect of PGR combinations to obtain the best combination.

Stock Solution Making

The stock solutions consist of macronutrient, micronutrients, iron, myoinositol, and vitamins. Each macronutrient stock consisted of NH_4NO_3 16.5 g, KNO_3 19 g, $CaCl_2.2H_2O$ 4.4 g, $MgSO_4.7H_2O$ 3.7 g, and KH_2PO_4 1.7 g. The reagent used is the Merck brand. Each reagent was dissolved in 10 ml of sterile distilled water and stirred using the IKA C-Mag HS brand stirrer 7. Micronutrient stock consisted of H_3BO_3 0.062g, $MnSO_4.4H_2O$ 0.223 g, $ZnSO_4.7H_2O$ 0.086 g, $NaMoO_4.2H_2O$ 0.00025 g, $CuSO_4.5H_2O$ 0.00025 g, $CoCl_2.6H_2O$ 0.00025 g and Kl 0.0083 g dissolved in 100 ml sterile distilled water. Iron stock consists of $FeSO_4.7H_2O$ 0.270 g, $Na_2EDTA.2H_2O$. 0.373 g which is dissolved in sterile distilled water. The myoinositol stock solution was made by dissolving 100 ml of sterile distilled water. The vitamin stock consists of nicotinic acid 0.005 g, pirodoxin HCl 0.005 g, Thiamin HCl 0.001 g, and Glisine 0.02 g dissolved in 100 ml sterile distilled water. The PGR used is the Merck brand. The PGR stock solution was made by dissolving 0.1 mg of PGR with 100 ml of sterile distilled water then stirred using a stirrer.

Culture Media

Culture media was made by mixing a stock solution of 10 ml with 30 g of sucrose, 0.01 g of PVP and 7.5 g of gelatine. Then the culture media was added with 1 liter of distilled water and heated using a hotplate and stirred using a stirrer. The pH of the culture medium was adjusted to 5.6 by adding NaOH to reduce acidity or adding HCl to increase acidity. Then the ZPT was added to the concentration that was previously determined. The culture media was sterilized in Autoclave brand of Hirayama HL-AE series Vertical Autoclave for 30 minutes at 121 °C and 1 atm pressure.

Explant Selection

The explants used were echinace leaves obtained from the greenhouse of the Center for Research and Development of Medicinal Plants and Traditional Medicine. The conditions for selecting explants are leaves that are young, growing healthy, free of pests and diseases.

Explant Sterilization

Echinace leaf was soaked in detergent for 3 minutes and rinsed three times using distilled water. The rinsed leaves were soaked in 0.5 g bactericide solution and rinsed three times using distilled water. The leaves soaked in 0.5 g fungicide and rinsed three times using distilled water. Then rinsed with distilled water three times and the explants were moved into Laminar Air Flow (LAF) ESCO brand Airstream vertical laminar Flow Clean Benches LVG. In LAF sterilization was soaked in 70% alcohol for 7 minutes then rinsed 3 times with distilled water. The explants soaked in 20% sodium hypochlorite for 15 minutes and rinsed. The explants soaked into 20% tween for 2 drops for 3 minutes and refracting. After sterile explants are ready to be planted.

Explant Transferring

Explants were cut into small pieces using a scalpel and then passed over the bunsen flame. Then the explants were transferred into a bottle that has been filled with culture media.

Incubation

The explants were incubated for 1 month in an incubation room with a temperature of 23 ° C and light for 24 hours. During incubation, observation of growth were carried out. The parameters used included growing time, number of shoots, number of leaves, plant height, callus color, number of callus, and number of roots.

Subculture

After incubation for 1 month, then subcultures were carried out with MS base media and ZPT combination in the form of IBA and BAP.

Data Analysis

All variables were tested statistically using the Analyze of Variant and if there were real or very real differences, it would be continued with the Duncan difference test at the 5% level.

RESULTS AND DISCUSSION

The results showed that BA 2 ppm + IBA 1 ppm treatment produced the fastest growth of callus for 8 days. Research conducted by Sudrajad and Saryanto (2011) using BAP can produce faster callus growth in an average of 5-7 days but no shoot growth occurs. At a lower concentration of BA, the results of long growth were 12.3 days while that of the highest BA treatment obtained callus growth at 10.33 days. Meanwhile, according to statistical tests, there was a significant difference between the growth of callus given the treatment of BA and IBA with controls (Table 1).

The combination treatment of BA 2 + IBA 1 produces the fastest callus growth. The combination PGR of BA2 + IBA1 is the most appropriate combination so that it gives the fastest growth result. In the low concentration BA, callus growth rapidly while in the high concentration callus grows slowly. Slow growth can be caused by excessive PGR concentrations that inhibit explant growth. According to Agustina (2015) at low concentrations, PGR can encourage growth, but at high concentrations, ZPT can inhibit growth and even cause death in plants.

Table 1. Growth of *Echinacea purpurea* (L.) Moench Callus with the combination of BA and IBA

Treatment	Growing time (in days)		
BA0 + IBA0	-	±	0.00
BA1 + IBA1	12.3	±	0.00
BA1 + IBA 2	9.67	±	2.52
BA2 +IBA 1	8	±	2.08
BA2 + IBA 2	10	±	2.00
BA3 + IBA 1	11	±	2.00
BA3 + IBA 2	13.66	±	2.65
BA4 + IBA 1	13.33	±	2.52
BA 4+IBA 2	10.33	±	2.52

Callus can form due to plant response to a wound. Callus formation comes from various types of cells that growth is stimulated by growth regulators and in subsequent growth can result in the formation of new organs or tissues (Ikeuchi et al., 2013). Callus initiation begins with the growth of parenchymal cells in the form of protuberances found in the epidermis or the bottom of the explant that is wounded. The bulge causes swelling of the tissue around the wound and grows into the middle of the explant. Furthermore, the tissue expands and the number is increasing (Hidayat 2007),

The results showed that callus was abundant in almost all treatments (Figure 1). Callus in small amounts was found in the treatment of BA1 + IBA1, BA2 + IBA2, and BA4 + IBA1. All the calluses were dark green (Table 2). Green callus arises from interactions between cytokinins and auxins that play a role in the formation of chlorophyll. Green callus shows that the callus contains a lot of chlorophyll while the white callus shows that the callus has begun to degrade chlorophyll but its growth is still good. A brown callus indicates that the cell has been physiologically degraded due to a lack of nutrients or growth hormones (Darmawati et al., 2013; Mahadi et al., 2013)

The highest number of shoots was found in the BA2 + IBA1 treatment while the least number was in the control treatment and BA 4+ IBA 1 with no shoot growth (Table 2). BA and IBA at high concentrations gave quite good results, with 2.67 buds while at low concentrations produced 1.67 buds . In the treatment of BA 4 + IBA 1 produced callus growth without shoot growth and slow callus growth. It is thought that this was caused by a combination of ZPT which was not appropriate for callus growth. According to Indah and Ermavitalini (2013) the slow formation of callus is due to inappropriate ZPT administration so that the endogenous and exogenous hormones present in the explants cannot stimulate callus growth quickly. High concentration cytokinin in BA4+IBA1 resulted in no shoot grow. The supraoptimal concentration of cytokinins causes the plant not to be affected or can be damaged. This is due to the use of PGR more than optimal concentrations of both cytokines and auxins that will inhibit growth (Dinarti et al., 2010; Rosmaina dan Aryani, 2015; Sukmadjaja dan Mulyana, 2011). According to statistical tests, there is a significant difference between the number of shoots that are given the treatment of BA and IBA with controls.

The plants that had the highest shoot were the BA1 + IBA2 treatment with a height of 1.56 cm

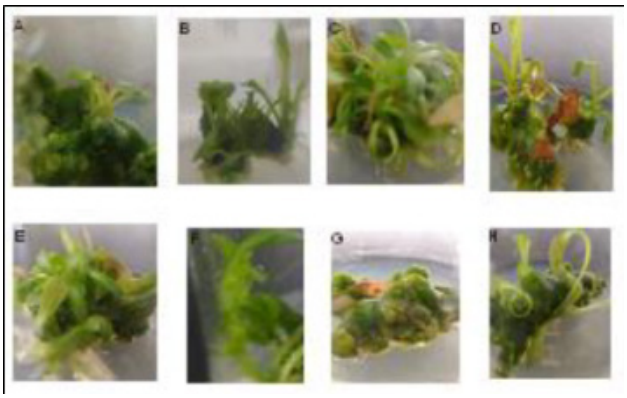

Figure 1. Results of shoot growth with BA and IBA treatment after 1 month. Results of Growth of Buds with BA and IBA treatment after 1 month with treatment a) BA1 + IBA1 b) BA1 + IBA2 c) BA2 + IBA1 d) BA2 + IBA2 e) BA3 + IBA1 f) BA3 + IBA2 g) BA4 + IBA1 h) BA4 + IBA2

while the lowest was found in the BA1 + IBA1 treatment with a height of 0,45 cm.. The results of shoot growth between BA with high concentrations and low concentrations did not differ so much. At the lowest BA and IBA concentrations obtained 0.45 cm height while at BA and IBA concentrations obtained a height of 0.67 cm. The high concentrations of IBA result in higher shoot sizes. Elongation of stems caused by division, elongation, and enlargement of cells in the apical meristem and stem segments so that plants grow taller (Widyastoety 2014). Auxin concentration gives effect to cell elongation. This hormone can stimulate protein synthesis and increase cell wall permeability,

Table 2. Shoot and Callus Growth of *Echinacea purpurea* (L.) Moench with a combination of BA and IBA after 1 month

Treatment	Number of shoot (shoot)	Plant height (cm)	Number of callus	Callus collor
BA0 + IBA0	0 ± 0,00	0 ± 0,00	0	-
BA1 + IBA1	1.67 ± 0.58	0.45 ± 0.05	++	Dark green
BA1 + IBA 2	3.33 ± 2.08	1.56 ± 0.14	+++	Dark green
BA2 + IBA 1	4.67 ± 0.58	0.8 ± 0.61	++	Dark green
BA2 + IBA 2	3.33 ± 1.15	1.36 ± 0.2	+++	Dark green
BA3 + IBA 1	1.6 ± 0.58	0.8 ± 0.9	+++	Dark green
BA3 + IBA 2	2 ± 1.00	0.6 ± 0.08	+++	Dark green
BA4 + IBA 1	0 ± 0.00	0 ± 0.00	++	Dark green
BA4 + IBA 2	2.67 ± 1.53	0.67 ± 0.31	+++	Dark green

Remarks : + Little
++ Medium
+++ Much

stimulate cell division and cell elongation so that it affects plant height. Stem elongation occurs due to division, elongation, and enlargement of new cells that occur in the apical meristem and stem segments so that plants grow tall (Rout et al., 2006; Santosa dan Soekendarsi, 2018). The BA1 + IBA1 treatment produces the smallest shoots. PGR concentration may be too low. According to statistical tests, there is a significant difference between the height of plants given the treatment and controls.The highest number of shoots was found in the BA2 + IBA2 treatment with 4.67 shoots. It is estimated that the treatment of BA 2 + IBA 2 is the optimal concentration for bud formation. The results showed that the increasing concentration of cytokinins, the number of shoot growth decreased. These results are consistent with research conducted by Tajuddin, et al., (2015) using sago explants with the addition of NAA and BAP. In the study, the increase in BAP resulted in a drastic reduction in the number of shots while at lower concentrations the percentage of shoots was higher. According to Menurut Moore (1997) dan Wattimena (1988) in Rahmi et al., (2010) PGR with high concentrations does not help growth but inhibits growth because there is no balance of exogenous growth regulators and endogenous hormones present in explants so cell division is inhibited. The process depends on the ability of explants to receive exogenous ZPT.

The highest number of leaves was found in the BA1 + IBA2 while the least amount was in the BA3 + IBA1 and BA4 + IBA2 (Table 3). BA at the highest and lowest concentrations did not show results that differed greatly. In the treatment of BA1 + IBA1, the number of leaves was 7.33 strands, while in BA4 + IBA2, the number of leaves was 6 strands. In the BA4 + IBA1 treatment, only callus growth and no shoot growth occurred. Whereas the control did not occur in any growth. The results of shoot growth can be seen in Table 2.

Table 3. Growth *Echinacea purpurea* (L.) Moench Leaves with Combination of BA and IBA

Treatment	Number of leaves		
BA0 + IBA0	0	±	0.00
BA1 + IBA1	7.33	±	3.06
BA1 + IBA 2	10.67	±	7.51
BA2 +IBA 1	9	±	5.57
BA2 + IBA 2	6.3	±	2.31
BA3 + IBA 1	6	±	5.29
BA3 + IBA 2	8.33	±	7.57
BA4 + IBA 1	0	±	0.00
BA 4+IBA 2	10.33	±	2.52

The highest number of leaves was found in the BA1 + IBA2 treatment with an average number of leaves of 10.67 strands. Auxin and cytokines in the right amount can increase cell division to form plant organs (Rahayu, Solichatun, and Endang 2003). According to statistical tests, there was no significant difference between the number of leaves given the treatment and controls.

After the emergence of shoot growth subcultures were carried out into BAP and IBA media to obtain shoot and root growth. According to research conducted by Salim et al., (2010), IBA can increase the number of secondary roots, root length, stimulate root formation and enlargement. The subculture with BAP and IBA treatments resulted in root growth only in the control treatment with 6.67 strands (Figure 2) while other treatments did not produce root growth. According to statistical tests, there is a significant difference between the number of roots given in the treatment of BA and

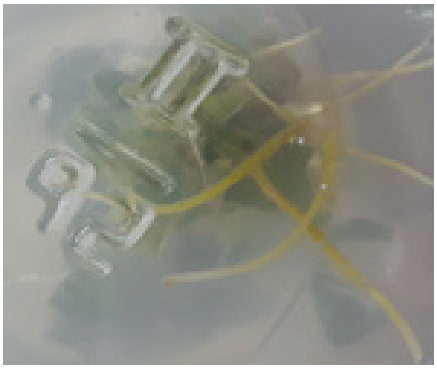

Figure 2. Root Growth after 1 Month in the Control Treatment

Table 4. Root Growth with IBA and BAP treatment after 1 month

Treatment	Number of roots (sheets)		
BA0 + IBA0	6.67	±	1.53
BA1 + IBA1	0	±	0.00
BA1 + IBA 2	0	±	0.00
BA2 +IBA 1	0	±	0.00
BA2 + IBA 2	0	±	0.00
BA3 + IBA 1	0	±	0.00
BA3 + IBA 2	0	±	0.00
BA4 + IBA 1	0	±	0.00
BA 4+IBA 2	0	±	0.00

IBA with controls. The results of the subculture are shown in Table 4.

Treatment without PGR (control) resulting in root growth. It caused by explants in the control that have high endogenous hormones and are sufficient for root growth without the need for additional growth regulators in culture media. According to Sulichantini (2016) explants can have a meristem tissue that is actively dividing and rich in endogenous growth-regulating substances so that it can trigger growth without the need for exogenous PGR.

Auxin can influence the root cell elongation process by initiating cell elongation. This hormone affects the flexing of the cell wall. Auxin affects the H+ ion pump to the cell wall by stimulating certain proteins in the plasma membrane. The H+ ion activates certain enzymes so that the hydrogen crosslinking that arrange the cell wall breaks. Cells are getting longer due to water enters by osmosis. Cells continue to growth by synthesizing the constituent material of the cell wall and cytoplasm (Kusumo (1990) in Yuliawan (2019). Although able to increase the number of roots, auxin can also inhibit root growth if the concentration is excessive. Excess auxin is toxic to plants because it disturbs the plant's cell division process. Abundant nitrogen found in media combined with various PGRs, especially auxin, will form amino acids that inhibit root growth (Putra and Shofi 2015). Treatment with PGR in the culture media did not produce root growth because explants could not absorb nutrients in the culture media so that it grew stunted.

CONCLUSION

The conclusion is BA and IBA influences the growth of shoots of Echinacea purpurea (L.) Moench). The most appropriate PGR treatments for the growth of these plant shoots were BA1 ppm+IBA 2 ppm and BA 2 ppm+IBA 1 ppm, whereas for root growth control produced the best growth. Further research is needed regarding tissue culture of Echinacea along with levels of flavonoids in callus at various concentrations of growth regulators.

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The Diversity of Rot Fungi from Cocoa Plantation and Its Ability to Grow on Carbon Source Media

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ABSTRACT

Rot fungi are microorganisms that can degrade biomass, especially biomass containing carbon. This fungus can decompose wood components (lignocelluloses) into simpler compounds. This research aimed to determine the diversity of rot fungi that have fruit body and grow in cocoa plantation as well as to observe their morphology and ability to grow on carbon source media. Fruiting body was taken from decayed cocoa stems from the farmers' cocoa plantation in Bila Village, Pitu Riase, Sidrap Regency, South Sulawesi. The fruiting body then was sterilized and grown on the PDA medium.. The isolates then were morphologically characterized and grown on a solid Czapek dox medium containing carbon source of lignin, chitin, cellulose, and pectin. The rot fungi from Basidiomycota found were Mycena spp, *Lycoperdon* spp, *Auricularia* spp, *Schizophyllum* spp, *Coprinus* spp, *Tremella* spp, *Crepidopus* spp, *Trametes* spp, *and Pleurotus* spp. *The different growth abilities were characterized by the large diameter of the colony formed. The highest colony diameter of Lycoperdon* spp *was on cellulose media, while that of Tremella* spp *was on the three other media. The results show that the rot fungi from cocoa plant have a large potential to be used as biodecomposer.*

Keywords: Biomass, Chitin,Lignin, Pectin, Tremella sp.

ABSTRAK

Cendawan pelapuk adalah mikroorganisme yang mempunyai kemampuan untuk mendegradasi biomassa, terutama biomassa yang mengandung karbon. Cendawan ini dapat menguraikan komponen kayu (lignosellulosa) menjadi senyawa yang lebih sederhana. Tujuan penelitian ini adalah untuk mengetahui keragaman cendawan pelapuk yang mempunyai tubuh buah dan tumbuh di pertanaman kakao. Selain itu untuk melihat karakterisasi morfologinya dan kemampuannya untuk tumbuh pada media sumber karbon. Tubuh buah cendawan pelapuk diambil dari batang kakao yang telah mati dari pertanaman kakao milik rakyat di Desa Bila, Kecamatan Pitu Riase, Kabupaten Sidrap, Sulawesi Selatan. Tubuh buah kemudian disterilkan dan ditumbuhkan pada media PDA secara aseptik dalam laminar air flow. Isolat kemudian dikarakterisasi secara morfologi. Isolat juga ditumbuhkan pada media Czapek dox padat yang ditambahkan sumber karbon berupa lignin, kitin, selulosa, dan pektin. Hasil penelitan menunjukkan cendawan pelapuk berasal dari Divisi Basidiomycota, yaitu *Mycena* spp, *Lycoperdon* spp, *Auricularia* spp, *Schizophyllum* spp, *Coprinus* spp, *Tremella* spp, *Crepidopus* spp, *Trametes* spp, dan *Pleurotus* spp. Cendawan-cendawan tersebut mempunyai kemampuan tumbuh yang berbeda-beda ditandai dengan besarnya diameter koloni yang terbentuk. Cendawan *Lycoperdon* spp mempunyai diameter koloni tertinggi pada media dengan selulosa sebagai sumber karbonnya. Sedangkan cendawan *Tremella* spp mempunyai kemampuan tumbuh tertinggi pada 3 media sumber karbon lainnya, yaitu Lignin, Kitin, dan Pektin. Hal ini menunjukkan bahwa cendawan pelapuk dari tanaman kakao berpotensi besar untuk dijadikan biodekomposer.

Kata Kunci: Biomassa, Kitin, Lignin, Pectin, *Tremella* sp.

INTRODUCTION

There are three groups of fungi in the nature that can decompose the components of wood (lignocellulose), namely brown rot, white rot, and soft rot. This grouping of rot fungi is based on the results of decomposing processes. Brown rot fungi produce brown residue, white rot fungi produce white residue, while the soft rot fungi produce residue which is like slimy. The three types of fungi have different characteristics. White rot fungi have the ability to degrade high lignin by causing a small loss of cellulose. The ability of white rot fungi to break down cell walls is better than other groups of organisms (Schmidt, 2006). The ability of the white rot fungi to degrade can be used to decompose the carbon contained in cocoa pod husk waste. Thus, it can be used as a source of plant nutrients.

White rot fungi are grouped based on microscopic characteristics of white pockets, white spots, and white filamentous. This is influenced by fungus species, wood species, and ecological conditions. In the decomposition process by white rot fungi, carbohydrates and lignin are degraded

at the same time and at the same level during all stages of decomposition. The decay of the cell wall begins by producing micro hyphae holes on the secondary wall (Schmidt and Liese, 1966), which flow together for larger wall openings by expanding decay. Hyphae grow in the lumen close to the tertiary wall. Hyphae are covered by a layer of mucus that secretes active substances within only a direct distance of hyphae. Thus, the lysis zone of the hypha develops below, and the hypha produces grooves in the wall, which gradually decrease in thickness, such as rivers eroding the soil (Liese, 1970).

Many white rot fungi produce extracellular phenol oxidase, which is produced in positive oxidase tests on nutrients so that tannic and gallic acids are used. Only 40% of white rot fungi produce a combination of lignin peroxidase and manganese peroxidase, while a combination of manganese peroxidase and laccase is more common. *Pycnoporus cinnabarinus*, in extreme cases, only produces laccase, not lignin and manganese peroxidase (Li, 2003 in Schmidt, 2006). This study aimed to determine the diversity of rot fungi isolated from cocoa plantations as well as to observe the ability of the rot fungi to grow on carbon source media, which is one indicator of the fungus ability decompose organic materials.

MATERIALS AND METHODS

Isolation and Screening of Rot Fungi Isolates

The fruiting body of the fungi growing on the decomposed cocoa plants was taken using tweezers and put into a paper envelope. The body of the fruit was brought to the laboratory then cut into pieces with a size of 1x1 cm. The fruit body was rinsed with sterile water then dipped in 70% alcohol for 2 seconds. The fruit body then was rinsed again with sterile water and then isolated on PDA media for screening. Screening was done by purifying and separating fungi based on their morphological characteristics.

The Growth of Rot Fungi Isolates on Carbon Source Media

This testing used solid Czapek dox media. The media were added with 0.1% Remazol Brillant Blue and divided into 4 parts. Each part was added with carbon source substrate, namely Carboxy Metil Cellulose (CMC) as source of cellulose, Lignin quaicol benomyl as source of lignin, crab shell as source of chitin, and sawdust as source of pectin. The media were homogenized on the hot plate stirrer for 15 minutes, then sterilized in autoclave for 2 hours. After cold, the media were poured on a sterile petri dish in Laminar Air Flow. Fungi isolates were cut using a cork borer, grown on the media, and incubated in a dark place for 7 days. There were 60 combinations of treatments (6 types of media and 10 types of isolates), each treatment combination consisted of 2 replications, resulting in 120 units of observation. Based on the method from Wirth & Wolf (1990), two to three days after cultivation, a bright colored zone around the culture will be formed. Growing ability was obtained by measuring the diameter of the colony 7 days after incubation.

RESULTS AND DISCUSSION

Isolation and characterization of Rot fungi from cocoa plantations

There were 10 isolates of rot fungi successfully isolated from cocoa plantations. Morphological characterization is shown in Table 1. Table 1 clearly shows that there were 10 types of isolates obtained from cocoa plantations. Each isolate was pure white to orange with a smooth to rough texture. The rot fungi found were included in the Basidiomycota. Basidiomycota have a large fruit body so they are easy to observe. The fruit body consists of a hood (pileus), blades (lamella), and stalk or stipe (Webster & Weber, 2007).

Colonies of rot fungi isolates varied, although all isolates were grown on the same medium, which was the potato dextrose agar (PDA) medium. The

Table 1. Morphological characterization of rot fungi isolates isolated from cocoa plantations

Rot Fungi	Morphological characterization				
	Colony color up	Colony color down	Shape	Texture	Exudate drops
Mycena sp	Lily-white	White	rounded compact	smooth	have
Lycoperdon sp	Yellowish white	White	edging serrated	slightly rough	not
Auricularia sp	smooth orange	yellowish	ring shape	smooth	not
Schizophyllum sp	yellow	yellowish	ring shape	smooth	have
Coprinus sp	white	white	rounded edges	smooth	not
Tremella sp	grey white	greyish	filamentous	slightly rough	not
Crepidotus sp	lily-white	white	dense edge	smooth	have
Tremetes sp	yellowish white	pale yellow	rounded	smooth	not
Pleurotus sp	white	white	filamentous	smooth	not

variety of color, shape, and texture of the rot fungi colonies obtained in this study were closely related to genetic factors. According to Baon, Wedhastri, & Kurniawan (2012), besides being influenced by genetics, variations in colonies may be caused by environmental conditions in the sample area and growth media, including carbon sources, temperature, and pH. Although the fungi were taken from the same cocoa plantation, the weather conditions and decayed wood media where the fungi grow were different. Color differences in colony can be influenced by temperature during the laboratory tests and the availability of nutrients in the medium (Ambar et al., 2010; Rozlianah & Sariah, 2006).

Growing Ability on Carbon Source Media

All rot fungi isolates obtained from cocoa plantations showed the ability to grow on carbon source media. This was indicated by the formation of a bright zone (Figure 1). The bright zone showed the presence of lignocellulosic enzymes produced by fungi to grow and degrade the carbon sources in the media. Enzyme is one of the secondary metabolites produced by fungi. Garraway and Evans (1984) state that the secondary metabolite production phase occurs due to unfavorable environmental conditions. Some reports showed that the growth of fungi on a solid substrate was different when grown in liquid media because of the presence of surface phenomena, moisture, and chemical composition of the substrate. This will affect the production of lignin peroxide (LiP) which is also a secondary metabolite (Hatakka, 1983).

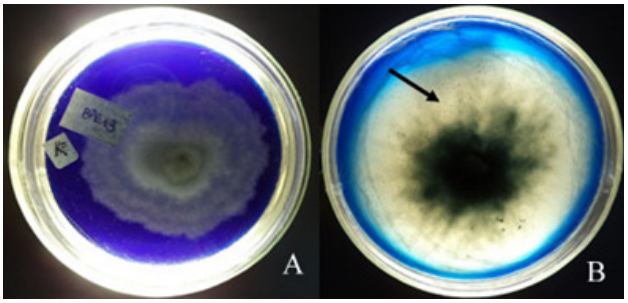

Figure 1. Bright zones (arrows) indicating the ability of fungi to grow on carbon source media (B) compared to without carbon sources as control (A).

The rot fungi isolates were grown on solid Czapek dox media added with carbon sources, namely lignin, chitin, cellulose, and pectin. The types of isolates and types of carbon source media had a very significant effect on the diameter of the rot fungi colonies. However, the interaction effect between the two treatments was not significant. There were no significant differences between the fungi grown on the media, but the diameter of the colony isolates of the fungi was the highest in *Tremella* sp, which was 6.96 cm (Table 2). *Tremella* sp has an orange, thin, and chewy fruiting body. This fungus is in the Agaricomycotina subdivision, Tremellomycetes, class and Tremellales order (Chen, 1998). The fruiting body is yellow, orange, or brownish. Intensity variations of fruiting body color can be caused by variations in carotenoids

Table 2. Colony diameter (cm) of various rot fungi isolates from cocoa plantations on carbon source media at 7 days after incubation

Rot Fungi	Diameter (cm) of colonies in carbon source media			
	Lignin	Chitin	Cellulose	Pectin
Mycena sp	6.08	5.03	4.83	4.00
Lycoperdon sp	6.12	6.78	7.22	3.13
Auricularia sp	3.47	2.95	2.20	3.67
Schizophyllum sp	4.23	4.30	4.20	3.73
Coprinus sp	3.83	4.48	5.27	2.93
Tremella sp	6.98	7.35	6.27	7.23
Crepidotus sp	3.83	5.60	5.18	3.00
Tremetes sp	2.72	1.10	1.10	1.10
Pleurotus sp	4.10	6.06	7.00	4.27
Average	4.61 ab	5.01 a	5.02 a	3.78 b

Remarks: Means followed by the same letters were not significantly different according to DMRT at the level of $\alpha = 0.01$.

by light intensity (Wong, Fares, Zimmermann, Butler, & Wolfe, 2003). Tramella sp also had the highest colony diameter on the Potato Dextrose Agar (PDA) media and filled up the petridish on the third day (data not shown).

Table 2 clearly shows that *Tremella* sp fungi has the higher colony diameter on lignin, chitin, and pectin media than other isolates. This was related to the ability of *Tremella* sp to produce high phytohormones than others. The concentration of IAA and GA3 produced by *Tremella* sp was 2.44 µgL-1 and 4.11 µgL-1, respectively, while the IAA and GA3 produced by *Pleurotus* sp fungi were 2.44 µgL-1 and 4.11 µgL-1, consecutively (Rahim, Nasruddin, Kuswinanti, Asrul, & Rasyid, 2018). The ability to produce phytohormones is related to the ability to grow in carbon source media. This causes *Tremella* sp to have the potential to become a biodecomposer for organic media from agricultural waste, which is mostly composed of carbon compounds.

The growth of rot fungi isolates on pectin media was not significantly different from the growth on lignin media. However, it was significantly different from the growth on chitin and cellulose media. The four media used to grow isolates of rot fungi were the carbon sources. This showed the ability of fungi to grow on these media. Carbon sources were degraded to be used as energy and structural sources of fungal cells (Chang and Miles, 1989). Carbon compounds that can be used by fungi include monosaccharides, oligosaccharides, organic acids, alcohol, cellulose, and lignin. The most easily absorbed carbon source is glucose (Hendritomo, 2002).

The best growth of rot fungi was observed on media containing cellulose. This was indicated by the highest diameter of the colony, which was 5.02 cm, although it was not significantly different from that on chitin media, which was 5.01 cm (Table 2). High cellulose content will increase cellulase enzyme production, and this has an important relationship with the formation of the fruiting body (Sivaprakasam et al., 1994; Sulistyarini, 2003). Meanwhile, chitin is the second largest polymer after cellulose which functions to regulate the growth and development of fungi by controlling lysis and synthesis of cell walls and skeletons (Hamid R et al., 2013; Ayes et al., 1994), therefor, the addition of chitin to the media has various influences on the existed organisms (Sharp, 2013).

CONCLUSION

There were 10 isolates of rot fungi isolated from cacao plantation, namely *Mycena* spp, *Lycoperdon* spp, *Auricularia* spp, *Schizophyllum* spp, *Coprinus* spp, *Tremella* spp, *Crepidopus* spp, *Trametes* spp, and *Pleurotus* spp. The fungi have different growth abilities indicated by the diameter of the colonies formed. *Lycoperdon* spp has the highest colony diameter in the media with cellulose as its carbon source. Meanwhile, *Tremella* spp has the highest growth ability in the three other carbon source media, namely lignin, chitin, and pectin.

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Physical Characteristics of Active Packaging Based on Methyl Cellulose with The Addition of Glutaraldehyde and Klutuk Banana (*Musa balbisiana* Colla) Leaf Extract

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ABSTRACT

Klutuk banana leaf is widely used as food packaging material since it has large size and not easily torn. Many traditional packaging materials are recently abandoned, thus it is necessary to develop an active packaging having an advantage of synthetic packaging. This study aimed to create active packaging from methyl cellulose (MC) added with glutaraldehyde (GA) and Klutuk banana leaf extract (EDPK), as well as to determine its properties and antioxidants. The casting method with GA as a crosslinker and EDPK as an antioxidant agent was used to make film/active packaging. Banana Klutuk leaves were dried using cabinet dryer for 24 hours and the leaf powder was extracted using maseration method with methanol 80%. The extract was concentrated with a rotary evaporator to be tested for its antioxidant capacity. The results showed that the film with EDPK addition exhibited dark green color. Tensile strength values increased when EDPK and GA were added at concentrations of 30 and 45%. However, EDPK film elongation was not affected by GA addition. Addition of EDPK decreased thermal stability by 25-45 °C, whereas the addition of GA improved thermal stability by 5-25 °C. Antioxidants in banana leaf extract can migrate to 10 and 50% simulant ethanol.

Keywords: Active packaging, Antioxidant, Banana Leaf, Glutaraldehyde, Methyl Cellulose.

ABSTRAK

Daun pisang Klutuk banyak digunakan sebagai bahan pengemas makanan karena memiliki ukuran yang lebar dan tidak mudah sobek dibandingkan dengan daun pisang jenis lainnya. Dewasa ini, pengemas tradisional banyak ditinggalkan masyarakat, sehingga perlu dikembangkan suatu kemasan aktif yang memiliki keunggulan dari pengemas sintetis. Tujuan dari penelitian ini adalah membuat kemasan aktif dengan bahan utama *methyl cellulose* (MC) yang ditambahkan *glutaraldehyde* (GA) dan ekstrak daun pisang Klutuk (EDPK), kemudian dipelajari sifat fisik dan antioksidannya. Metode yang digunakan pada pembuatan film/kemasan aktif menggunakan metode *casting* dengan GA sebagai *crosslinker* dan ditambahkan EDPK sebagai bahan antioksidan. Daun pisang Klutuk dikeringkan menggunakan *cabinet dryer* selama 24 jam. Bubuk daun pisang diekstraksi dengan metode maserasi menggunakan methanol 80%. Ekstrak dipekatkan dengan *rotary evaporator* dan dianaisa antioksidannya. Dari penelitian yang dilakukan dapat disimpulkan bahwa warna film EDPK menunjukkan warna hijau tua seiring dengan penambahan EDPK. Nilai *tensil strength* meningkat ketika ditambahkan EDPK dan GA pada konsentrasi 30 dan 45% namun *elongation* film EDPK tidak dipengaruhi oleh penambahan GA. Penambahan EDPK menurunkan stabilitas termal sekitar 25-45 °C, sedangkan penambahan GA dapat meningkatkan stabilitas termal sekitar 5-25 °C. Antioksidan pada ekstrak daun pisang Klutuk dapat bermigrasi kedalam simulan etanol 10 dan 50%.

Kata Kunci: Antioksidan, Daun Pisang Klutuk, Glutaraldehydel, Kemasan Aktif, *Methyl Cellulose*

INTRODUCTION

Many regional foods in Indonesia use leaves such as banana leaves, teak leaves, guava leaves, "simpor" leaves, and others as packaging materials. Banana leaves are widely used as food packaging. Only a few types of banana leaves are commonly used as packaging, especially by Javanese people, including "Klutuk"-, "Kepok"-, "Raja Bandung/ Uter"- banana leaves. Food will have a certain taste when it is wrapped and steamed in banana leaves (Mohapatra et al., 2010). According to the previous study, banana leaf has antimicrobial and antioxidant activity. Banana leaf extract contains gallic acid type of catechin. Catechins are included in polyphenol group, and it is one of the antioxidant compounds (Sahaa et al., 2013). Since banana leaf extract contains antioxidant compounds, it can be used as an active compound that can be inserted into active packaging.

Traditional packaging has been largely abandoned because it is impractical and hard to find

in the modern market. People begin to switch to plastic as a packaging. However, plastic does not contribute flavors and active compounds to packaged food. Besides, plastic is unbiodegradable, making it not environmentally friendly. Therefore, natural polymers which are substituted with extract or active compound can be used for active packaging that are environmentally friendly.

The active packaging based on methyl cellulose (MC) is widely used because it is thermo-gelated, and it has good film making properties, such as oxygen efficiency, lipid barrier, good water vapor permeability, increased tensile strength and good solubility (Ayana and Turhan, 2009; Gracia et al., 2004; Hauser et al., 2015). According to Dicastillo et al. (2016), MC is a biopolymer that has environment friendly properties. The crosslinking material that is widely used in MC-based packaging is glutaraldehyde (GA). According to Hernandez-Munoz et al. (2004), crosslinking is one method that is often used to modify water-soluble polymers to achieve the desired properties. Some characteristics of polymers can be enhanced by crosslinking, such as increased permeability, increased swelling, and mechanical properties such as tensile strength and elongation (Aiedeh et al., 2006; Dicastillo et al., 2016). GA reacts quickly with amine groups at neutral pH, and it is more efficient than other aldehydes in terms of stability as a crosslinker thermally and chemically (Nimni et al., 1987; Okuda et al., 1991).

Active packaging is a package supplied by a compound in packaging or headspace packaging materials to improve the performance of the packaging system (Robertson, 2006). Some previous research results state that active packaging can actively respond to product changes or packaging environments, such as oxygen scavenger, CO2 absorbers or CO2 generators, ethanol emitters, ethylene absorbers, water absorbers, materials antimicrobial, flavor enhancer and photochromic (Vermeiren et al., 1999; Park et al., 2001; Kerry et al., 2006; Mahalik et al., 2010; Appendini and Hotchkiss., 2002). The natural active ingredients of maqui berry extract, chilean berry, murta fruit and leaves, β-tocopherol, tea leaf catechins and olive leaf extract have been incorporated into MC-based active packaging so that they can improve the performance of the active packaging system (Ayana and Turhan, 2009; Dicastillo et al., 2015; Dicastillo et al., 2016; Hauser et al., 2015; Noronha et al., 2014; Yu et al., 2014).

The objective of this study was to create active packaging with methyl cellulose (MC) as the main ingredient, which was added with glutaraldehyde (GA) and Klutuk banana leaf extract (EDPK), as well as to determine its properties and antioxidants. This effort is a form of packaging material development by utilizing local wisdom.

MATERIALS AND METHODS

Materials and Instruments

Klutuk banana leaves were harvested from banana orchard in Gamping, Yogyakarta. Methyl Cellulose (Sigma Aldrich, USA) and Glutaraldehyde (Merck, USA) were used for making active packaging. Several chemicals such as Polyethylene Glycol 400 (Merck, USA), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma Aldrich, USA), Gallic acid (Sigma Aldrich, USA), Quercetin (Sigma Aldrich, USA), and Folin Ciocalteu (Merck, Germany) were prepared for further analysis.

The apparatus used were Cabinet dryer (EYELA NDS-601D; Japan), Rotary vacuum evaporator (IKA RV 06-ML 1-B), UV-VIS Spectrophotometer (Spectronic 200), Chromameter (Konika Minolta CR-400), Universal Testing Machine (Zwick ZO.5; USA), Thermogravimetric Analysis (Diamond TG / DTA Perkin Elmer).

Klutuk Banana Leaf Extraction

The extraction of Klutuk banana leaves was carried out based on the method by Fitriani (2016). Banana leaves with good quality were taken (the leaves are green, not torn, clean and undamaged) from the second and third stems from the top of the trees. Banana leaves were washed using tap water and cut into 3x4 cm using scissors. Banana leaves were dried using a cabinet dryer at 50°C for 24 hours then mashed with a blender. A 100 g Klutuk banana leaf powder was soaked in 800 ml of 80% methanol for 48 hours at room temperature and filtered using filter cloth and Whatman paper number 41. Then, the supernatant was concentrated with a rotary vacuum evaporator at 40°C.

Active Packaging Preparation

Active packaging was prepared using the film casting method based on the method of Dicastillo et al. (2015 and 2016) with modifications. The main material used was methyl cellulose. A 0.75 g of methyl cellulose was dissolved in 100 ml of 70% ethanol at 70oC for 1 hour. Then glutaraldehyde 15, 30 and 45% (w/w) and 1 M HCl were added to reach pH 3 and then cooled. After the temperature of the solution reached 40o C, 50,000 ppm of Klutuk banana leaf extract (5, 10 and 15% (v/v)) and polyethylene glycol 400 (PEG 400) 25% (w/w) were added. The solution was poured in a glass tray 15x21x2 cm and put into a cabinet dryer at 50o C for 12 hours. The film obtained is called EDPK film (Klutuk banana leaf extract).

Characterization of Active Packaging

Optical Properties

The film color was determined using chromameter (Konika Minolta CR-400). The film was placed on top of the plate reader, then placed on the top of the chromameter. The plate reader was shot with light on the tool used. The color was expressed in L (lightness), a (appearance), and b (blueness). The color measurement of the film was carried out three times.

Mechanical Properties

Mechanical analysis of the film included tensile strength and elongation with Universal Testing Machine (Zwick ZO.5; USA). The specimen was placed on the center of the plate until it was locked. The speed was set to 10 mm/min then the machine was turned on. After the sample was broken, the machine stopped and graphical data appeared on the monitor. Three specimens were used to determine the average of mechanical parameters.

Thermogravimetric Analysis (TGA)

Thermogravimetric analysis was tested using TG/DTA (Diamond TG/DTA; Perkin Elmer). The sample was heated at a temperature range from 30 to 600°C with an increase of 10°C/minute to determine the evaporation process of volatile components and or degradation in the sample. For samples in the form of sheets or chunks, particle size reduction was carried out by measuring the cross-sectional area of the pan sample.

Release Studies of Active Packaging

A study of release on active packaging was based on Dicastillo et al. (2015). The films were soaked in food simulants. Two types of food simulants were used, namely 10% ethanol (a) as a simulation of aqueous foods and 50% ethanol (b) as simulation for fatty foods. The film was cut into 3 cm2 and placed in a vial than added with 5 ml simulant a and b. Migration studies was carried out at 40°C. Food simulants a and b were taken periodically 0, 2, 4, 6, 8, 12, 24, 48, and 72 hours. Analysis of antioxidant activity was performed using DPPH method to find out the active components of the released film into food simulants.

Data Analysis

The data obtained were statistically analyzed with IBM SPSS Statistics 20 and MS Excel 2007. Two-way ANOVA was used to compare the effect of Klutuk banana leaf extract and glutaraldehyde. The differences between treatments were tested using Duncan's Multiple Range Test (DMRT) with $p<0.05$.

RESULTS AND DISCUSSION

Optical Properties

EDPK films with higher concentration of Klutuk banana leaf extract showed increasingly darker , more red and more blue color. The result showed that the L and b value decreased significantly with the increase of Klutuk banana leaf extract (Table 1). Meanwhile, a value increased significantly with the increase of Klutuk banana leaf extract. Overall, EDPK films had a green to dark green color because Klutuk banana leaf extract was greenish black.

Table 1. The color of methyl cellulose-based film with the addition of glutaraldehyde and Klutuk banana leaf extract in various combinations

Sample	Color		
	*L	*a	*b
DP5_GA0	66.51 ± 0.14^{c1}	2.45 ± 0.03^{a1}	61.46 ± 0.09^{c2}
DP5_GA15	65.44 ± 0.48^{c1}	2.23 ± 0.04^{a2}	65.96 ± 0.19^{c23}
DP5_GA30	67.86 ± 1.13^{c2}	2.51 ± 0.06^{a3}	65.36 ± 0.15^{c3}
DP5_GA45	66.98 ± 0.42^{c3}	2.36 ± 0.05^{a4}	61.38 ± 0.46^{c1}
DP10_GA0	55.39 ± 0.42^{b1}	7.35 ± 0.05^{b1}	51.42 ± 0.17^{b2}
DP10_GA15	55.42 ± 0.33^{b1}	7.47 ± 0.02^{b2}	50.24 ± 0.43^{b23}
DP10_GA30	55.12 ± 0.10^{b2}	7.32 ± 0.06^{b3}	49.45 ± 0.12^{b3}
DP10_GA45	56.64 ± 0.08^{b3}	7.87 ± 0.07^{b4}	48.39 ± 0.18^{b1}
DP15_GA0	44.56 ± 0.24^{a1}	9.10 ± 0.01^{c1}	43.79 ± 0.22^{a2}
DP15_GA15	44.76 ± 0.06^{a1}	9.58 ± 0.06^{c2}	41.53 ± 0.09^{a23}
DP15_GA30	45.01 ± 0.64^{a2}	9.87 ± 0.06^{c3}	43.47 ± 0.95^{a3}
DP15_GA45	46.38 ± 0.35^{a3}	9.74 ± 0.05^{c4}	42.90 ± 0.52^{a1}

Remarks: Values followed by the same letters in the same column are not significantly different as affected by Klutuk banana leaf extract (DP) ($p < 0.05$) . Values followed by the same number codes in the same column are not significantly different as affected by glutaraldehyde (GA) ($p < 0.05$). DP5, DP10 and DP 15 are the addition of Klutuk banana leaf extract as many as 5, 10 and 15%. GA0, GA15, GA30 and GA45 are the addition of glutaraldehyde by 0, 15, 30 and 45%.

The dark color of the EDPK film can be an advantage. Also, dark colors of the packaging can protect food from light causing damage, one of which is oxidation. According to Choe and Min (2006), oxidation begins with a catalyst reaction involving heat, light and oxygen. Oxidation will result in the formation of free radicals which release hydrogen. Haile et al. (2013) reported that cooked ham wrapped with foil and kept in light showed higher discoloration than cooked ham wrapped with foil and kept in dark. With the presence of green color on the film, the EDPK film can protect packaged foods from being exposed to light so that it can reduce the formation of free radicals.

Mechanical properties

The film with the addition of 10% Klutuk banana leaf extract had a significantly higher elongation at break (EB) than the film added with 5 and 15%Klutuk banana leaf extract (Table 2). In overall, the addition of glutaraldehyde (GA) significantly increased tensile strength (TS) but had no effect on the EB. Besides GA, plasticizers also had an important role to play in the mechanical properties of film or packaging. TS and EB in a film are also influenced by the thickness of the film (Akhtar et al., 2012).

The increase in TS is in line with the research by Park and Ruckeinstein (2001) which reported that TS on methyl cellulose (MC) increased with the increase of GA and HCl concentration. On the contrary, the value of EB decreased. It can be explained that the addition of GA increases the network structure so the polymer chain mobility decreases. Therefore, material that experiences crossing becomes rigid. However, if the GA concentration increases to the maximum concentration, GA can function as a plasticizer which causes softening

Tabel 2. The mechanical properties of methyl cellulose-based films with the addition of glutaraldehyde and Klutuk banana leaf extract in various combinations

Sample	Tensil strength (N/mm²)	Elongation at break (%)
DP5_GA0	12.75 ± 0.86^{b1}	61.59 ± 4.97^{a1}
DP5_GA15	13.60 ± 0.18^{b2}	59.90 ± 3.98^{a1}
DP5_GA30	15.73 ± 0.58^{b3}	52.49 ± 4.67^{a1}
DP5_GA45	16.46 ± 0.37^{b3}	56.70 ± 3.68^{a1}
DP10_GA0	16.23 ± 0.67^{c1}	59.60 ± 6.04^{b1}
DP10_GA15	15.80 ± 1.62^{c2}	75.91 ± 5.29^{b1}
DP10_GA30	15.35 ± 0.87^{c3}	68.28 ± 3.94^{b1}
DP10_GA45	16.35 ± 0.89^{c3}	65.46 ± 3.82^{b1}
DP15_GA0	4.73 ± 0.13^{a1}	65.82 ± 1.96^{a1}
DP15_GA15	8.16 ± 0.70^{a2}	45.44 ± 3.37^{a1}
DP15_GA30	15.63 ± 0.24^{a3}	66.41 ± 4.37^{a1}
DP15_GA45	13.78 ± 0.68^{a3}	64.23 ± 5.17^{a1}

Remarks: Values followed by the same letters in the same column are not significantly different as affected by Klutuk banana leaf extract (DP) ($p < 0.05$) . Values followed by the same number codes in the same column are not significantly different as affected by glutaraldehyde (GA) ($p < 0.05$). DP5, DP10 and DP 15 are the addition of Klutuk banana leaf extract as many as 5, 10 and 15%. GA0, GA15, GA30 and GA45 are the addition of glutaraldehyde by 0, 15, 30 and 45%.

of the crossed film. The higher the excess GA, the higher the plasticizing effect. Also, an increase in TS value was caused by the formation of a more stable network because of crosslinking among MC, EDPK and GA as a crosslinker agent. The increase in TS and decrease in EB indicate a relationship between polymer chains and GA (Benbettaieb et al., 2015; Rimdusit et al., 2008).

Thermogravimetric Analysis (TGA)

The thermogram in Figure 1 shows the maximum decomposition in the sample at temperatures of 220, 225, 175 and 200°C for DP5_GA0, D5_GA45, DP15_GA0 and DP15_GA45. Based on Figure 1, it can be stated that the addition of Klutuk banana leaf extract could lower the thermal stability by 25-45°C, while the addition of GA could increase thermal stability around 5-25°C. A decrease in thermal stability caused the film to become brittle when heated at high temperatures.

When compared with the research by Dicastillo et al. (2016), the maximum decomposition value in EDPK films shows a lower value. Thermograms in the film owned by Dicastillo et al. (2016) showed a maximum decomposition value at temperatures around 361oC. The low thermal decomposition of MC-based films with the addition of GA and Klutuk banana leaf extract was caused by the crosslinking between the three components (MC, GA, and Klutuk banana leaf extract) which did not produce new bonds which thermally having better heat resistance MC network.

Release Studies of Active Packaging

Overall, the antioxidant activity of EDPK films has the same release profile, which increases at 2 hours and will be constant or will decrease at 72 hours (Figure 2). Research on antioxidant release by Calatayud et al. (2013), Dicastillo et al. (2011) and Dicastillo et al. (2015) showed an antioxidant release curve in the form of the maximum exponential curve profile, although the samples had a different area, kinetics and GA content. In line with the research of Calatayud et al. (2013), Dicastillo et al. (2011) and Dicastillo et al. (2015), in this study, the resulting curve is also a maximum exponential curve.

The antioxidant release was influenced by the presence of MC crosslinking with EDPK and GA as a crosslinker agent. In the study of Dicastillo et al. (2015), the release of phenolic compounds in food simulations was slightly influenced by interactions between MC, GA and extract of maqui berry. Balaguer et al. (2011), Khalil et al. (2015), Mi et al. (2006) and Yu et al. (2014) stated that natural compounds from plants such as cinnamaldehyde, eugenol, citric acid, geniposidic acid and catechins from green tea extract may act as crosslinking additives.

Figure 2 shows that the DP15_GA0 film has the highest antioxidant activity on 10% and 50% etha-

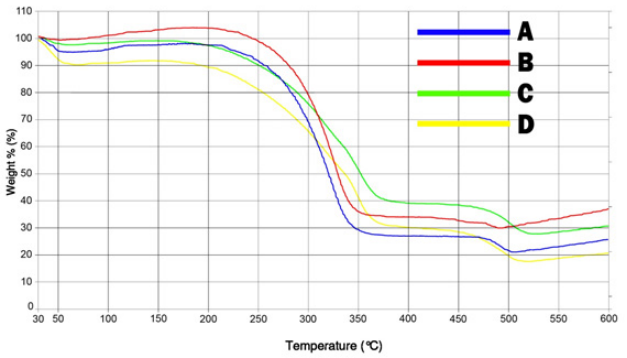

Figure 1. TGA in methyl celluloce-based films with the addition of glutaraldehyde and Klutuk banana leaf extract. (A) DP5_GA0 (B) DP5_GA45 (C) DP15_GA0 (D) DP15_GA45. (DP5 and DP 15 are the addition of Klutuk banana leaf extract 5 and 15%. GA0 and GA45 are the addition of glutaraldehyde 0 and 45%).

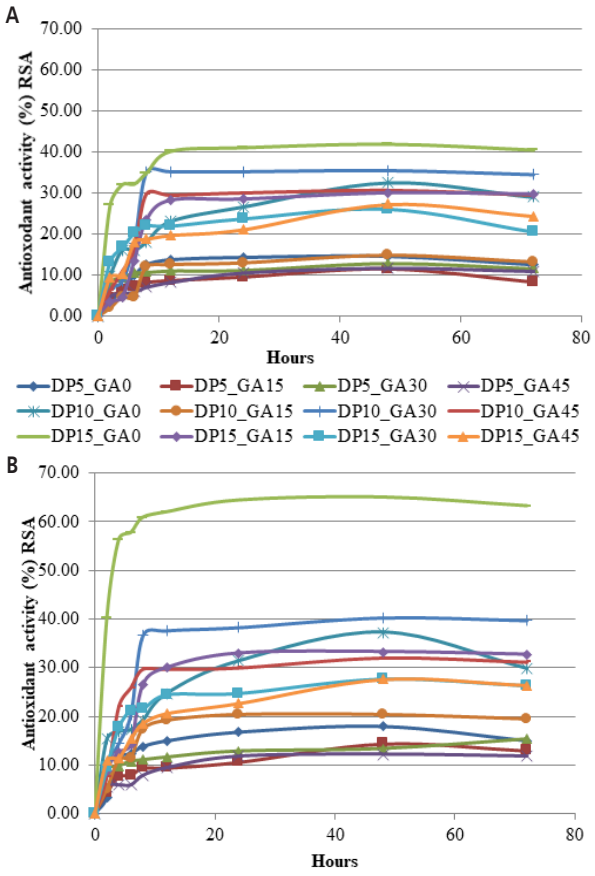

Figure 2. Release of antioxidants in EDPK films. (A) EtOH Simulations 10% (B) EtOH Simulations 50%. (DP 5, 10 and 15 are the addition of Klutuk banana leaf extract 5, 10 and 15%. GA0, 15, 30 and 45 are the addition of glutaraldehyde 0, 15, 30 and 45%).

nol simulations. By decreasing the concentration of GA, the active component of Klutuk banana leaf extract cannot react with the MC polymer chain to form a crosslinking. Therefore, EDPK can release into food simulation optimally.

CONCLUSIONS

This study provides a method to prepare active packaging with methyl cellulose (MC) as the main ingredient, which was added with glutaraldehyde (GA) and Klutuk banana leaf extract (EDPK). The active packaging was also then studied for its properties and antioxidants. The result showed that the addition of Klutuk banana leaf extract caused the color of the film to be green to dark green. Films with the addition of Klutuk banana leaf extract and GA caused the film to be stronger, marked by an increase in TS and EB. The heat resistance of EDPK films was low so that the film became easily brittle when heated. Klutuk banana leaf extract added to MC-based active packaging can migrate into food simulations, therefore EDPK films may act as antioxidants.

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Parasitization and Identification of The Red Guava Fruit Fly Parasitoids in The Deli Serdang District

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ABSTRACT

Deli Serdang District is one of the regions producing red guava fruit in Sumatra Utara. Cultivation of fruit trees is never separated from pest disorders, which can cause a decrease in the quality and quantity of fruit. Then proper control is needed so that it can maintain the balance of insect populations in the field. This study aims to determine the type of parasitoid and parasitoid parasitic level in red guava cropss. Identification morphology of parasitoid's fruit flies rearing from red guava fruit that had been attacked by fruit flies was taken from several locations of red guava crops in Deli Serdang District. Two species of parasitoid Psytalia sp. the parasitic fruit fly in the red guava crop. The parasitoids that have been found then identified at LIPI, Cibinong, Bogor morphologically has many similarities with Psytalia walker and Psytalia walkeri so that identification of species is only made close to the morphology of the species. Only in two locations were found parasitoid's rearing from infected fruit, namely Parasitization rate of 6.9% in Sei Beras Sekata village, and Kolam village of 3.6%.

Keywords: Identification; Morphology; Parasitization; Psytalia sp.

ABSTRAK

Kabupaten Deli Serdang merupakan salah satu wilayah penghasil buah jambu biji merah di Sumatera Utara. Budidaya tanaman buah tidak pernah lepas dari gangguan hama, yang dapat menyebabkan penurunan kualitas dan kuantitas buah. Maka perlu dilakukan pengendalian yang tepat sehingga dapat menjaga keseimbangan populasi serangga di lapangan. Penelitian ini bertujuan mengetahui jenis parasitoid dan daya Parasitisasi parasitoid di pertanaman jambu biji merah. Identifikasi morfologi parasitoid lalat buah hasil *rearing* buah jambu biji merah yang telah terserang lalat buah diambil dari beberapa lokasi pertanaman jambu biji merah di Kabupaten Deli Serdang. Ditemukan dua spesies parasitoid *Psytalia* sp. yang memparasit lalat buah di pertanaman jambu biji merah. Parasitoid yang telah ditemukan kemudian diidentifikasi di LIPI, Cibinong, Bogor secara morfologi memiliki banyak kesamaan dengan *Psytalia walker* dan *Psytalia walkeri* sehingga untuk identifikasi spesies hanya dibuat mendekati morfologi spesies tersebut. Hanya pada dua lokasi ditemukan parasitoid hasil *rearing* dari buah yang terserang, yakni tingkat Parasitisasi sebesar 6.9% di desa Sei Beras Sekata, dan desa Kolam sebesar 3.6%.

Kata Kunci: Identifikasi; Morfologi; Parasitisasi; *Psytalia* sp.

INTRODUCTION

Fruit farming is inseparable from the Plant Disturbing organisms, which can reduce production and become a barrier to trade between countries (Kardinan et al., 2009). One of them is fruit fly (Bactrocera sp.) which is a concern in the world because it is an important pest in the fruit. This pest has also been a problem in fruit commodities in Indonesia (Suputa et al., 2007).

The productivity of red guava in Deli Serdang District has reportedly decreased since 2010, red guava production amounted to 35,261 fell to 12,661 tons in 2014 (Badan Pusat Statistik, 2016). Reduced productivity of red guava one of which can be caused by fruit fly attacks that cause damage to fruit and reduce the quality and quantity of yields (Amin, 2015).

All ways to control fruit flies have been done, among others, fruit wrapped, biological control, pesticide use, etc. (Dhillon et al., 2005). The use of pesticides has proven effective but leaves chemical residues, therefore it is necessary to control environmentally friendly and have been proven effective namely the use of methyl eugenol as an attractant (Vargas, 2007). Biological control by utilizing the role of parasitoids from the family Branconidae (Hymenoptera), namely *Fopius* sp. and *Biosteres* sp. also able to suppress fruit fly populations in the field (Siwi et al., 2006).

Drew & Romig (2012) states that identification of insect species is very important, because some groups of insect taxa have almost the same variation in morphological characters. For example, the difference in body shape of insects with one another between *B. carambolae* and *B. papayae* is due to the genetic relationship closeness so that from the shape of the abdomen and the wing pattern looks almost the same, in other species the direct difference can be seen only from the pattern of the wings (Pramudi et al., 2013). Study about fruit fly parasitoids in Deli Serdang District is urgently needed so that control can be carried out using parasitoids that are suitable for the target pest.

MATERIALS AND METHODS

Collecting Fruit Attacked

We collected 5 attacked fruits by purposive random sampling as much as 4x with an interval of 2 weeks at each sample location. The fruit is placed into a jar that has been filled with sand.

Rearing of Fruit Fly Parasitoid

To get fruit flies pupa, the sand was sifted every two days for 2 weeks. The collected fruit flies were placed in another plastic container then use gauze as a cover. Fruit flies Imago and parasitoids were seen given feed in the form of a solution of honey until the imago was 3 days old, after enough age the imago was turned off and stored in bottles that had been filled with 70% alcohol and identified.

Morphological Identification

The parasitoid that has been found was identified morphologically including caput, thorax, wings, abdomen, using a microscope and assisted with the book identification of Hymenoptera parasitoid, entitled Hymenoptera of the World An Identification Guide To Families (Goulet & Huber, 1993), in the Research Center Laboratory Biology, LIPI Cibinong Bogor.

Parasitic Level

Calculation of the level of Parasitization of each parasitoid associated with the red guava crop, using the formula (Buchori et al., 2010).

$$TP = \frac{\sum A}{\sum B + \Sigma A} X\ 100\%$$

Remark:
TP = Parasitic level
A = The number of parasitoids that appear
B = The number of fruit fly imago

RESULTS AND DISCUSSION

The dentification of parasitoids at LIPI were obtained 2 species, which were *Psytalia* sp. near walker and *Psytalia* sp. near walkeri found in fruit fly imago at red guava crops of the Sei Beras Kata village and the Kolam village, shown in Table 1.

Morphology of *Psytalia* sp. near walker, the antenna has 52 segments. It has a medial dark 2RS front wing, anterior-posterior infumate band through the middle of the front wing. The abdo-

Table 1. Morphology of Parasitoid Fruit Flies

Character	Caput	Wings	Abdomen	Imago
Psytalia sp. near *walker*				
Psytalia sp. near *walkeri*				

men is oval with black lines that are not entirely full. the body is brownish yellow, the legs are brown.

Morphology of *Psytalia* sp. near walkeri is the Abdomen with full black lines. The m-cu and subdiscal distal front wing arches are enlarged. The antenna has 50 vertebrae, brownish-yellow bodies, there is an occipital carina that extends the height of the back more than the height of the head.

In Table 2, the effectiveness of the parasitoid in controlling fruit flies in these two locations can be measured by parasitic level, ie in the village of Sei Beras Sekata has a parasitic level of 6.9%, and the village of Kolam is 3.6%. Based on the parasitic level, it can be assessed the ability of natural enemies in regulating the balance of fruit fly populations at both locations is very small. One of the low parasitic level is thought to be due to the use of insecticides in the field by farmers and how to cultivate that is not in accordance with environmental rules (e.g. too tight spacing), thus adversely affecting the presence and parasitic level of parasitoid in the field. According to Herlinda (2007) and Berryman (1981), factors that influence the development of parasitoids are (a) the amount of food, food suitability, nutrient content, appropriate water content and host plants suitable for growth and development, (b) temperature, good humidity, light and aeration for mass breeding, (c) the extent to which pest control measures have been carried out by manipulation of host plants, crop rotation or control with pesticides, (d) insects are able to create resistance naturally so that insects are able to adapt to physiological changes in the host or food so that the insect is able to maintain its life.

Table 2. Parasitoid Parasitic Level

Locations	Kolam Village	Sei Beras Sekata Village
Number of fruit	20	20
Number of fruit fly	27	54
Number of parasitoid	1	4
Parasitic level	3.6%	6.9%

From Table 2, it can be seen the difference in level parasitic in the two locations, namely in the village of Sei Beras Sekata (6.9%) having a higher parasitic power level than in the village of Kolam (3.6%), this is presumably because of the red guava crops in Sei village Sei Beras Sekata is next to the corn crop land, where it is known that the pollen of the corn plant can be a source of additional food for parasitoids. According to Russell (1989) states that higher flora diversity provides more niches and habitat for insect species, and according to Herlinda (2005) that Tetrastichus and O. sokolowskii are only found in the rainy season because in that season the caisin crop area is wider and species other plants that live are also more diverse than in the dry season.

CONCLUSION

The type of natural enemy found in this study was very low, only 2 species of parasitoid were found. This amount is certainly less effective in controlling fruit flies in the field. The results of the identification morphologically parasitoid namely *Psytalia* sp. near walker and *Psytalia* sp. near walkeri came from Sei Beras Kata village and Kolam village, with the highest parasitoid parasitic level of 6.9% found in Sei Beras Sekata village.

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Methane Emissions and Rice Yield in Rainfed Bed System (*Surjan*) as Affected by Manure and Zeolite Treatment

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ABSTRACT

Rainfed area as one of rice production areas is facing drought due to climate change. Management of rainfed area is needed due to its contribution, in addition to the production of rice, in producing methane as a contributor to greenhouse gas emission. This research aimed to investigate the methane emission status and yield from rainfed rice system with manure and zeolite treatment on the bed system (surjan). The doses of manure were 5, 15 and 30 tons/ha and the zeolite was 1 and 2.5 tons/ha. The result showed that all treatment had no significant effect on daily methane fluxe and grain yield in surjan system. However, the combination of manure at 15 tons/ha with zeolite at 1 ton/ha promoted higher methane emissions (63.43 kg CH_4/ha/season). In addition, the combination treatment of manure at 5 tons/ha with zeolite at 2.5 tons/ha contributed to obtain higher grain yield (6.9 tons/ha).

Keywords: Methane emission; Rainfed; Surjan

ABSTRAK

Sebagai salah satu areal produksi padi, lahan tadah hujan menghadapi cekaman kekeringan karena perubahan iklim. Manajemen lahan tadah hujan diperlukan karena selain sebagai lokasi produksi padi namun juga sebagai lokasi yang menghasilkan emisi gas rumah kaca khususnya metana. Penelitian ini bertujuan untuk menginvestigasi emisi metana dan hasil gabah padi dari sistem pertanaman surjan dengan perlakuan pupuk kandang (pukan) dan zeolit. Dosis pukan yang digunakan yaitu 5, 15 dan 30 ton/ha sedangkan dosis zeolit yang digunakan yaitu 1 dan 2.5 ton/ha. Hasil penelitian menunjukkan bahwa semua perlakuan tidak berpengaruh terhadap fluks metana harian dan gabah kering panen dari lahan surjan. Kombinasi perlakuan pukan dosis 15 ton/ha dengan zeolit 1 ton/ha mengemisikan metana lebih tinggi dibandingkan kombinasi perlakuan lain sebesar 63.43 kg CH_4/ha/musim. Gabah kering panen (GKP) maksimum didapatkan pada kombinasi perlakuan pukan 5 ton/ha dengan zeolit 2.5 ton/ha seberat 6.9 ton/ha.

Kata Kunci: Emisi metana; Tadah hujan; Surjan

INTRODUCTION

Rainfed is one of the rice production systems that contribute to provide rice yield. However, rainfed known as a suboptimal area facing drought. The characteristics of rainfed area are low soil fertility level and unpredictable rainfall pattern that promotes risk under drought condition (Mulyadi and Wihardjaka, 2014). Regarding the climate change issue, the rainfed area is getting marginalized. Concerning on this issue, farmers from rainfed area adopt bed farming system (*surjan*) to develop soil productivity and obtain the diverse crop yield while as an adaptation action to climate change.

Bed farming system is common local wisdom in the coastal area that manages the rice field due to the bad drainage system. The bad drainage system is caused by the geomorphology rainfed area that is a fluviomarine plain and a former of a black swamp (Marwasta and Priyono, 2007). According to Aminatun et al. (2014), the bed farming system is called *surjan* since the rice field pattern looks like the lines pattern on the traditional clothes of Javanese (*surjan*). These lines are formed from terrestrial at high level and aquatic grooves at a low level. The terrestrial parts are planted with secondary crops or horticulture, while the aquatic grooves are planted with rice. Therefore, the *surjan* ecosystem is different from the general rice field. The great function of *surjan* is to store water from rainfall and runoff for water supply system during rice growth.

Zeolite is a naturally crystalized aluminosilicate used as ameliorant in the rice field to develop cation exchange capacity that promotes yield and support nutrient efficiency (Ramesh and Reddy,

2011). Moreover, zeolite treatment is able to increase protein quality of rice, develop nitrogen efficiency and, in the long-term application, promote recovery of soil nitrogen level (Sepaskhah and Barzegar, 2010). The application of manure as organic fertilizer is an effort to develop the carbon sequestration for climate change mitigation scenario, to increase fertility, chemical, physical, and biological properties of the soil, to develop agronomic performance and to increase the yield as well as to enhance the soil organic nitrogen content (Diacono and Montemurro, 2011; Mulyadi and Wihardjaka, 2014). However, manure and other organic material as a soil amendment in rice field contributes to the increase in methane emissions (Dendooven et al., 2012).

Agriculture sector is one of the sources of greenhouse gas (GHG) emissions especially methane (CH_4), dinitro oxide (N_2O) and carbon dioxide (CO_2), in which each gas contributes 15%, 6% and 55% of the total emissions, respectively (Mosier et al., 1994). Rainfed as part of agriculture ecosystem also plays a role as a source of emission releasing the GHG to the atmosphere. Appropriate technology is needed to reduce GHG emissions from rainfed rice system. This study aimed to determine the level of CH_4 gas emissions from rainfed rice field in the *surjan* system treated with manure and zeolite.

MATERIALS AND METHODS

The research was conducted in the Indonesian Agricultural Environment Research Institute field trial during the rainy season in 2012. The experiment was carried out on a plot trial with the plot size of 6 m x 46.5 m using rice cv. Ciherang grown at the aquatic grooves of *surjan*. Meanwhile, the terrestrial area of *surjan* with a size of 2 m x 46.5 m was used to grow mango (Figure 1). The *surjan* cross-section consisted of the aquatic grooves as a subsoil (*tabukan part*) in a high bulk density, planted with rice, and the terrestrial part/topsoil

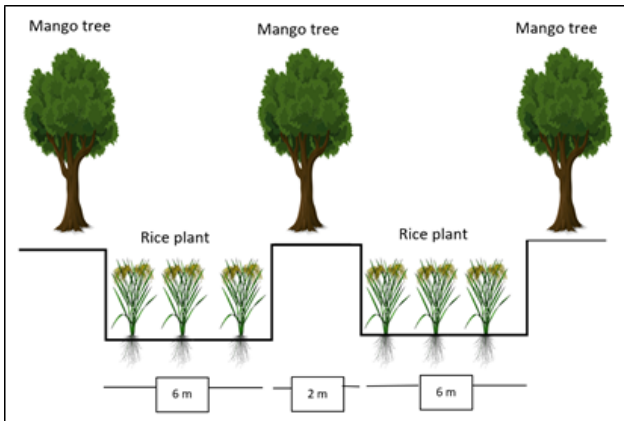

Figure 1. Agricultural Plan of the Surjan System of Rice with Mango Trees in Rainfed Areas

(guludan part), planted with mango (Wihardjaka dan Indratin, 2002).

There were combination treatments between manure and zeolite. The manure application rates were 5, 15, 30 tons/ha, while the zeolite treatment rates were 1 and 2.5 tons/ha. Manure and zeolite were applied at the beginning of planting time. The basalt fertilizer, such as urea was applied at a dose of 250 kg/ha in each plot with leaf color chart as guidance. The dose of. P_2O_5 was 36 kg/ha applied at the beginning of planting along with the application of manure and zeolite. Meanwhile, K_2O was applied at a dose of 60 kg/ha, twice in one planting season. The first application of K2O fertilizer was 30 kg/ha at the beginning of planting along with P_2O_5, manure and zeolite application, while the second application was at 39 Days After Transplanting (DAT). The variables observed were grain yield ((*gabah kering panen (GKP)*) at 14% water content and methane emissions from *surjan* in the rainfed system. The grain yield was obtained by using harvest sampling area with a size of 2.5 x 2.5 m.

The sampling of CH_4 emissions was performed by capturing the air samples using a closed chamber method with a dimension of 50 cm × 50 cm × 103 cm. The three-time interval for gas sampling were 10, 20, and 30 minutes. The gas was taken from the chamber using a 10 ml of syringe then the methane was analyzed by Gas Chromatography 8A which

has an FID detector (Flame Ionization Detector) to analyze CH_4 concentration. The CH_4 gas was observed 3 (three) times in 1 (one) growing season according to the growth development phase of rice plants. The Global Warming Potential (GWP) of methane was calculated using the CO_2 equivalent weight (kg CO_2eq/ha). The potential radiative value of methane, as a relative value to CO_2, was used at 25 (Houghton et al., 2001).

According to Khalil et al. (1991), the methane emissions from methane concentration can be calculated using the equation:

$$F = \frac{dc}{dt} \times \frac{Vch}{Ach} \times \frac{mW}{mV} \times \frac{273,2}{(273,2+T)}$$

Annotation:

F : Flux of methane (mg/m^2/minute)

dc/dt : Slope concentration of methane/time sampling (ppm/minute)

Vch: Volume of the chamber (m^3)

Ach : Base area of the chamber (m^2)

mW : The molecule weight of methane (g)

mV : The molecule volume of methane (22.41 l)

T : Average temperature during gas sampling (°C)

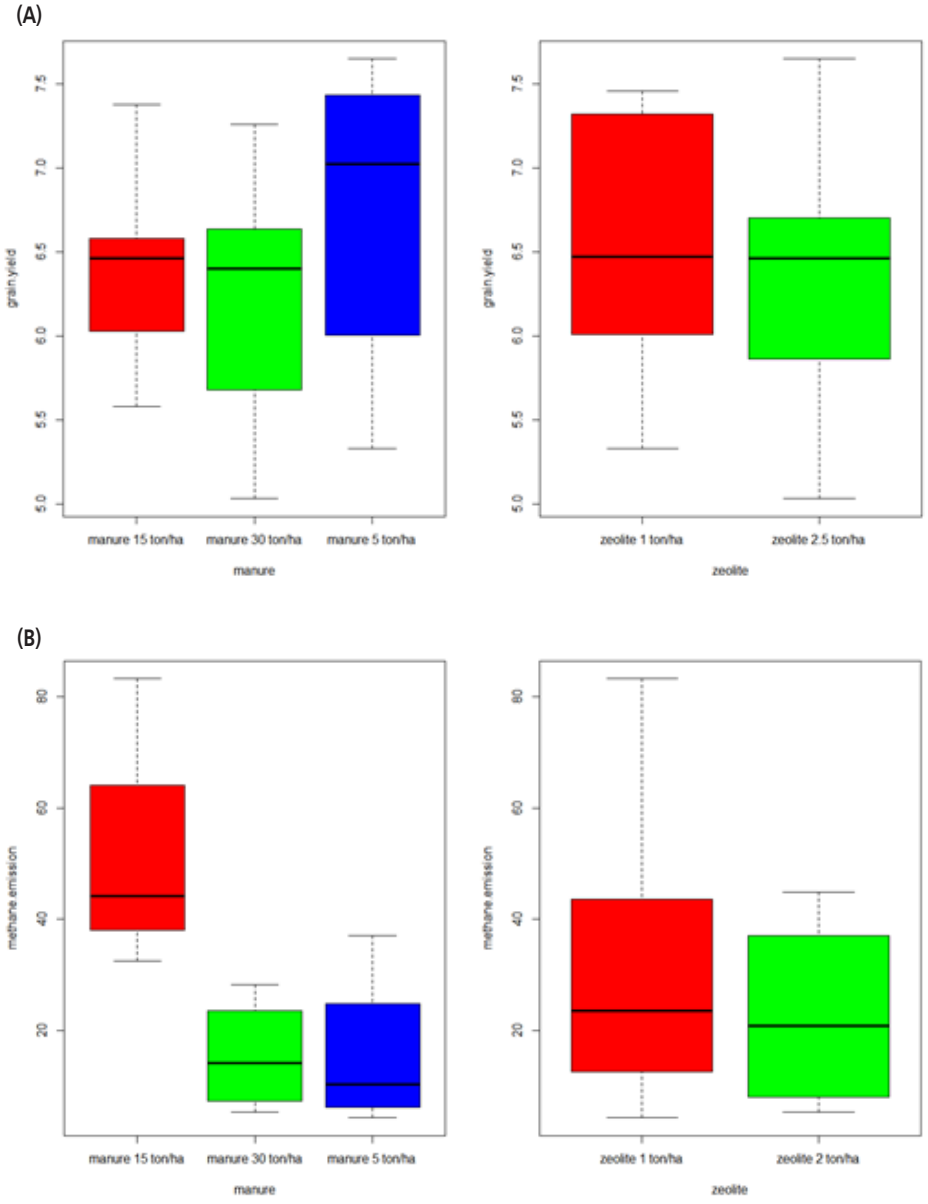

Figure 2. Boxplot FF ANOVA of the Effect of Manure and Zeolite Treatment on Methane Emissions (A) and Grain Yield (GKP) (B)

Data Analysis

For statistical analysis, the R Studio (version 3.2.1) was used to analyze the data. The Levene test and Shapiro-Wilk was used to analyze the homogeneity and normality distribution of the parametric data. Meanwhile, FF ANOVA was used to analyze methane emission and yield. Post hoc test was used to examine the differences between the treatment.

RESULS AND DISCUSSION

There was no significant interaction effect between manure and zeolite on the methane emissions (F = 1.8; df = 2; P = 0.24). Based on the statistical tests (FF ANOVA), manure had a significant effect on CH_4 emissions (F = 7.39; df = 2; P = 0.24), while zeolite did not significantly affect CH_4 emissions (F = 1.06; df = 1; P = 0.34) (Figure 2a). The post hoc test at 95% level showed that manure treatment at 5 tons/ha (P = 0.01) and at 30 tons/ha (P = 0.03) significantly affected CH_4 emissions from the rice fields with *surjan* planting system.

The application of manure and zeolite did not significantly affect the daily CH_4 flux (P> 0.05) (Table 1). At 69 DAT, the addition of 2.5 tons/ha of zeolite showed a smaller CH_4 flux compared to the addition of 1 ton/ha of zeolite. The addition of 2.5 tons/ha zeolite was able to suppress CH_4 flux by 80%, 46% and 24% in the treatment of 5 tons/ha, 15 tons/ha, 30 tons/ha of manure at 69 DAT, respectively.

There was no significant interaction effect between manure and zeolite on the grain yield (F = 1.94; df = 2; P = 0.17). Manure treatment (F = 1.08; df = 2; P = 0.36) and zeolite treatment (F = 0.37; df = 1. P = 0.55) had no significant effect on the grain yield (*GKP*) (Figure 2b). The harvested grains in all treatments were between 5.7 to 6.9 tons/ha (Table 2). The manure treatment at 5 tons/ha combined with zeolite at 2.5 tons/ha produced grain yield 21% greater than the treatment of 30 tons/ha combined with zeolite at 2.5 tons/ha that produced the lowest grain yield. Treatment of zeolite at 2.5 tons/ha combined with 5 tons of manure tended to increase the grain yield (GKP) significantly than the treatment of zeolite at 1 ton/ha. Similarly, the research result from Al-Jabri, (2009)stated that the application of zeolite combined with manure will increase the grain yield (GKP).

The post hoc test showed that manure treatment at 15 tons/ha combined with Zeolite at 1 ton/ha produced a CH_4 emission level that was significantly different from all treatments except the treatment of manure at 15 tons/ha combined with zeolite at 2.5 tons/ha (Table 3). It showed that the treatment of zeolite can reduce methane emissions. Zeolite can be used as an addictive substance to inhibit methane emissions (Mukesh et al., 2016), moreover, zeolite is a cheap ameliorant as a mitigating agent for reducing methane emission (Hui and Chao, 2008). Zeolite, as a stable material, has a capability of storing methane (Joseph et al.,

Table 1. Flux of CH_4 during three rice plant growth periods as affected by the application of manure and zeolite at various doses

Flux (mg/m²/day)	40 DAT	55 DAT	69 DAT
Manure 5 ton/ha + Zeolite 1 ton/ha	10.65	6.65	70.65
Manure 5 ton/ha + Zeolite 2.5 ton/ha	27.15	3.25	14.15
Manure 15 ton/ha + Zeolite 1 ton/ha	59.90	5.20	76.40
Manure 15 ton/ha + Zeolite 2.5 ton/ha	58.70	3.50	40.95
Manure 30 ton/ha + Zeolite 1 ton/ha	29.80	3.55	35.25
Manure 30 ton/ha + Zeolite 2.5 ton/ha	3.30	8.90	26.95

Table 2. Grain yield (*GKP*) with 14% water content as affected by the application of manure and zeolite at various doses

Flux (mg/m²/day)	Grain yield 14% (ton/ha)
Manure 5 ton/ha + Zeolite 1 ton/ha	6.461
Manure 5 ton/ha + Zeolite 2.5 ton/ha	6.992
Manure 15 ton/ha + Zeolite 1 ton/ha	6.465
Manure 15 ton/ha + Zeolite 2.5 ton/ha	6.292
Manure 30 ton/ha + Zeolite 1 ton/ha	6.668
Manure 30 ton/ha + Zeolite 2.5 ton/ha	5.761

Table 3. Methane emission, Global Warming Potential (GWP), Global Warming Potential-Yield (GWPy) as affected by the application of manure and zeolite at various doses

Flux (mg/m²/day)	CH_4 kg CH_4 / ha / season	GWP kg CO_2-eq/ ha / season	GWPy kg CO_2-eq/ ton/ season
Manure 5 ton/ha + Zeolite 1 ton/ha	17.2 b	430.3	67
Manure 5 ton/ha + Zeolite 2.5 ton/ha	22.5 b	563.8	81
Manure 15 ton/ha + Zeolite 1 ton/ha	63.4 a	1585.8	245
Manure 15 ton/ha + Zeolite 2.5 ton/ha	38.6 ab	966.5	154
Manure 30 ton/ha + Zeolite 1 ton/ha	23.6 b	590	88
Manure 30 ton/ha + Zeolite 2.5 ton/ha	7.4 b	185.3	32

Remarks: Means followed by the same letters in the same column are not significantly different according to post hoc test at a 95% level.

1983; Eckhard and Matthias, 1997; Myrsini et al., 2014). Therefore, manure amendment to the soil as an organic fertilizer and as a substrate of methanogenesis to produce methane has no significant effect on methane emissions.

CONCLUSION

All treatments had no significant effect on the daily methane flux and harvested grain yield in the rice field with *surjan* system. The application of manure at 15 tons/ha combined with zeolite at 1 ton/ha promoted higher methane emission at 63.43 kg CH4/ha/season than the combination of other treatments. The great grain yield (GKP) was obtained in the application of manure at 5 tons/ha combined with 2.5 tons/ha of zeolite, reaching 6.9 tons/ha of rice grain yield.

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The Effect of Light Color Variation in Simple Light Traps on the Number of Fruit Flies (*Bactrocera* sp.)

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ABSTRACT

Fruit flies (Bactrocera sp.) are the most common types of plant pests attacking fruit plants. The pest attacks the fruit in the plantation. The control of fruit flies is quite difficult, which is usually done by using eugenol. Fruit flies are insects that are sensitive to light with wavelengths of 300-650 nm. The light trap is a method commonly used yet it is rarely used to control the fruit flies. This research was conducted to determine the effect of the color variation in light traps on the number of trapped fruit flies. This study used quasi-experimental research methods. The data were analyzed descriptively and continued with one-way ANOVA statistical testing using SPSS 25.0. The results showed that the highest average number of fruit flies was in light traps with the addition of blue lights of 17.22. Post hoc tests showed that blue lights were more effective in attracting flies into light traps.

Keywords: Bactrocera sp.; blue light lamp; fruit flies; light trap; pest control

ABSTRAK

Lalat buah (*Bactrocera* sp.) merupakan jenis hama tanaman yang paling umum menyerang tanaman buah. Hama ini menyerang buah di perkebunan. Pengendalian lalat buah cukup sulit dilakukan, biasanya pengendalian dilakukan dengan menggunakan eugenol. Lalat buah merupakan serangga yang peka terhadap cahaya dengan panjang gelombang 300-650 nm. Penggunaan *Light trap* adalah metode yang sudah sangat umum digunakan namun penggunaannya untuk pengendalian lalat buah masih sangat jarang sehingga penelitian ini dilakukan untuk menguji penggunaan variasi warna lampu pada *Light trap* terhadap jumlah lalat buah yang terperangkap. Penelitian yang dilakukan termasuk dalam eksperimen semu dengan metode penelitian *post test with control group design.* Data di analisis secara deskriptif dan dilanjutkan dengan pengujian statistik menggunakan SPSS 25.0 dengan uji *One-way Anova.* Hasil analisis dengan menggunakan *One-Way Anova* menunjukkan rata-rata jumlah lalat buah paling tinggi adalah pada light trap dengan penambahan lampu biru yakni sebesar 17,22. Uji *post hoc* menunjukkan bahwa lampu biru lebih efektif untuk menarik lalat ke dalam light trap. Penelitian ini diharapkan dapat memberikan Informasi baru mengenai cara pengendalian lalat buah di lapangan.

Kata Kunci: Lalat Buah; Light trap; Lampu cahaya biru; Bactrocera sp.; Pengendalian Hama

INTRODUCTION

Fruit and vegetable commodities have a high prospective to be developed because they have high economic value, even the market demand for these commodities covers the domestic and foreign markets (Sarjan, Yulistiono, & Haryanto, 2010). Indonesia's agricultural land reaches millions of hectares since it is an agricultural country (Mukhlis, 2016). The land is used by farmers to handle the increased demand for fruit and vegetable commodities (Sarjan et al., 2010). The value of Indonesian fruit imports is high (Rofika Rochmawati, 2017). The price of imported fruit and vegetable is more expensive than that of local varieties (Syahfari & Mujiyanto, 2013). This provides opportunities for local varieties of fruit and vegetable to compete on the market. However, the quality of fruits and vegetables must be considered so that opportunities can be realized well (Sarjan et al., 2010).

The low quality of local fruits and vegetables is due to the attack of fruit fly pests (*Bactrocera* sp.) (Siregar & Agus Sutikno, 2015). This type of fly is one of the main pests of horticultural crops, especially fruit plants. More than 100 types of fruit plants are the target of fruit fly attacks. In high populations, the intensity of attacks reaches 100%. Crops that are frequently affected by these pests are oranges, papayas, cantaloupe, mangoes and starfruit as well as rice (Ruswandi, 2017; Susanto et al., 2017; Wulansari et al., 2017). This fruit fly pest attack causes substantial losses reaching 30-60%. The attack on the old fruit causes the fruit to become wet rot due to larvae attack. The

attack of fruit fly populations will increase in a cool climate, high humidity and moderate winds. Fruit fly attacks are increasing so that the need for control techniques is highly expected, especially in producing effective, efficient, and environmentally-friendly control techniques (Muryati, Hasyim, & de Kogel, 2007).

Controlling fruit flies is difficult despite a lot of efforts that have been carried out, including mechanical, technical, and biological methods (Patty, 2012). Fruit flies (*Bactrocera* sp.) are one of the pests that belong to the class of insects. One of the characteristics of insects is having an interest in light (Mukhlis, 2016). Fruit flies like dim light compared to dark places (Oktary, Ridhwan, & Armi, 2015). The use of light as an insect trap has traditionally been used for a long time, for example, the use of a petromax lamp to catch larvae (insects), the use of striking colors to capture fruit flies and flies, and the use of ultraviolet to catch mosquitoes (Mukhlis, 2016). Light traps are one of the most common methods for collecting insects (González et al., 2016). Although light traps are commonly called "CDC light traps", various light trap models equipped with incandescent or UV lamps have been developed (Gaglio et al., 2017). Recently, light traps have been modified by replacing incandescent lamps into light-emitting diodes (LEDs) (Gaglio et al., 2018; Müller et al., 2011; Silva et al., 2016). Various types of insects, including fruit flies, can respond to light at wavelengths of 300 - 650 nm with ultraviolet to red color spectrum.

Wavelengths that can be received by insects are varied due to the differences in retinal cells in the insect's eyes (Munandar, Hestiningsih, & Kusariana, 2018). Flies can also sense ultraviolet frequencies in the spectrum of light that are invisible to humans (Prasetya, Yamtana, & Amalia, 2015). Based on various experiments, it can be proven that insects can recognize and distinguish different types of colors. Insects can see ultraviolet light clearly. In general, insects have two sensitivity peaks, namely the blue-green color. This is also reinforced by previous research (González et al., 2016) using a light trap with five light-emitting diodes (LEDs) (white, green, red, blue, ultraviolet) run for 15 consecutive nights. The results showed that a higher number of Culicoides (fies) was trapped in traps with green, blue or ultraviolet (UV) lights compared to red and white LED traps (Gaglio et al., 2017). Differences in the results of these studies lead to the necessity to conduct studies on Light Traps with various lamp colors.

Factors of quality, price, brand, location of purchase, source of information (preference), physical quality, product packaging, and promotion influence consumer behavior to buy fruit. Fruit flies do not only attack plantations but also attack fresh fruit in the market. Many fruit sellers complain that there are fruit flies in the place where they sell the fruits. This is because one fruit fly can attack other fruits, especially if the fruit is papaya and sapodilla because the fruits do not need to be peeled before it can still attract fruit flies (Oktary et al., 2015). Thus, alternative fruit fly control is needed with a simple method and an affordable price. Alternative control of fruit flies in Indonesia that has prospects to be developed is an active ingredient with methyl eugenol (Petrogenol 80 L). However, a further research is still needed (Susanto et al., 2017). The use of light traps can also be used as an alternative in controlling fruit fly pests. Several previous studies applying the Light Trap method to catch flies reported that flies were also trapped in yellow light (Mustikawati, Martini, & Hadi, 2016), and the number of flies trapped was higher in red light traps (Munandar et al., 2018). Meanwhile, the study of Prasetya et al. (2015) showed that flies were trapped in blue light.

Physical-mechanical and physiological control of flies is also commonly carried out by applying glue adhesives and various color stick traps (Ardiansyah

et al., 2019). In this research, control was carried out by installing fly adhesive glue by adding TL (tubular lamp) lamps to the traps with color variations according to the wavelength preferred by the flies. The aim of this study was to determine the effect of the light color in a simple light trap and to find the most effective light to be used in this flytrap. The light trap is expected to attract fruit flies to perch since the fly is highly attracted to light.

MATERIALS AND METHOD

Research Site

This research is a quasi-experimental study using a post-test with control group design research method. The population in this study were all fruit flies in the sampling area. The study was conducted in April for 3 days in Sleman Regency, Yogyakarta. The location of this research was in the surrounding of fruit sellers, allowing the existence of fruit flies. The temperature and humidity of the research location were the same and appropriate for the activities of the surrounding population.

Simple Light Trap Design

The tools and materials used in designing simple light traps are easily obtained. The tool used was a TL lamp (Tubular lamp), which was chosen because this type of lamp can emit ultraviolet light preferred by insects including fruit flies. The color of the lamp used was based on the wavelength preferred by the insects including fruit flies, which was at a wavelength of 300-650 nm. In this study, the chosen lamp colors were red, blue, green and white. The white color was chosen because, the previous studies reported that the highest number of flies was trapped in this color compared to other colors, as well as control treatments with no TL (light) lamps. The other tools used were five plastic boxes, flies glue and an electrical socket to turn on the lights. The materials used were several types of fruits such as guava, papaya, banana, and longan, which will be placed in each box with the same amount.

Setting Traps in The Field

The simple Light Trap was designed and made from 5 plastic boxes, consisting of four light trap boxes with TL lamps as a treatment group and one box without lights as a control. TL lights then were installed and fly glue was added to the box and the fruits were put inside in equal quantities. The light trap treatment and control box were placed in the same place at the same temperature and humidity, and the TL (Tubular lamp) lamp was connected to the socket. The exposure was carried out for 8 hours, starting at 21:30 with 9 repetitions both in control and treatment group. The trapped fruit flies were counted directly. Fruit flies have a size of 3-4 mm with brownish-yellow body (some are gray) and red eye. The samples in this study were the fruit flies trapped in light traps. The independent variable in this study was the color of the lamp and the dependent variable was the number of trapped fruit flies. This study referred to the method of previous research conducted by Prasetya et al. (2015) and González et al. (2016) in which the researchers created a varied light color in the traps of flies and mosquitoes in house. The simple light trap designed by the researchers in this study is shown in Figure 2.

Data Analysis

The data obtained were grouped in tables and then analyzed descriptively and continued with statistical tests using the SPSS version 25.0 program. The statistical test began with the data normality test using the Kolmogorov Smirnov test as an initial test. Normal distributed data were then proceeded to the statistical test using one-way ANOVA (5%). A Post Hoc test was performed to determine the most effective light color to trap flies with a significance level of 5%.

RESULTS AND DISCUSSION

Data retrieval was carried out 9 times, showing that light traps with blue lights had a higher number of trapped fruit flies compared to red, green and white lights. An average of 17 fruit flies were trapped in the blue light trap. The fewest trapped fruit flies were found in Light traps without TL lights (control), which were 5 fruit flies in average (Table 1).

The data in Table 1 were tested for normality using SPSS 25.0 then continued to be analyzed using one-way ANOVA. Data normality test was performed using the Kolmogorov-Smirnov Test. Based on the results of normality tests, it can be seen that the data has p = 0.200 (p> 0.05) so that it can be concluded that the data of the number of fruit flies trapped in the light trap both the treatment and control group are normally distributed. One-Way ANOVA was performed to determine differences in variance on each factor. The factors in this study were the color variations of the lamps used in the light trap. Statistical test with one-way ANOVA resulted an average number of flies trapped in the light trap control (without lights) of 4.89, while the average number of flies trapped in the light trap with green, blue, red, and white light was 8.44, 17.22, 11.22, and 9.67, respectively. Based on the test results obtained descriptively, it can be concluded that the highest average number of trapped fruit flies was in light traps with blue light, which was 17.22. The result of ANOVA also showed a significant difference in the average number of fruit flies based on the color variation of the light trap lights, thus, further tests (Post Hoc Test) was carried out. According to the homogeneity test, the Post Hoc test used was the Games-Howell test since the variance of the data was not homogenous.

Table 1. Number of fruit flies (*Bactrocera* sp.) in each light trap

Repetition	Control	Treatments			
	No lamp	Green lamp	Blue lamp	Red lamp	White lamp
1	0	6	11	8	3
2	7	6	12	7	5
3	4	4	16	10	2
4	5	7	12	3	8
5	9	8	13	11	10
6	7	10	20	8	5
7	4	11	25	17	19
8	5	13	23	19	18
9	3	11	23	18	17
Average	5	8	17	11	10
Difference		3	12	6	5

The Games Howell test was performed to determine the treatments giving significantly different effect. The results showed that control group (without light) had significantly different number of trapped fruit flies compared to blue light. Meanwhile, the control group did not show significant difference in the number of trapped fruit flies compared to green, red, and white lights. These results indicate the blue light is more effective in attracting flies into the light trap

Fruit flies trapped in a simple light trap were calculated by looking at the general characteristics of fruit flies. The morphological characteristics of fruit flies that can be observed are brownish yellow and gray body with thin and flat wings, an abdomen with black bands, and a size of 3-4 mm. There were certain types that have red eyes (Indriyanti, Insnaini, & Priyono, 2014) The average number of trapped fruit flies shown by descriptive data showed that the control group (Light trap without light (Figure 1) was the group that had the lowest average number of trapped fruit flies compared to the treatment group (Light trap with light variations (Figure 2). This is because the fly is an insect that has phototrophic properties, which means that the insect is attracted to the color of light so that fruit flies like bright places over the dark places (Oktary et al., 2015). Symptoms that arise because an object reflects light and has the nature of light as well as having different wavelengths are called

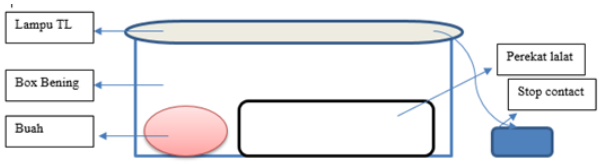

Figure 1. Simple light trap design

Figure 2. The simple light trap (control and treatment)

colors. The colors used in this study ranged from 300 nm to 650 nm, consisting of red, green and blue. This study also used white, although this color does not belong to the wavelength range of 300-650 nm, because it is an object that can reflect all light.

Light traps with blue lights obtained the highest average number of trapped flies. The wavelength of blue color is in the range of 450-495 nm. These results are supported by previous studies, which stated that the highest number of trapped flies was found in blue lights (Prasetya et al., 2015). The sensitivity range of flies' eyes is between 300-650 nm. The blue color has a smaller wavelength than the red and green color. Light trap with the second highest number of trapped flies was the red light. According to previous research, red is included in the wavelength range that can attract insects, included in the range from ultraviolet to red. This is also supported by the research of Munandar, Hestiningsih, and Kusariana (2018) reporting most flieas were attracted by red color.

Light traps with green lights obtained the least results in trapping fruit flies among the colors included in the wavelength range of 300-650 nm. This happened because the green light cannot emit ultraviolet light. Even the green light had fewer trapped fruit flies compared to white. This is in line with the research of Munandar et al. (2018) and Wulandari, Bey, & Tindaon (2014) which showed that flies were still attracted to the color of white light. Light traps without the addition of lights obtained the lowest results in trapping fruit flies. This fact shows that the addition of light colors to the light trap has an effect on increasing the number of trapped fruit flies. The control was still visited by flies even though it did not reflect light at all. A further research on the effect of ultraviolet light showed that Traps with UV lamps trapped more house flies than without UV lamps and open trap types (Puspitarani, Sukendra, & Siwiendrayanti, 2017).

The use of lights in controlling fruit flies is based on the physiological aspects of insects. There are so many types of insects that can detect aphrodisiacs in low doses. In fruit flies, the commonly used aphrodisiac is eugenol. However, the application of light traps in trapping the fruit flies, in particular, is still less optimal. Insects have a high sensitivity to the stimulation of smell, hearing, and vision. Flies are usually attracted by lights due to their sensitive eyesight. The lamp used in the study emits light that has been adjusted to the sensitivity of the visual senses of fruit flies and insects in general, namely in the range of light spectrum of 300-650 nm or the range of purple, blue and green to red color. The Post Hoc Test results showed that light trap with blue lights was the most effective in trapping fruit flies.

In line with the results of Prasetya's study (2015) stating that the sticky trap glue with blue light had the highest number of trapped fruit flies of 14.67. Meanwhile, in this study, the percentage of trapped fruit flies was 16% for green, 33% for blue lights, 22% for red lights, 19% for white lights, and 10% for control groups (Figure 3). Based on these results it can be concluded that the blue light is the most effective color to attract fruit flies. The results of this study also support the reasons why many insect traps on the market have bluish-colored lights.

Some difficulties in this research were controlling fruits, glue thickness, box color and also the smell of glue. However, these things can be over-

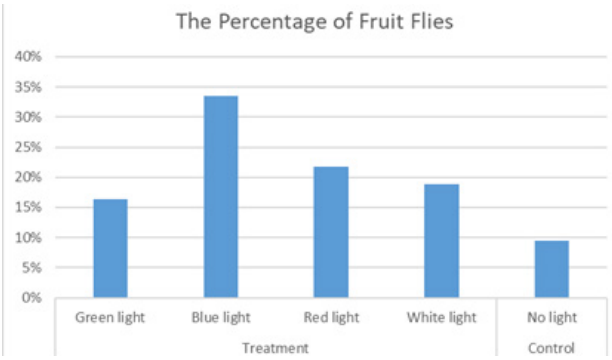

Figure 3. Percentage of number of fruit flies (treatment and control)

come by putting the same amount of fruits in each box. Instant sticky trap glue that has been provided on a paper sheet shape was used because if using manual fly glue, it will be difficult to measure the thickness of each sticky trap glue. Instant glue has the same odor, which is the smell of durian fruit, and white is used to control the color of the box. Simple light traps designed in this study were able to help the community, especially fruit sellers, control fruit flies that perched on the fruits they sell, and maintain the quality of fruit.

CONCLUSION

Green, blue, red and hite light variations influenced the number of trapped fruit flies. The most effective lamp color to be used in light trap application was blue. The difficulties in this research were controlling the fruit, the thickness of the glue, the color of the box, and the smell of the glue. Simple light traps can be used as an alternative for the community, especially fruit sellers to control fruit flies that perch on fruit.

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