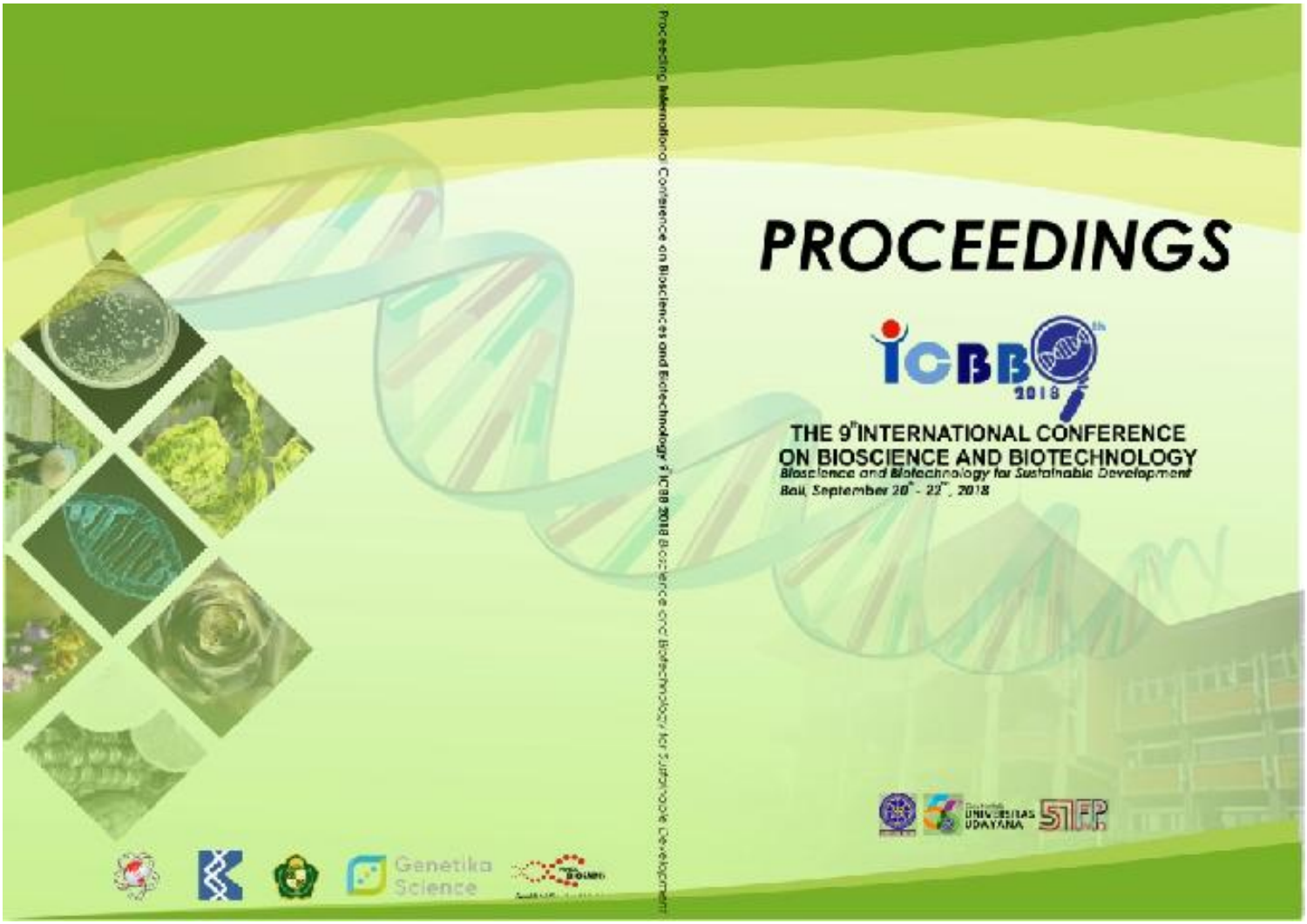
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WELCOME NOTE

It has been an honor for faculty of Agriculture Udayana University to host the international conference on biosciences and biotechnology for the nine times. The ICBB is a yearly conference initiated by Asia Oceania Biosciences and Biotechnology Consortium (AOBBC). This year, the 9th ICBB is held at the same time with the celebration of the anniversary of Udayana University and the anniversary of Faculty of Agriculture Udayana University. The theme for the ICBB 2018 is "Biosciences and Biotechnology for Sustainable Development".

The conference had been attended by approximately 165 participants. They come from various science background, such as agriculture, health and medicine, veterinary, and animal husbandry. Participant from Australia, Japan, Korea and Indonesia itself – Java, Flores, Kupang, Sulawesi, and Bali. Their contribution on the advancement of the biosciences and biotechnology are documented partly in this proceeding book.

At this good moment, I especially would like to thank Rector of Udayana University for the financial support given. Thank you for all keynote and invited speakers, persenters, and especially writers whom have contributed their knowledge, science research and experience to the wider audience through this proceeding. To all participants for your enthusiasm during the conference that make this conference a success. I also would like to thank the conference organizer team and student volunteers for their untiring efforts to make this conference as one of the memorable ones. My best wishes to all of you.

Denpasar, 6 November 2018
Dean of Faculty of Agriculture Udayana University
Prof. Dr. Ir. I Nyoman Rai, M.S.

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RELATION OF CARBON, NITROGEN AND BACTERIA IN SEDIMENT OF BADEK AND MEWEK RIVER, MALANG, INDONESIA

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Abstract

Total bacteria and avaibility of total carbon and nitrogen in sediment from Badek and Mewek River, Malang, East Java was observed in this study. This study was aimed to observed the total bacteria number in both river, and observed the relation between total bacterial number and the avaibility of carbon and nitrogen in the sediment. This study was carried out by observing the total bacterial number by using slow stirring method, total carbon was measured by using total carbon analyzer, while total nitrogen was measured by using indophenol blue method. The result showed that total bacterial number in the sediment of Badek River was lower (3.80×10^8 cell/g) than Mewek River (4.83×10^8 cell/g). There is relation between total bacterial number with total carbon ($R^2 = 0.8873$) and Total Nitrogen ($R^2 = 0.7955$). From that result it is indicating higher ecological pressure in Badek River than in Mewek River. Total bacteria and avaibility of total carbon and nitrogen in sediment from Badek and Mewek River, Malang, East Java was observed in this study. This study

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Keywords: bacterial, carbon, nitrogen, badek, mewek

Background

River ecosystem is one of aquatic ecosystems with dynamic characteristic influenced by its water flow. Interaction between microorganisms and other aquatic environmental components become interesting topic to be studied. Biogeochemical cycle in the aquatic ecosystem involves several kinds of microorganisms, such as bacteria and archea. However, about 70% of microorganisms in the sediment are bacteria. Several bacteria are involved in the decomposition of organic materials, including organic carbon and organic nitrogen, to inorganic materials through various mechanisms. A disturbance on this mechanism may cause instability in the river ecosystem. The input of pollutant to the river may, which is caused by anthropogenic activities, causes degradation of water quality in the river (Agustiningsih et al, 2012; Fransisca, 2011; Yeany, 2010).

Badek River and Mewek River is one of 20 rivers located in Malang City (Poetranto, 2018). These rivers plays important role on the aquatic ecosystem in Malang City. However, the increase of human activities around the river causes environmental degradation regarding to the pollutant input to the river (Santoso, 2016; Hidayat, 2018). Some factors causing the environmental degradation on these rivers are the disposal of domestic and industrial wastes. The environmental degradation in the Badek River is related with the existence of tannery industry, while Mewek River is influenced by domestic activities. This causes different characteristic of water and sediment properties in both

rivers. The high loading of this waste may increase the accumulation of suspended materials in the sediment (Auliyani and Wijaya, 2017). Therefore, it is important to perform an effort to recover the environmental quality for those rivers.

In order to know the way to recover these rivers condition, it is important to observe the characteristic of its environment (Widodo et al, 2010). Analysis on the sediment properties are crucial to be performed to know the sediment condition. The main reason to perform this way is whether sediment plays important role on the nutrient cycle in the river environment. Organic carbon and nitrogen are needed by the bacteria to perform these biogeochemical processes. Carbon is known to be used as energy for several aerobic bacteria (genus *Bacillus*, *Pseudomonas*, *etc.*) during the bacterial respiration (Jonsson et al, 2001; Orji et al, 2016), while nitrogen is used by α, β, and γ proteobacteria during dentrification process (Rodríguez et al, 2011; Pei et al, 2010). Therefore, it is important to perform a study to observe the characteristic of sediment in the Badek and Mewek River, and also the relation between total bacterial number with carbon and nitrogen.

Materials and Methods

Site location and sediment sampling

Sediment sampling was carried out in Badek and Mewek River, Malang City, East Java Province, Indonesia from July to August 2017. Sediment sample was taken from 3 different sites in Badek River (SB1, SB2, dan SB3) and Mewek River (SM1, SM2, dan SM3) (Figure 1). Sediment sample was taken from the bottom of the river by using Ekman Grab, and kept in a plastic bag. The samples, then, was kept in a refrigerator until analysis.

Measurement of TC, TN, and total bacterial number of sediment

Measurement of TC was performed using total carbon analyzer. A 0.5 to 1.0 g of sediment sample was put on the ceramic boat of the equipment, and placed on the TC analyzer machine (Adhikari et al, 2016). TN of sediment was measured using indophenol blue method after digested in a kjeldahl digester (Donald Nicholas dan Nason, 1957).

A 0.5 g of sediment sample was placed on a kjeldahl tube, mixed with H_2SO_4 dan H_2O_2 in equal volume (5 ml). Sediment sample was heated up to 420^oC for 1.5 hours in the kjeldahl machine, followed by cooling step at room temperature for 30 minutes. The extract sample was filtered by using filter paper no 6 (ADVANTEC). TN was measured by mixing 1 ml of extract with indophenol solution (0.4 ml) and sodium hypochlorite (0.6 ml). The mixture was incubated at room temperature for 45 minutes for color development. TN value was obtained from observation of mixture absorbance at 635 nm using UV visible spectrophotometer.

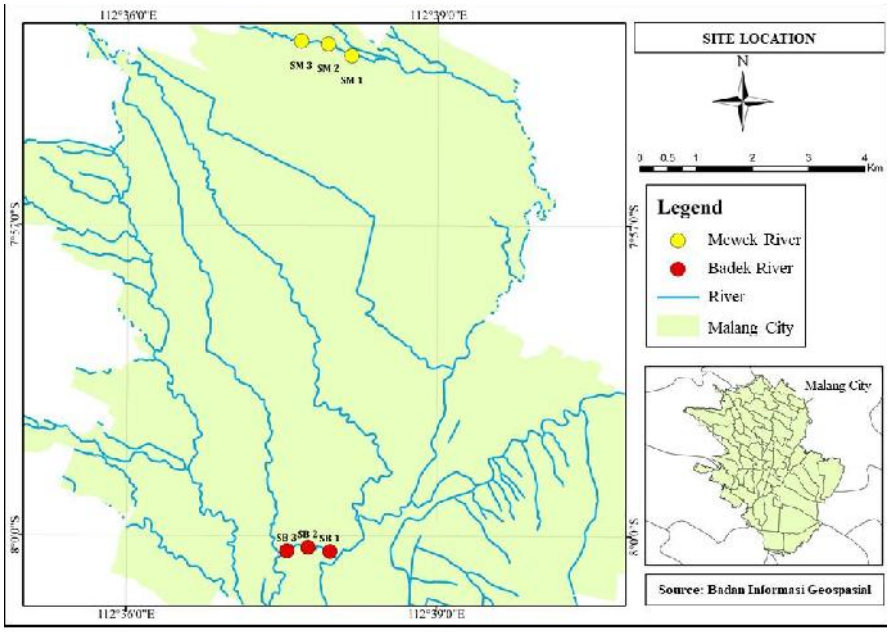

Figure 1. Site location

Total bacterial number in the sediment was estimated using slow stirring method (Aoshima et al., 2006). A 1 g of sediment sample was mixed with 8 ml of eDNA extraction buffer (37,22 g/L EDTA; 10,0 g/L CTAB; 87,66 g/L NaCl; 12,0 g/L H_6NaO_6P; and 12,11 Tris(hydroxymethyl)aminomethane) with pH 8,0) and 1 ml of 20% sodium dodecyl sulfate (SDS) solution. Next, the suspension was agitated at 1,500 rpm for 20 minutes, followed by centrifugation of that suspension at 6000×g for 10 minutes. The yielded supernatant was mixed with chloroform-isoamylalcohol (24:1) solution in equal volume (0.7 ml)

and centrifuged at 18000×g for 10 menit. To precipitated the DNA, 0.5 ml of aqueous phase was mixed with 0.3 ml of isopropanol solution, and centrifuged at 18000×g selama 20 minutes. Next, the precipitated DNA was rinsed using etanol 70% and mixed with TE Buffer 1×. Quantification of total bacterial number was obtained from electrophoresis of DNA extract in 1% gel agarose. Smartladder was used as the marker.

Results

Characteristic of sediment in Badek and Mewek River

The result showed that TC of sediment in the Badek river was lower (6,700 mg/kg) than that in the Mewek River (7,600 mg/kg). The TN of sediment in the Badek River was also lower (380 mg/kg) than that in the Mewek River (400 mg/kg). Based on this result, C/N ratio of sediment in Badek River was lower (C/N = 18) than that of sediment in Mewek River (C/N = 22). Total bacterial number in the sediment of Badek River was ranging from 3.09 to 4.03 × 10^8 cells/g, while in the sediment of Mewek River was ranging from 3.88 to 6.36 × 10^8 cells/g. Based on this result, the total bacterial number of sediment in the Badek River was lower (3.80 × 10^8 cells/g) than that in the Mewek River (4.83 × 10^8 cells/g) (Table 1). This result indicates the higher bacterial activities in the sediment of Mewek River than that in the Badek River. The high carbon source seems to enhance the bacterial number in the sediment of Mewek River.

Table 1. TC, TN, and total bacterial number of sediment

Site location	TC (mg/kg)	TN (mg/kg)	C/N Ratio	Total bacterial number (×10^8 cells/g)
SB1	5,700	310	18	3.09
SB2	5,800	370	16	3.49
SB3	8,700	470	19	4.83
Mean	6,700	380	18	3.80
SM1	6,800	330	21	4.25

SM2	11,500	510	23	6.36
SM3	8,300	370	22	3.88
Mean	7,600	400	22	4.83

Relation of TC and total bacterial number in the sediment

A simple analysis of correlation showed that there was correlation (positive correlation) between TC and total bacterial number in the sediment of river (Badek and Mewek River) (R^2 = 0.8873) (Figure 2). This result indicate that the availability of total carbon in the sediment might influence the density of bacteria in the environment.

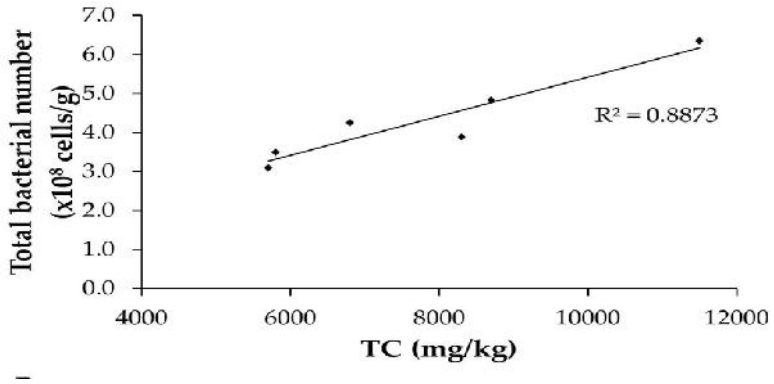

Figure 2. Relation of TC and total bacterial number in the sediment

Relation of TN and total bacterial number in the sediment

There are high correlation between TN and total bacterial number in the sediment of Badek and Mewek River. The analysis of correlation showed that value of R^2 was high (R^2 = 0.7955) (Gambar 3). This result indicates that nitrogen was needed by bacteria in the environment. Together with carbon, nitrogen might have high influence on the bacterial activities in the sediment.

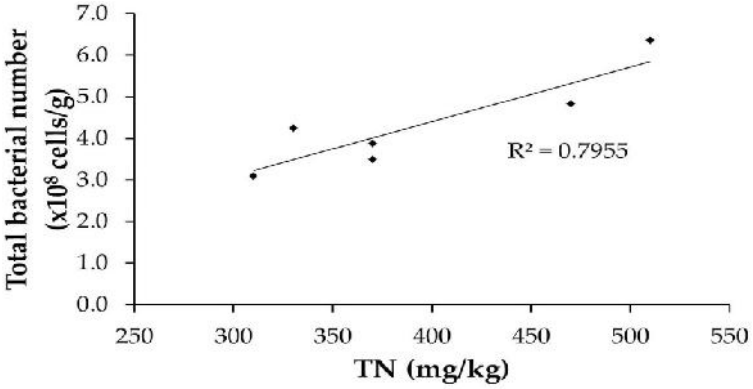

Figure 3. Relation of TN and total bacterial number in the sediment

Discussions

In a river ecosystem, the value of organic materials are varied depend on their hydrological characteristic (Aspetsberger et al, 2002; Hein et al, 2003; Schiemer et al, 2006; Besemer et al, 2009). Domestic waste sourced from anthropogenic activities tends to supply higher organic materials than that of industrial wastes. Waste disposal from industrial activity usually contain several kinds of pollutant, such as heavy metals. The high loading of heavy metals in the river may suppress and inhibit the bacterial activity. In case of Badek River, the lower bacterial number (Table 1) might be caused by the high loading of pollutant from industrial waste around the river. There are several tannery industries located around this area which may cause pollution to the Badek River.

There is high relation between total bacterial number and total carbon and total nitrogen, as shown at Figure 2 and Figure 3. According to Sobczak et al (1998), the key factors influencing the bacterial activity are dissolved organic carbon (DOC) and particulate organic carbon (POC). Organic carbons, such as cellulose, hemicelluloses and lignin are decomposed to CO_2 form by involving aerobic bacteria such as *Bacillus* sp. and *Pseudomonas* sp. through respiration (Jonsson et al., 2001; Orji et al., 2016). Regarding to the nitrogen content in the sediment of the river, the bioavailability of nitrogen in the river depends on the conversion of nitrogen containing compounds into utilizable form. In case of Badek and

Mewek River, bacteria in the sediment might be able to convert a broad range of N compunds into various forms (Madigan et al, 2006). According to Peterson et al (2001), the most available nitrogen forms in a river are nitrate and ammonium. This might involve bacteria responsible to perform nitrification (nitrifying bacteria) and denitrification (denitrifying bacteria). Therefore, the high relation between total nitrogen and total bacterial number might be due to these processes.

Conclusions

Badek river showed lower total bacterial number than that in the Mewek River, which might be caused by high loading of pollutant to the river. The result showed high correlation between total carbon with the total bacterial number. This might be due to the aerobic mechanism such as microbial respiration. Then, the relation between total nitrogen and total bacterial number might be due to the nitrogen conversion processes, such as nitrification that involving nitrifying bacteria and denitrification that involving denitrifying bacteria.

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PREVALENCE AND INTENSITY ECTOPARASITE *Trichodina* sp. ON NILE TILAPIA (*Oreochromis niloticus*) IN BADUNG RIVER, BALI PROVINCE FOR BIOMONITORING OF ECOSYSTEM

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Abstract

Badung river is one of the biggest river located in Bali Province. The river located in the Denpasar City, which is characterized as dense population and high industrial activities. As a result, pollutant also affect the biota's health in the ecosystem such as the Tilapia Fish (*Oreochromis niloticus*). This study was aimed to observe the prevalence and intensity level of ectoparasite *Trichodina* sp. in the Nile Tilapia. This study was conducted in April 2017. The research method was carried out with the descriptive method. Twenty five fishes were taken randomly from the Badung river. The parasite of the fishes was observed at the Fisheries Laboratory, Udayana University. The result showed that there were 21 fishes were infected by *Trichodina* sp. Total number of *Trichodina* sp. found was 181 individual from the infected fish. The prevalence and intensity of *Trichodina* sp. were 84% and 8.61 individual/fish.

Keywords: Nile Tilapia, Badung river, Prevalence, Intensity, Trichodina sp.

Background

Badung River is the main river that crosses Badung regency and Denpasar District. Tilapia is one of the fish that lives on the Tukad Badung river. Nile tilapias are among the most important commercial freshwater fish species in the world (Sulaiman and Al

Harbi, 2016). The health of fish is also affected by human activities such as anthropogencic and industrial activity that can influence change of water quality in the aquatic environment and can cause fish diseases and also mortalities (Poulin,1992). Protozoan parasites such as *Trichodina* sp. are most common fish diseases in fish, especially in Nile Tilapia. Investigations of parasites present in a habitat can provide good information about the health of the environment (Palm & Dobberstein 1999). The present study aimed to investigate the occurrence of prevalence and intensity of ectoparasites *Trichodina* sp. on Nile Tilapia in Badung River, Bali.

Materials and Methods

This study was conducted in April 2017. The research method was carried out with the descriptive method, 25 fishes were taken randomly from the Badung river. Furthermore, fish samples were taken and identified in the Fisheries Laboratory of the Faculty of Marine and Fisheries, Udayana University, Bali. Fish samples were sacrificed by a blow to the head immediately, skin scraped and gills filament were dissected and put on a glass slide with covered by cover glass and examined under microscope. The total number of parasites from the body surface (skin) and gills from each fish were counted. The intensity (I) was determined as the ratio between the total number of parasites in a sample and the number of infested fish in a sample. The prevalence (%) of the *Trichodina* sp. was estimated as the ratio between the number of infested fish and the number of examined fish in percentages. The data of prevalence and intensity were calculated following Bush *et al* (1997).

Results

Based on the research, prevalence and intensity of *Trichodina* sp. in Nile Tilapia in Badung River, Bali as presented in Table 1.

Table 1. Prevalence and Intensity of *Trichodina* sp. in Nile Tilapia in Badung River, Bali

Number of examined fish	Number of infested fish	Number of parasite observed	Prevalence (%)	Intensity (Ind/fish)
25	21	181	84	86,1

The result showed that there were 21 fishes were infected by *Trichodina* sp. Total number of *Trichodina* sp. found was 181 individual from the infected fish. The prevalence and intensity of *Trichodina* sp. were 84% and 8.61 individual/fish. Morphology of *Trichodina* sp. (figure 1) was observed from the body surface and gill fillament of fish. The key characteristics of *Trichodina* sp, were circular rings of cilia on oral and basal region (Aksit et al., 2008), round shape when seen from top of the dorsal of parasite and a ring with hook like denticles (Kabata, 1985).

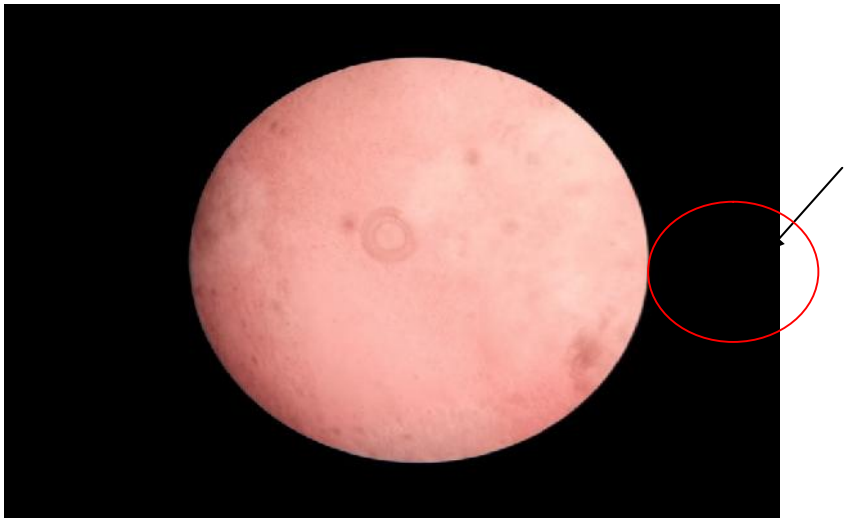

Figure 1. *Trichodina* sp. in Nile Tilapia in were taken from Badung River, Bali

Discussions

Trichodina sp. is one of fish protozoan parasites. *Trichodina* sp. can cause stress and damage fish morphology and also cause mortality in fish. *Trichodina* sp. are one of protozoan ectoparasite that are typically found on body surface, gills, and fins of fishes (Bason and Van As, 2006). The results of this research showed that the highest predilection of the *Trichodina* sp. was found on the gill filament compared to the body surface (skin) of fishes. The high number of parasites found on the surface of the gills filament of *Trichodina* sp. because on the gill filament contains a lot of blood vessels which are a good and supports parasites life. When the fish breathes the operculum is open, allowing the opportunity for *Trichodina* sp. enter the gills and can penetrate the gill filament into the blood vessels and cause massive destruction of the gill epithelium (Enayat et al., 2008). Trichodina sp. attached to the host epithelium with the sharp rim of the border membrane bites into the surface of the host epithelial cells, these activities are the main cause of host irritation and Trichodinids feed on the disrupted cells and of the hosts gills and skin of fish (Lom and Dykovà, 1992).

Although, the body's surface of fish is directly related to the environment, making it easier for parasites to stick compared to the gills covered by overculum. On the other hand, this maybe there is environmental stressor that affect water quality condition and caused low abudance of ectoparasite in the body surface. The presence of ectoparasites is directly related to water quality

(Moraes and Martins, 2004). Parasites could be used to determine the effects of pollutants on ecosystems, as an alternative to studying other parameters (Marcogliese & Cone, 1997). In addition, if the prevalence of *Trichodina* sp. is high also supported by water quality conditions, it is possible to accelerate the breeding process of ectoparasite. This parasite has ability to multiply by dividing rapidly and always moving actively (Woo, 2006; Basson, 2010). The high intensity of ectoparasite can be influenced by water quality condition because it can support the life cycle of the ectoparasite. *Trichodina* sp. has a wide spread and can multiply quickly (Basson dan Van As 2006). Polluted condition can make parasite populations more abundant and therefore *Trichodina* sp. could be used as a pollution indicator (Palm & Dobberstein 1999).

Conclusions

The results of the observation in this research showed that the highest predilection of the *Trichodina* sp. was found on the gill filament compared to the body surface (skin). The prevalence and intensity of *Trichodina* sp. in this study were 84% and 86,1 individual/fish.

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UTILIZATION Of *Trichoderma* sp. AS A BIOLOGICAL AGENT OF FUSARIUM SCREEN DISEASE ON *Capsicum Annum* PLANTS IN LUWU DISTRICT

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Abstract

Chili pepper is one of the leading vegetable commodities that have comparative and competitive advantages that are cultivated by farmers in various scales of farming. However, in the effort to cultivate in Sulawesi - South are still many obstacles encountered, and one of them is the existence of disease attack. Attack of diseases that often attack chili planting is fusarium wilt disease caused by fungus Fusarium oxysporum. The effects of fusarium wilt disease have resulted in decreasing yield of harvests even to crop failure. One of the alternative efforts to control fusarium attacks is by utilizing the fungal biological agent Trichoderma sp so that it can increase the yield of chilli crops in South Sulawesi. This research is expected to be a reference for farmers, especially in Luwu District to implement control of biological agents that are environmentally friendly in terms of suppression and spread of fusarium in chili plantations. The research design used in this study was a randomized block design consisting of 5 treatments including P1 Control, P2 Trichoderma sp 70 grams of rice media, P3 Trichoderma sp 80 grams medium of rice, P4 Trichoderma sp 90 grams of rice medium and P5 Trichoderma sp 100 grams rice media. Furthermore, this research was repeated 3 times to obtain 15 units of experimental unit. The results showed that the combination of 100 grams of trichoderm treatment (P5), sp + 10 kg of organic fertilizer had a significant effect on the intensity of fusarium wilt attack on chili plants with an attack intensity of 3.33%. This research can be concluded that the use of Trichoderma biological agents in combination with 100 grams of organic fertilizer + 10 kg of organic fertilizer can reduce the intensity of fusarium attacks on chili plants.

Keywoord : Chili, Fusarium, Trichoderma sp.

Background

The red pepper plant is one of the horticultural plants quite important, both for domestic consumption and as a commodity export. The consumption needs of red chili each year increases and Until now the red pepper plant is one of the plants considered potential to be developed. Red chili plants can grow and producing in the lowlands to the highlands, both on paddy fields and moor, in the lowlands to the highlands. (Hodiyah and Hartini, 2014)South Sulawesi is one of the centers for chili cultivation in Indonesia with the average yield of chili crops in 2014 amounting to 20.79 thousand Ton / Ha (Central Statistics Agency, 2014). But that number is stillan not meet the needs of existing markets in South Sulawesi because in the cultivation of red chili always faces obstacles, wrong the other is fusarium wilt attack caused by fungi Fusarium oxysporum. Fusarium oxysporum f.sp.capsici (Foca) is a major obstacle in the cultivation of chili plants. This pathogenic fungus can attack plants Red chili starts from germination to adulthood, with characteristics Symptoms of leaf attack experience wilting from the bottom, yellowing and spread up and to the young branch. Fungus F intensity. oxysporum if not seriously controlled will have an impact on the decline harvest pruduction with a percentage of yield loss reaching 80%. The high rate of yield loss due to F.oxysporum fungus attacks demanding farmers to take control measures. Control measures which is often done by using synthetic fungicides.

But thus, excessive use over a long period of time will have a negative impact on human health and environmental pollution. Addition tothe application of the use of synthetic fungicides that is not wise can trigger the emergence of pathogens that are resistant to synthetic fungicides used. Alternative control of the spread of fungi F. oxysporum on plants red chili is the use of biological agents in controlling the organism plant pest (OPT) currently biological agents are growing because has advantages compared to pesticide-based controls. Wrong one utilization of biological agents that can be used is the use of mushrooms Trichoderma sp to control the spread and attack of Fusarium wilt on chili plants. Trichoderma sp is one of the fungi that is antagonistic to other fungi. Pathogen antagonist fungus mechanism plants in suppressing population or plant pathogen activity can in the form of hyper parasitism, competition for space and nutrients, and antibiosis and lisis. Its effectiveness can be seen by not developing the disease (Herman. et al 2014).

Some members of the genus Trichoderma produce trichodermin toxin, this toxin is produced by fungi when alive on living plants. The presence of high metabolic hyphae in the material organic can also attack and destroy existing pathogen propagules surrounding. Trichoderma viridae produces 2 types of antibiotics, gliotoxins and viridian that can protect seed plants from disease attacks (DKP, 2011). The results of research by Prabowo et al. (2006) prove that with addition of T. harzianum can suppress the development of the fungus F. oxysporum Schelect. f.sp.

Zingeberi Trijillo in kencur plants with results ranging from 7.9% to 56.3%. Based on this, it needs to be done research to find out the right number of doses in mushroom applications Trichoderma sp, in terms of controlling the spread of fusarium wilt environmentally friendly based on chili plants.

Materials and Methods

The study will use a randomized block design (RBD)consists of 5 treatment applications and 3 replications so as to obtain 15 units of unitstrial.Variations in treatment applications that will be used are as follows:

P1 = Without using Trichoderma sp (control)

P2 = Trichoderma sp 70 grams of rice medium

P3 = Trichoderma sp 80 grams of rice medium

P4 = Trichoderma sp 90 grams of rice medium

P5 = Trichoderma sp 100 grams of rice media

A. Sample Search

The isolate collection was carried out for 2 days in the chilli planting center Luwu Regency, namely Balo-balo Village and Cilallang Village, each village 4 samples of chili plant were attacked by wilted disease with characteristics symptoms typical of F.oxysporum attacks. that is, the leaves turn yellow, occur withering unilaterally or completely, the rootstock turns brown and if the stem is split longitudinally or transversely, the network is visible xylem is brown, reddish, blackish or yellowish. Part plants

taken as samples come from the base of the stem, collection of parts diseased plants are used as a source of isolate.

B. Making PDA Media (Potato Dextrose Agar)

A total of 200 grams of potatoes were put into 1000 ml of distilled water and heated to boiling, then add 20 grams of sugar as a source of nutrition. Furthermore, as much as 15 grams to be added as compactor. The media is sterilized on autoclaves with a temperature of 121ºC for 15 minutes. Before pouring on petri dishes, antibiotics were added Clorompenicol to avoid contamination or growth Other microorganisms including bacteria. Then the PDA media is removed 12 poured on a sterile cup 10 ml each and left untilfreezes.

C. Making Fusarium oxysporum culture

The making of F. oxysporum culture is done by selecting plant parts(the base of the stem) is diseased and then cut about 10 cm, then given a sign and stored in the cooler. Sporulation of diseased plant parts in the box tray to accelerate hyphae The purification of isolates is carried out with take part of the plant that has been stored and then cut 0.5 cm, after the cutting process of the part is planted on PDA media that has been added with anti-bacterial. The culture was incubated at room temperature for 2-3 days. After growing, identification is done by taking fungus end part using ose needle, then isolated on New PDA media that has

been sterile. This process is carried out in an isolation box aseptically then incubated. Purification is done 2-3 times
obtained pure isolate.

D. Preparation of Propagation of Fungi Trichoderma sp

Trichoderma sp fungus used is the result of multiplication Laboratory of Installation, Forecasting and Controlling of Organism Plant Distractors (OPT IP3) Region I Luwu District developed on rice media.

E. Application of Trichoderma sp

Trichoderma sp is given when transplanting, accordingly with the dose tested by sprinkling the mushroom inoculum on the surface of the planting medium just before planting chilli seeds. Fungus F inoculat oxysporum is carried out together with the administration of a mushroom inoculumTrichoderma sp. In each planting hole, F. oxysporum sp as much as 100 ml with a concentration of 1% (10 grams / liter of water).

F. Harvest

Harvesting is done with mature physiological characteristics on the changing fruit become a reddish color The cayenne plant can be harvested after age 2.5-3 months after sowing. The next harvest can be done 1-2 weeks depending on the health and fertility of the plant.

G. Observation Variable

The parameters observed in this study include:

a. Percentage of fusarium wilt attacks, using the formula:

$$P = \frac{n}{N} \times 100\%$$

P = Percentage of fusarium wilt attacks

n = Number of petioles that show fusarium symptoms

N = The total number of petiole. (Hidayat, 1993).

Observations made every 1 week include:

a. Vegetative growth of chili plants once a week up to 10 week after planting (MST), consisting of: plant height growth and the growth of the number of leaf stalks.

b. Generative growth and yield of chili plants which consist of:

- Growth in the amount of interest, starting at 8 MST to 16 MST.
- Growth in the amount of interest, starting 12 MST to 18 MST.
- The number of weights to start from 16 MST to 18 MST.

Results

A. Plant Height (cm)

The results of the study for the character height of plants from several combinations Trichoderma sp and organic fertilizer are presented in Table 1. Combinations administration of the treatment of this study shows that the combination Trichoderma sp and organic fertilizers give effect significant growth of chili plants. Treatment of 100 grams Trichoderma sp + 10 kg of organic

fertilizer has a significant effect on height plants and significantly different from other treatments with a mean of 19.90 cm.
cm at 63 days.

Table 1. Average effect of the dose of Trichoderma sp and organic fertilizer onplant height.

Dosis Trichoderma sp + pupuk organik	**Tinggi Tanaman (hst)**			
	42	49	56	63
P1 (Kontrol)	7.80c	9.23b	10.10b	10.67c
P2 (70 gram *Trichoderma* sp + 10 kg pupuk organik)	8.67c	9.27b	10.30b	11.83c
P3 (80 gram *Trichoderma* sp + 10 kg pupuk organik)	9.07c	10.63b	11.43b	12.67c
P4 (90 gram *Trichoderma* sp + 10 kg pupuk organik)	13.40b	14.83a	15.50a	16.53b
P5 (100 gram *Trichoderma* sp + 10 kg pupuk organik)	17.63a	18.37a	18.83a	19.90a

The administration of trichoderma sp combined with organic fertilizer is able to supply nutrients for the needs of the plant so that it can stimulate maximum plant growth. Bertham et al., (1996) reported that the provision of organic matter decomposed by saprophytic fungi Trichoderma sp was able to stimulate the number of stems and plant growth in line with this opinion Trichoderma sp was also able to degrade organic matter into nutrients that support plant growth well. In addition to stimulating the growth of trichoderma plants, they are also antagonistic fungi that can suppress various soil-borne diseases.

Trichoderma sp. also known to produce growth hormones such as cytokines and auxins. Organic material as a carrier material for biocontrol agents has dual benefits because in addition to being a carrier material as a source of nutrition (food base) for biocontrol agents (Hoitink and Boelim, 1999). The application of the use of organic fertilizer as an additional nutrient element can support the process of growth and development optimally because it has macro nutrient content of Fe, Mn, Cu, Zn and B which will help the process of soil nutrient needs.

Trichoderma spp. able to increase the growth of high plants because it is able to maintain soil fertility, increase the activity of indigenous microorganisms as well as being a decomposer of nutrients that were previously unavailable to be available from organic and mineral materials. Trichoderma spp. if it has infected the roots of the host plant, it will be able to help the host plant absorb certain nutrients, especially phosphorus (Harrison and van Buuren, 1995; Bryla and Koide, 1998).

B. Number of Leaves.

The response of plant growth in this case the number of leaves is presented in Table 2. The character of the number of leaves showed a significant effect on the BNT follow-up test. Chili plants treated with Trichoderma sp and biological fertilizers showed the best number of leaves, namely for treatment P5 with an average of 20,00 strands. This is because organic fertilizer can improve the physical properties of the soil so that the soil becomes loose. Raharjo and Pribadi (2010) reported that organic fertilizers

increase the availability of nutrient elements N, P, and K which are nutrients that are most absorbed by plants, so that if there is a shortage of nutrients it will cause a decrease in plant growth and production activities

Table 1. Average effect of the dose of Trichoderma sp and organic fertilizer Number of Leaves.

***Trichoderma* sp + pupuk organic**	**Number of Leaves (hst)**			
	42	49	56	63
P1 (Kontrol)	10.33d	9.23c	7.67d	12.00d
P2 (70 gram *Trichoderma* sp + 10 kg pupuk organik	12.67c	10.83c	9.67cd	14.33b
P3 (80 gram *Trichoderma* sp + 10 kg pupuk organik)	15.00b	12.83bc	11.67bc	17.33b
P4 (90 gram *Trichoderma* sp + 10 kg pupuk organik)	16.00b	15.83ab	12.00ab	18.00b
P5 (100 gram *Trichoderma* sp + 10 k pupuk organik)	18.33a	18.37a	14.00a	20.00a

Trichoderma sp causes the formation of new leaves and increases in root fibers. According to Sepwanti et al. (2016) Trichoderma sp. serves to break down organic materials such as N contained in complex compounds, nitrogen is used by plants to stimulate plant growth and give a green color to the leaves. The results of Bryla and Koide (1998) research on chilli plants grown in soil types associated with Oxisols and Alluvial showed that the administration of Trichoderma fungi increased the P leaf content. Trichoderma sp. able to maintain soil fertility, increase the activity

of indigenous microorganisms and become decomposers of nutrients that were previously unavailable to become available. In addition organic fertilizer has a role but this type of fertilizer has another function that can improve soil physical properties such as soil permeability, porosity. Organic compounds in the form of crop residues or animals are composed of complex carbohydrates, simple sugars, flour, cellulose, hemicellulose, pectin, proteins, fats, waxes, resins, alcohols, aldehydes, ketones, organic acids, lignin, phenols, tannins, hydrocarbons, alkaloids, pigments, and other products.

Addition of Trichoderma sp. on land before planting is one way of manipulating land biologically. Trichoderma sp is a useful microorganism and is a symbiotic fungus that is not dangerous, even mutually beneficial for plants because it can colonize plant roots. Trichoderma fungi help mother plants absorb certain nutrients (Poulton et al., 2011), especially phosphate (Harrison and van Buuren 1995).

Conclusions

The development of chilli commodity is carried out through the application of a cultivation system using biological agents and organic fertilizers that aim to increase the growth of chili plants. The results of the interim study showed that the combination of treatment (P5) 100 grams of trichoderma, sp + 10 kg of organic fertilizer had a significant effect on plant height and number of leaves. The average plant height and the best number of leaves is

19.37 cm and 20.00 leaves. This study can be concluded that the use of biological agents combined with organic fertilizer as much as 100 grams + 10 kg of organic fertilizer can increase the growth of chili plants.

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THE POPULATION AND INTENSITY OF THE PEST ON SEVERAL RICE VARIETIES IN SUB DISTRICT TURIKALE REGENCY OF MAROS

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Abstrack

Rice plants (*Oryza sativa* L.) are annual crops that play an important role in meeting the food sources of the Indonesian population. Types of pests that attack rice plants include *Scirpophaga innotata, Nephotettix virescens, Leptocorixa acuta, Nezara viridula, Nilaparvata lugens, Nephotettix apicalis, Cnaphalocrocis medinalis, Nephotettix apicalis, Nephotettix nigropictus, Pachydiplosis oryza, Nymphula depunctalis, Tryporiza innotata, Sesamia inferens,* and *Rattus argentiventer*. This study aims to determine the population and intensity of pest attacks on several varieties of rice plants. The time of this research was started from January to March 2018. The location of this research was the rice field area of Turikale District, Maros Regency. This study uses a survey method of the location of rice fields with each field size of 20 x 15 m. The results showed that pest populations in Inpari 30 variety rice plants with the highest average population were 3.13 tails and the lowest Ciliwung 0.88 tails. The highest pest intensity was observed at week I observations with the highest intensity average of 20.06% and the lowest in week III which was 17.71%. The Inpari 30 variety has the highest attack intensity of 33% and the lowest is Ciliwung variety, which is 10.15%.

Keywords: population, intensity of attack, pest, rice

Background

Rice plants (*Oryza sativa* L.) are annual crops that play an important role in meeting the food sources of the Indonesian population. According to BPS data from the Maros Regency (2015) most of the rice production in Maros Regency is produced by wetland rice. This type of rice contributes 98.49% of all rice production or 322,429.44 tons. While 1.51% is produced by field rice.

At present the government continues to make efforts to meet food needs. However, one of the obstacles in the effort to fulfill this is an attack of pests that can reduce the quantity and quality of yields and can even cause crop failure. The main pests of rice crops include *Scirpophaga innotata, Nephotettix virescens, Leptocorixa acuta, Nezara viridula, Nilaparvata lugens, Nephotettix apicalis, medinal Cnaphalocrocis, Nephotettix apicalis, Nephotettix nigropictus, Pachydiplosis oryza, Nymphula depunctalis, Tryporiza innotata, Sesamia inferens,* and *Rattus argentiventer* (Pracaya , 2007).

Various ways that have been done in suppressing greater yield losses caused by pests, however, still found some obstacles due to information about the existence of pest population dynamics, causes of damage and effective and efficient control methods. One effective pest control is to implement an IPM system (integrated pest control) which is a pest control system in the relationship between population and environmental dynamics of a pest, and uses a variety of compatible control techniques to keep pest

populations always below the economic threshold (Baehaki, S.E. 2009). This study aims to determine the population and intensity of pest attacks on several varieties of rice plants in Turikale District, Maros Regency.

Materials And Methods

Materials and tools used in this study include killing bottles, meters, collection bottles, insect nets, label paper, plastic bags, bamboo stakes, scissors and writing instruments. This research was conducted at the rice field location in Turikale District, Maros Regency. This study uses a survey method for the location of rice fields to determine where the sampling took place. Each field plot was determined with a size of 20 x 15 m and then divided into 3 observation sub-plots which were spread with each of the 10 clumps of plants. This sampling was carried out by performing 10 double swings in the predetermined paddy field sub-plot. Pests netted in a swing then put into a bottle killing then observed and calculated the number of individuals. The level of damage caused by a pest attack is determined by the formula:

$$I = \frac{n}{N} x100\%$$

Information :

I = intensity of attack (%)

n = Number of clusters attacked

N = Number of clumps observed

Results

Pest Population in Some Rice Plant Varieties

Table 1. Average Pest Population on Inpari 30 Varieties of Rice

Sunday observation (mst)	Average pest population in the sample (tail)							Average
	Scirpophaga innotata	*Cnaphalocrocis medinalis*	*Nephotettix virescens*	*Nilaparvata lugens*		*Leptocorixa acuta*	*Rattus argentiventer*	
I	1.9	6.3	0.5	-	-	-	-	2.90
II	0.9	10.4	0.9	-	-	-	-	4.07
III	0.8	5.8	0.7	-	-	-	-	2.43
Averag e	1.2	7.5	0.7	-	-	-	-	3.13

Table 2. Average Pest Population in Mekongga Varieties of Rice

Sunday observation (mst)	Average pest population in the sample (tail)							Average
	Scirpophaga innotata	*Cnaphalocrocis medinalis*	*Nephotettix virescens*	*Nilaparvata lugens*	*Leptocorixa acuta*	*Rattus argentiventer*		
I	0.9	1.1	-	-	-	-	-	1.00
II	0.5	0.2	-	-	-	-	-	0.35
III	0.4	3.5	-	-	-	-	-	1.95
Average	0.6	1.6	-	-	-	-	-	1.10

Table 3. Average Pest Population in Ciliwung Varieties of Rice

Sunday observation (mst)	Average pest population in the sample (tail)							Average
	Scirpophaga innotata	*Cnaphalocrocis medinalis*	*Nephotettix virescens*	*Nilaparvata lugens*	*Leptocorixa acuta*	*Rattus argentiventer*		
I	0.5	1.5	-	-	-	-	-	1.00
II	0.3	0.9	-	-	-	-	-	0.60
III	0.4	1.7	-	-	-	-	-	1.05
Average	0.40	1.37	-	-	-	-	-	0.88

Table 4. Average Pest Population in Ciherang Varieties of Rice

Sunday observation (mst)	Average pest population in the sample (tail)							Average
	Scirpophaga innotata	*Cnaphalocrocis medinalis*	*Nephotettix virescens*	*Nilaparvata lugens*	*Leptocorixa acuta*	*Rattus argentiventer*		
I	0.8	2.8	-	0.1	-	-	-	1.23
II	0.1	2.4	-	-	-	-	-	1.25
III	1.7	2.8	-	-	-	-	-	2.25

Average	0.87	2.67	-	0.1	-	-	-	1.57

From the results of the observation, the highest pest population can be seen in Inpari 30 varieties with an average of 3.13 tails (Table 1). The second highest pest population is the Ciherang variety with an average of 1.57 tails (Table 4.) when compared to the average pest population in Mekongga Varieties (Table 2.) and Ciliwung Varieties (Table 3.)

Intensity of Pest Attack on Some Rice Crop Varieties

Table 5. Average intensity of pest attack on some rice varieties

Sunday observation (mst)	Pest attack intensity in several varieties (%)				Average (%)
	Inpari 30	Mekongga	Ciliwung	Ciherang	
I	26.54	14.98	12.22	26.48	20.06
II	39.59	7.36	9.56	11.34	16.96
III	32.86	15.87	8.67	13.45	17.71
Average (%)	33.00	12.74	10.15	17.09	18.24

From table 5 it can be seen that the intensity of the attack on observation week I had the highest average intensity of 20.06% compared to observations of week II and week III. The varieties with the highest attack intensity can be seen in the Inpari 30 variety which is 33% and the lowest is Ciliwung variety which is 10.15%.

Discussion

Pest Population in Some Rice Plant Varieties

From the results of observations of sampling in several varieties of rice plants it was found that pest populations differed

from observational data for week I week II and week III. For Inpari 30 varieties with the highest average population of 3.13 compared to Mekongga varieties with an average population of 1.10, Ciliwung 0.88 tail, and Ciherang 1.57 varieties. This is because the cultivation factors carried out in carrying out a rice cultivation business such as spacing, still depend on the use of pesticides. According to Georghiou (in Pratiwi, 2014) one of the negative impacts caused by the use of pesticides such as insecticides is the emergence of resistance in insect pests. Resistance causes a pest insect to become resistant to insecticides. This situation usually arises as a result of the continuous use of one type of insecticide for a long time.

In addition, the use of high Nitrogen chemical fertilizers can increase pest attacks. This is consistent with the opinion of Baehaki, S.E (2009) that excessive use of chemical fertilizers - N can increase pest populations, therefore giving fertilizer tailored to the needs of plants is one way to reduce the development of pests and diseases. Leaf color chart technology (BWD) developed by IRRI is a breakthrough in improving fertilizer efficiency due to the use of nitrogen fertilizer (urea) tailored to the needs of plants. The Agricultural Research and Development Agency has also developed P and K fertilization technology based on soil nutrient status.

The third factor is the environmental factor in which around the location of the research site a sanitation technique is not carried out so that the number of weeds that grow in the field of rice fields,

because in addition to attacking rice plants, these pests also make weeds as alternative hosts to survive. According to Manwan (in Manopo, 2013) host plants also play an important role in regulating the high and low population of insects. Depending on the level of resistance of a new variety can cause pests to become more important.

Intensity of Pest Attack on Some Rice Crop Varieties

The highest pest attack intensity can be seen in week I observations have the highest average intensity of 20.06% compared to observations of week II and week III. This is due to climatic factors that make the pest population decrease when observing. This is consistent with the opinion of Baehaki, S.E (2009) that rice pests will not explode throughout the season and the increase in population only occurs during the rainy season. In the dry season, pest populations, such as leafhoppers, tend to be low, except in a rainy dry season or in the basin.

It can also be seen that the highest intensity of attack can be seen in the Inpari 30 variety, which is 33% and the lowest is Ciliwung variety, which is 10.15%. This is because the cultivation technique of regulating techniques or crop rotation is not carried out, causing high intensity of pest attack on rice plants. This statement is supported by the opinion of Baehaki, SE (2009) that setting in-season cropping is also needed to counteract the attacks of brown planthopper and rice-stem borer, ie at the beginning of the rainy season planting resistant varieties that are short lived and in

the middle of the season until the end of the rainy season planting varieties who are not resistant or resistant to brown plant hopper and long lived.

Conclusion

1. Pest population in Inpari 30 variety rice plants with the highest average population of 3.13 tails and the lowest Ciliwung 0.88 tails.
2. The highest intensity of pest attack on observations of week I with the highest average intensity of 20.06% and the lowest in the third week of 17.71%.
3. Inpari 30 varieties have the highest attack intensity of 33% and the lowest is Ciliwung variety which is 10.15%

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An Increase of Plant Growth of Vanda Orchid Using Various Concentrations of Auksin and Giberelin *In Vivo*

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Abstract

This study aims to determine (1) the effect of growth concentration of growth regulator auksin and giberelin on the growth of Vanda orchid and (2) the proper concentration of the growth regulator for the optimum growth of Vanda orchid. This research was conducted at Rumah Anggrek, Campus 2 Faculty of Agriculture Universitas Cokroaminoto Palopo in February to April 2018. The method used in this study is a Completely Randomized Design (CRD) with 4 treatments consisting of 3 replications. P0 = 0 mg/L auxin + 0 mg/L giberelin (control), P1 = 50 mg/L auxin + 250 mg/L giberelin, P2 = 100 mg /L auxin + 300 mg/L giberelin, and P3 = 150 mg/L auxin + 350 mg/L giberelin. The results showed that the administration of auxin and giberelin at each concentration gave results that were not significantly different for the character of plant height, leaf length, leaf width, and number of roots. The treatment of P0 shows the best root length with an average of 18.43 cm. However, unlike P3 which shows the plant height, leaf length, leaf width, the number of leaves and the best number of roots with an average of 10.13 cm, 22.90 cm, 2.30 cm, 8.33 strands and 7.33 strands.

Keywords: Auxin, gibberellins, growth orchid, growth regulators, vanda orchid

Background

Orchids have a high economic value when compared to other ornamental plants, both for cut flowers and for potted flowers. Indonesia's tropical climate in addition to suitable orchid life is also very potential to produce quality orchids. Orchid production in 2011 reached 15,490,256 stalks, in 2012 it reached 20,727,891 stalks, 2013 as many as 20,277,672 stalks and 2014 as many as 19,739,627 stalks. The export of orchids in 2012 to several countries such as Taiwan, Singapore, Malaysia, Australia, Korea and Japan was 69,353 kg, a decrease in 2013 of 58,656 kg and 2014 of 52,651 (BPS, 2014 *dalam* Pusdatin, 2015). The magnitude of this fluctuating acquisition shows that there is a need to increase the quality and quantity of orchids in order to improve the economic value of orchid plants (Puspitasari, 2006 *in* Krishardianto, 2016). Agus (2001) *in* Utari (2015) states that aside from the aspect of beauty, the attraction of orchids is the endurance of flower blooms, and the scarcity of its species. All this attraction makes orchid collectors or breeders look for it so the demand for orchids as cut flowers and potted plants is increasing.

According to Rupawan et al. (2014) one type of orchid that is in great demand by the community and has high economic value is Vanda orchids. Vanda popular because of the beauty and the beauty of the flowers so that the market demand for this type of orchid is increasing, and the number of requests to meet these needs, Vanda *sp* has high economic value and has the potential to

be developed commercially. During this time the fulfillment of the demand for the orchid market was carried out using conventional techniques and tissue culture techniques. Conventional techniques can usually be in the form of stem cuttings, clumping of clumps, or separation of tillers (split) (Gunawan, 2007 *in* Meilani et al., 2017). However, in an effort to fulfill the demand for orchids by using conventional techniques, it has several disadvantages including requiring a long time, is not practical, and is not commercially profitable because the number of tillers obtained is very limited (Ning, 2013 *in* Meilani et al., 2017).

Orchid plants have a fairly slow type of growth, with different growth rates depending on the type (Nesiaty and Maloedyn, 2007 *in* Krishardianto, 2016). The type of slow growth requires a long time for flowering to occur. The low productivity and quality of orchids is an obstacle that hinders the progress of orchid development in Indonesia to be able to compete in the international market. Quality and quantity enhancement of orchids needs to be done by increasing growth and accelerating flowering of orchids. Orchid flowering is influenced by internal and external factors. Internal factors that influence flowering are plant age, physiological conditions, and hormonal availability. While external influencing factors are climatic conditions, media conditions and availability of nutrients. The provision of fertilizer and growth regulating substances (ZPT) is one effort to optimize both growth and flowering (Sandra, 2007 *in* Hasan et al., 2012).

There are several ZPTs that can be used to stimulate flowering initiation processes, namely Giberelin, Auxin, Cytokines, Bnine Inhibitors (Alar) and Paclobutrasol. According to Heddy (1996), Miryam et al. (2008) *in* Kurnianti (2011) auxin is able to stimulate the process of cell elongation in plants. The type and concentration of auxin were able to induce roots in shoots, root length, and number of roots in Dendrobium sp. (Sulasiah et al., 2015). The combination of auxin along with giberelin can stimulate the development of vascular tissue and encourage cell division in the vessel cambium so that it can support stem diameter growth. Giberelin itself affects the development and germination of embryos. Bey et al. (2005) stated that the administration of giberelin has a positive effect on the germination of the moon orchid seeds. According to Gunadi (1985) in Hasan et al. (2012) leaf fertilization is more efficient for orchids, because fertilizers can enter plant cells through the mouth of the leaves (stomata) that are on the leaf surface. In orchid plants, the leaves can absorb fertilizer approximately 90%, while the roots can only absorb approximately 10% (Sessler, 1978 *in* Hasan et al., 2012).

The purpose of this study are (1) to find out the effect of concentrations of auxin and giberelin growth regulators on the growth of Vanda sp orchid plants, and (2) to find out at what concentration the growth regulating agent gives the best effect on the growth of Vanda sp. Based on the description above, it is necessary to do research on the administration of auxin and gibberelline ZPT to the growth of orchid plants.

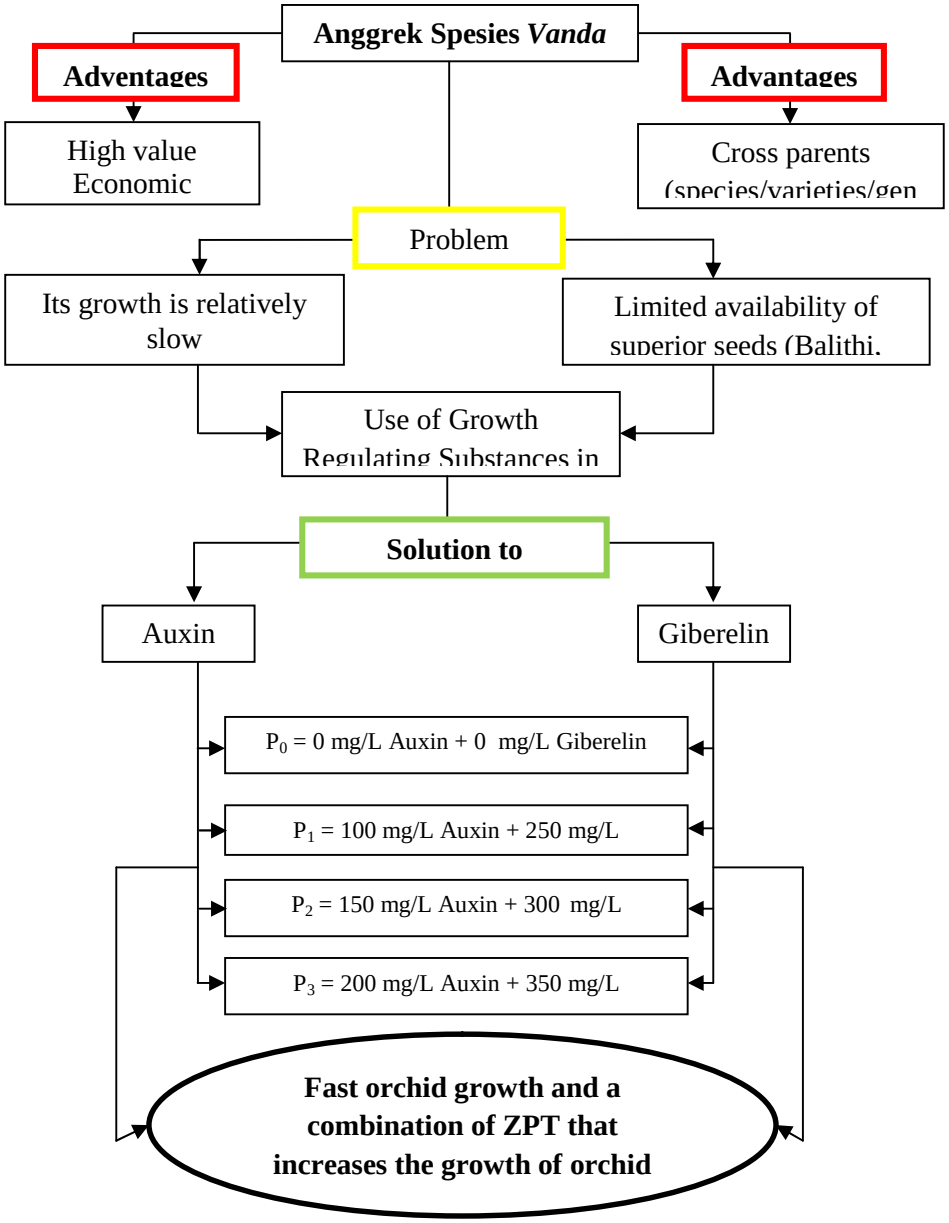

Figure 1 Research framework

Materials and Methods

This research was conducted at the Home of Orchid Campus 2 Faculty of Agriculture, University of Cokroaminoto Palopo. The study time is from February 2018 to April 2018. he materials used are Vanda species orchid plants, fern media, auxin and giberelin growth regulators, and water. The tools used in this study are pots, handsprayers, labels, markers, trophies, rulers, cameras, pens, books and calipers. This study used a Completely Randomized Design (CRD) with 4 treatments and 3 replications so that there were 12 experimental units. The treatments used in the study were P0 = 0 mg/L Auxin + 0 mg/L Giberelin (Control); P1 = 100 mg/L Auxin + 250 mg / L Giberelin; P2 = 150 mg/L Auxin + 300 mg/L Giberelin; P3 = 200 mg/L Auxin + 350 mg/L Giberelin.

Before planting, the Vanda sp orchid selection phase was carried out which will be used by selecting orchids that are 4 months old. The selected orchids are grown in a pot containing the chopped fern media. The characteristics that are superior to fern rod media are due to its properties which are easy to bind to water, have good aerase and drainage, and are soft textured so that they are easily penetrated by plant roots. After the orchids are able to adapt, then auxin and geberelin treatments are prepared according to their concentration.

Spraying is done every evening in accordance with the concentration level for 1.5 months (6 times the application) which is aimed at all parts of the orchid plant. Observations are carried

out every 2 weeks to see the effect of treatment that has been given based on the character of the observations that have been determined. Meanwhile, for observations carried out every 2 weeks to see the effect of treatment that has been given based on the character of the observations that have been determined.

The parameters measured and observed were plant height (cm) measured from the pangkar stems to the end of the stem, the length of the leaves (cm) measured starting from the base of the leaf to the tip of the leaf, the width of the leaf (cm) measured in the center of the leaf, the number of leaves (fruit) calculation of the stem, the number of roots (fruit) is calculated from the number of shoots per plant, and the root length (cm) is measured starting from the base of the shoot to the tip of the bud.

Data analysis used ANOVA (Analysis of Variance), if there were significant differences followed by the Smallest Significant Difference Test (BNT) at 95% confidence interval ($\alpha = 0.05$).

Results

Based on the results of research that has been carried out and processing data through analysis of variance on the administration of auxin and giberelin combinations with various concentrations on the growth of Vanda sp orchid plants.

Plant Height (cm)

The average plant height was not significantly different from the various combinations of auxin and giberelin concentrations shown in Figure 2. Figure 2 shows the P_3 treatment

produces the best plant height with an average of 10.13 cm and the lowest in P_1 treatment with an average of 8.30 cm. This is because the concentration of ZPT used is effective enough to stimulate the growth of orchid plants. In addition, zpt is used to play a role in cell division and elongation which affects plant growth.

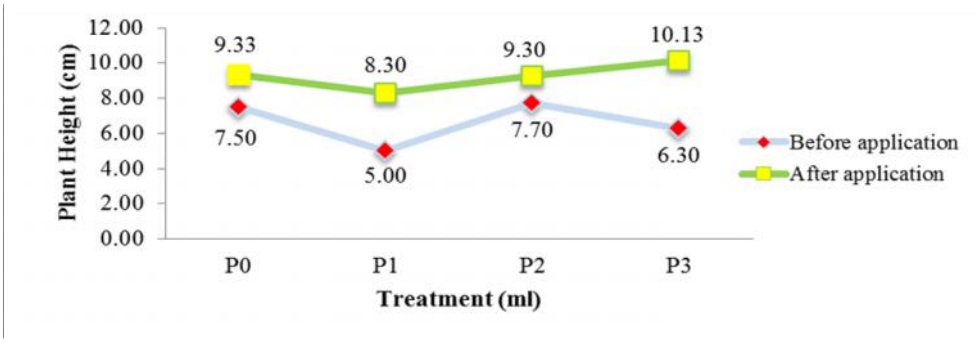

Figure 2 Average diagram of the height of the vanda orchid plant on various combinations of auxin and gibberellin concentrations to plant growth.

Leaf length and leaf width (cm)

The results of the analysis of variance showed that the data were not significantly different in the length and width of the leaves of the orchid plants presented in Figures 3 and 4. Figure 3 shows the best average leaf length in P3 treatment with an average of 22.90 cm and the lowest in P1 treatment with an average of 17.80 cm. While Figure 4 shows that the best leaf width in treatment P0 with a difference of 0.47 cm and the lowest in treatment P1 with a difference of 0.23 cm. Application of 200 mg/L auxin + 350 mg/L giberelin has not been effective on the character

of the length and width of the leaves because considering the growth of orchid plants is relatively slow.

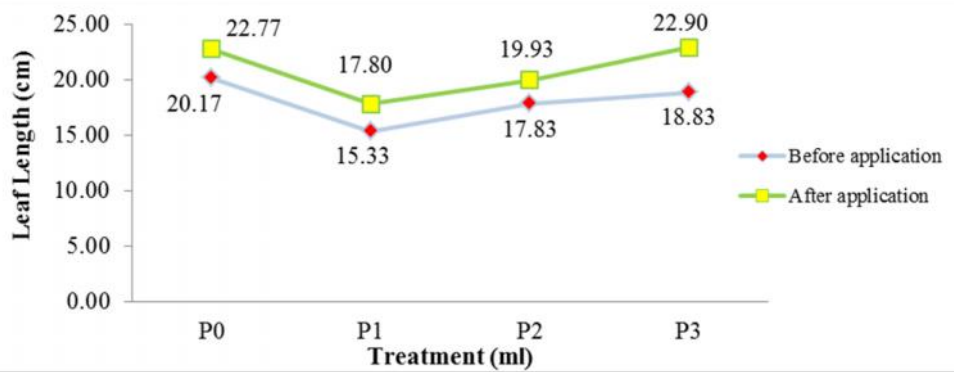

Figure 3 The average diagram of the length of vanda orchid leaves on various combinations of auxin and gibberellin concentrations to plant growth.

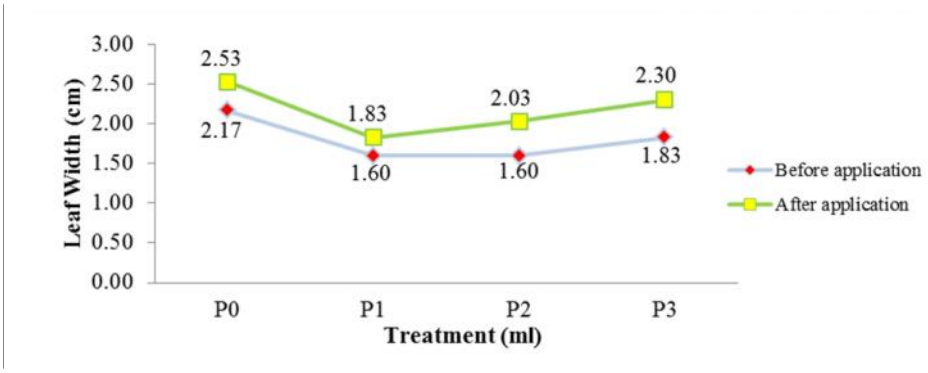

Figure 4 The average width diagram of vanda orchid leaves on various combinations of auxin and gibberellin concentrations to plant growth.

Number of Leaves (strands)

Data from observations on the number of leaves analyzed based on variance showed no significant differences in the various combinations of auxin and gibberellin concentrations to the growth of orchid plants. The average number of leaves is presented in Figure 5.

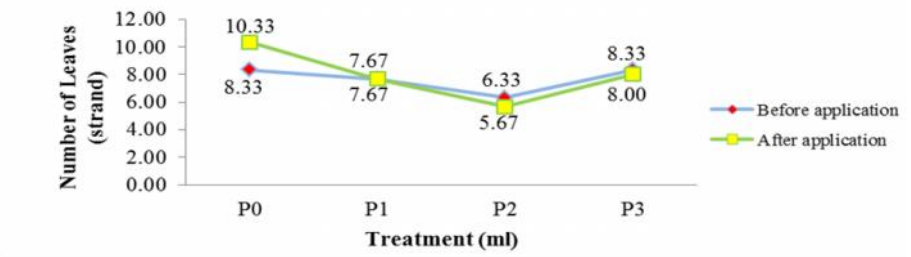

Figure 5 Average diagram of the number of vanda orchid leaves on various combinations of auxin and gibberellin concentrations to plant growth.

Number of Roots (Strands)

The results of analysis of variance showed that the combination of auxin and giberelin significantly different in the number of roots of orchid plants are presented in Figure 6. The average for the best number of root parameters was obtained in treatment P_3 with an average of 12.67 strands and the lowest in P_2 treatment with an average 3.67 strands.

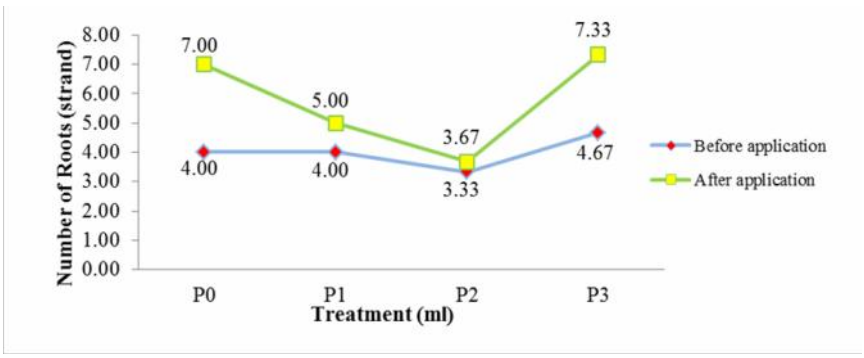

Figure 6 Average number of orchid roots at various combinations of auxin and gibberelline concentrations for plant growth.

Root Length (cm)

The administration of auxin and giberelin gave results that were not significantly different in the root length of vanda orchid plants with an average root length as presented in Figure 7 below. The best root length treatment is shown by P_0 with an average of 21.10 cm and the lowest in P_3 treatment with an average of 13.70 cm.

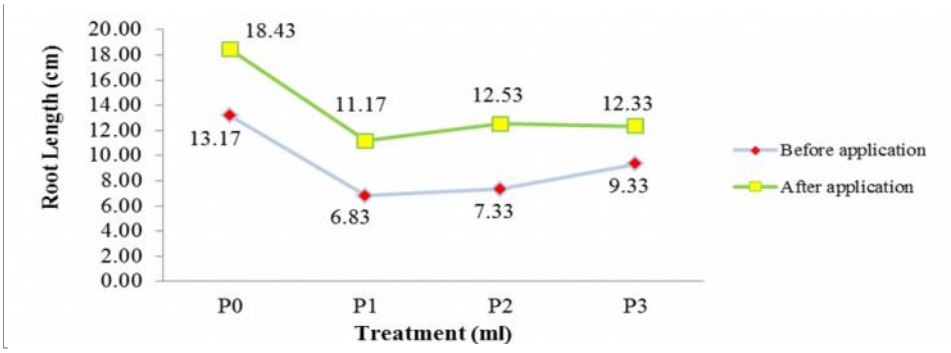

Figure 7 Average length of vanda orchid roots in various combinations of auxin and gibberelline concentrations to plant growth.

Discussion

Obtained results that have no real effect for all the characters that have been determined. This is because the application of auxin and gibberellin types used contains low active ingredients which is less than 15% so that the growth of orchid

plants does not increase significantly. his statement is in accordance with the opinion of Wudianto (2004) who said the use of growth regulators is only effective in certain amounts because the concentration is too high can damage parts of the plant while the hormone concentration below optimal becomes ineffective.

Plant Height (cm)

Tetuko et al (2015) that the ability of the gibberellins to increase plant growth is stronger than the influence caused by auxin if given alone. Giving giberelin with a concentration of 350 mg / L is able to stimulate the growth of orchid stems so as to trigger the increase in plant height. Stem lengthening occurs due to the process of division, elongation and enlargement of new cells that occur in the apical meristem and stem segments, this is what causes the increase in the height of the orchid plant as in P_3. The effect of the giberelin not only encourages the extension of the stem, but also in the regulatory process of plant development including the roots as well as auxin so that the right combination of auxin and giberelin can stimulate high growth and stimulate the formation of orchid roots in P_3 treatment. This is in line with the statement of Tetuko et al. (2015) that auxin and giberelin if applied together cause a high increase in cambium cleavage and xylem and phloem differentiation.

Leaf length and leaf width (cm)

The length and width of the leaf are related to the direction of division, enlargement, number and distribution of cells. The

greater the leaf area, the stomata that play a role in the absorption of nutrients for the metabolic process of plants is increasing. Therefore, the combination of the highest concentrations in P_3, namely auxin 200 mg/L and 350 mg/L giberelin have a better leaf length and width than P_1 and P_2, although the average difference is not much different from P_0.

Number of Leaves (strands)

The average number of orchid leaves is the best in treatment P_0 with an average of 10.33 strands and the lowest in treatment P_2 with an average of 6.33 strands. Based on data on the number of leaves produced the difference in the average number of leaves for each treatment is quite high. It is indicated that the use of low auxin and giberelin concentrations is more effective against leaf growth rate.

Rahayu (2011) reported that the use of growth regulators for low concentrations of 25 ppm was able to produce more leaves compared to the use of ZPT for a higher concentration of 100 ppm. n addition, treatment without the administration of ZPT is able to experience an increase in the number of leaves due to each plant having a natural hormone that is active auxin produced in meristem tissues.

Number of Roots (Strands)

The development of the number of roots is the initial process of plant growth and development. Root is one organ that plays a role in the absorption of nutrients and nutrients contained in the soil. The number of roots formed in plants will provide benefits

in maximizing nutrient absorption for these additions. Giving with a combination of 200 mg / L Auxin + 350 mg / L Giberelin produces the best number of leaves and is significantly different from other treatments. This is because auxin plays a role in cell division in meristem tissues and the development of cells in the meristem area. These cells increase in number as happened to the character.

Putra (2015) reported that auxin affects the development of cell walls which results in reduced cell wall pressure on protoplast. Growth is the increase in the number of cells in an organism followed by the development process. Roots have parts or components that make up the roots, one of which is the root cap that is found at the growing point in the form of meristem cells that are actively dividing.

Root Length (cm)

Based on the results of the study, it was seen that the use of auxin in high concentrations produced a low number of roots compared to those who did not use auxin. The resulting diagram can be seen higher or increase in auxin concentration (P1 to P3) so the average root length is lower. The resulting diagram can be seen higher or increase in auxin concentration (P1 to P3) so the average root length is lower. Thus, it can be concluded that the use of auxin are better able to inhibit the growth of roots. The use of auxin with high concentration (above optimum) is able to break the crosslinking of hydrogen carbon chains of cellulose molecules

which make up the cell wall. Auxin excess can inhibit root elongation which is characterized by an increase in the amount of ethylene causing a peghunting effect on the root extension (Salguero, 2000).

Physiological effects of auxin on plant root growth usually inhibit cell elongation, except at very low concentrations. The provision of auxin as a type of synthetic auxin has been proven to increase roots (Ibrahim, 2004). Auxin stimulates certain proteins in the plasma membrane of plant cells to pump H^+ ions into the cell wall. This H^+ ion activates certain enzymes so that cells can elongate and cause water to enter by osmosis. After this lengthening process, the cell continues to grow by synthesizing the cell wall and cytoplasmic material (Taiz, 2012).

Conclusion

Giving several concentrations of Wauxsin and Gibro to increase the growth of Vanda species orchid plants did not significantly increase growth. This is because orchids generally have relatively slow growth. In addition, in this study Wauxsin and Gibro had no significant effect on the character of plant height, leaf length, leaf width, number of leaves, number of roots and root length caused by the use of growth regulating substances which sometimes only contain low active ingredients plus concentration treatment which is low. Of the several concentrations used, the best concentration is obtained which can affect the growth of orchid plants. The treatment of P_3 with a concentration of 200 mg/L auxin + 350 mg/L gibgro produced plant height, leaf length, leaf width,

plant height, leaf length, leaf width, number of leaves and the best number of roots with an average of 10.13 cm, respectively. 22.90 cm, 2.30 cm, 8.33 strands and 7.33 strands.

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An Increase of Roselle Seed Germination (*Hibiscus sabdariffa* L.) Through Various Dormancy Breaking Techniques

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Abstract

This study aimed to determine the response of dormancy breaking techniques in increasing roselle seed germination and obtaining effective dormancy breaking techniques to increase seed roselle germination. This research was conducted at the Research Institute for Industrial Crops and Fiber Highway Ngijo Karangploso, Kepuharjo, Karangploso, Malang, East Java, which took place in July and August 2017. This study uses a three-factor factorial design consisting of seed factor (B), the growing medium (M), and the technique of breaking dormancy (P). Seed factor consists of AC 1301 (B_1) roselle and Madura 1614 rosela (B_2), the media factor consists of medium paper (M_1) and sand media (M_2). While the dormancy breakdown technique consisted of 5 minutes of hot water immersion (P_1), 10 minutes (P_2), 15 minutes (P_3), 12 hours (P_4) cold water immersion, 16 hours (P_5), 20 hours (P_6), and scarification (P_7). The results showed that the interaction between seeds, media, and dormancy breaking techniques significantly affected the high character of plumula and radicular length. B_2P_7 treatment produces an average of germination, growing simultaneity, the maximum growth potential, and the percentage of the seeds do not grow best with their respective 84, 80, 90 and 6%. The treatment of $B_1M_1P_7$ produced the best plumula length with an average of 11 cm, $B_2M_1P_7$ treatment produced the best radicle length with an average of 10 cm, B_1P_7 treatment produced the best sprout wet weight character with an average of 0.40 g, B_2P_7 treatment produced the best sprout dry weight character with on average 0.06 g and seed treatment of Madura rosela 1614 (B_2) showed the best moisture content with an average of 8.93%.

Keywords: dormancy breaking technique, germination, medium, roselle (Hibiscus sabdariffa L.), seed

Background

Rosella (Hibiscus sabdariffa L.) is an outdoor ornamental plant from the hibiscus family from western India. Rosella ferrous secure consumed humans and have benefits including fruit rosella can be used as a salad, syrup, pickles., Extracts of petals rosella helpful for lowering high blood pressure, the leaves of roselle useful for treating wounds and seeds of roselle efficacious as a diuretic and tonic (Noor et al. 2010).

Rosella plants have been known by the Indonesian people since 1922 as ornamental plants, hedges and sklerenkim fiber-producing plants. Rosella is currently a plant that is in demand by the public because of various products that can be produced from flowers and sklerenkim fibers, so that it experiences a high increase in cultivation. There are 2 types of plant varieties belonging to the Malvaceae family, namely roselle with yellow flower petals (*Hibiscus sabdariffa* var. Altisima) and roselle with red flower petals (*Hibiscus sabdariffa* var. Sabdariffa). Rosella is usually used as a stem fiber as a material to make rope and burlap sacks. In terms of health, rosella has benefits for disease prevention. The high content of vitamin C can function as an antioxidant in the body. Antioxidants can inhibit the accumulation of free radicals of chronic diseases, such as kidney damage, diabetes, coronary heart disease and cancer (Maria and Sulastri, 2008).

Harvesting of the crops that are developed for seed usually depends on the maturity of the fruit or seed and is carried out in stages. f done in a non-gradual way, there may be risks including fruits that ripen first will be scattered, seed germination is still bound in the fruit, and vigor and viability decreases for the seeds (Kartasapoetra, 2003). In this case, based on the results of a study by Syarovy (2012), the maturity of roselle at the age of 33 days after anthesis. Productivity of roselle petals abroad far exceeds domestic productivity. For example, the production of flower petals in California reaches 1.3 kg per plant. n Puerto Rico around 1.8 kg per plant. Whereas in Indonesia, precisely on the island of Java, each new roselle tree can produce 0.2-1 kg per plant (Mardiah et al., 2009).

One of the factors causing the low productivity of crops is the low quality of seeds. Low quality seeds have low viability and vigor. Another thing that causes low seed quality is dormancy. Dormancy is defined as the status where the seeds do not germinate even under ideal environmental conditions for germination. Some dormancy mechanisms occur in both physical and physiological seeds, including primary and secondary dormancy. The intensity of dormancy is influenced by the environment during seed development. Duration (persistence) of dormancy and the mechanism of dormancy differ between species and between genotypes.

Seed dormancy is a state of seed that does not have the ability to germinate in a certain period of time even though it is in

an environment that meets the requirements for germination (Baskin & Baskin, 2004). Dormancy in certain species results in seeds not germinating in the soil for several years (Ilyas 2010). According to Razavi and Hajiboland (2009), some species have dormancy as a strategy to defend themselves and disseminate their adaptation areas. In addition, the germination is low or below 80% and the germination period reaches 5 days after planting (Baliitas, 2016) is thought to be caused by a hard seed skin structure because it is composed of dense sklerenkim tissue (Dianxiang & Hartley, 2008).

Solving dormancy is one of the important efforts in increasing germination so that it facilitates the technical implementation of planting. Solving dormancy can be done through seed immersion with water temperatures of 80 ° C and 60 ° C, as well as through mechanical scarification. Soaking seeds with 80 ° C water for 10 minutes can increase yute seed germination up to 77% (Velempini, 2003). Soaking seeds with water temperature of 80 ° C and continued with mechanical scarification can improve Sesbania sesban seed viability up to 94% (Wang & Hanson, 2008). Yute seed immersion in 2006 which was soaked in water temperature of 80 ° C until cold for 3 hours was able to increase the percentage of simultaneity to grow to 90.5%, germination power to 90.1%, reduce the number of hard seeds to 8.75%, produce the best plumula and radicle 3.88 cm and 3.89 cm (Hidayat & Marjani, 2017). Soaking the seeds with a temperature of 60 ° C and left to

cool for 24 hours is also a potential to increase germination and shorten andaliman seed germination time (Siregar, 2010).

Materials and Methods

This research was conducted at the Research Institute for Industrial Crops and Fiber at Jalan Raya Ngijo Karangploso, Kepuharjo, Karangploso, Malang, East Java, which was held on July to August 2017. Tools and materials used are paper, oven, germinator, analytic scales, envelopes, clear plastic, rubber, germination tanks, desiccators, rulers, scissors, press tools, aluminum containers, nail clippers, sand, AC rosel seeds 1301 2005 (B_1), the seed of the Madura rosela 1614 of 2004 (B_2). This study uses a completely randomized design with 3 (three) factorials. The first factor is seed (B), the second factor is media (M) which consists of medium of paper (M_1) and sand (M_2), and the third factor is the dormancy break (P) technique. Seed treatments used were control (P_0), soaking hot water for 5 minutes (P_1), soaking hot water for 10 minutes (P_2), soaking hot water for 15 minutes (P_3), soaking cold water for 12 hours (P_4), soaking cold water for 16 hours (P_5), soaking cold water for 20 hours (P_6), scarification (P_7).

AC 1301 (B_1) roselle seeds and madura 1614 rosela (B_2) were weighed as much as 500 grams then put into different containers. Furthermore, counted as many as 200 seeds for the treatment (four replications) in medium grain rice paper and 100 for a single treatment (four repetitions) in the sand media.

Each group of seeds is grouped into 8 according to the treatment to be carried out, starting from P_0, P_1, P_2, P_3, P_4, P_5, P_6

and P_7. Then testing the initial germination power, and testing the initial moisture content. Parameters observed and measured were germination (%), simultaneous growth (%), maximum growth potential (cm), percentage of seed not growing (%), height of plumula (cm), length of radicle (cm), wet weight of sprouts (g), dry weight of sprouts (g), and moisture content

Results

Investigation Analysis of Rosella Germination

Based on the variance fingerprint in table 1 shows that the type of seed gives a very real influence on all parameters of observation except for the parameters of simultaneous growth. The media also gave a very significant influence on the parameters of simultaneous growth, plumula height, radicle length, wet weight of sprouts and dry weight of sprouts, gave a significant effect on the germination and did not give effect to the observation parameters of maximum growth potential and the percentage of seeds did not grow.

The treatment of the seeds gives a very real influence on all observation parameters. The interaction between the seeds and the media gave a very significant effect on the height of the radicle and the length of the radicle, the real effect on the parameters of the sprout wet weight and no effect on germination, simultaneous growth, maximum growth potential, percentage of seeds not growing and dry weight of sprouts. The interaction between the seeds and the treatment gave a very real influence on all

observation parameters except the parameters of the dry weight of the sprouts which were significantly affected.

Table 1. Variety Analysis of the Effect of Seeds, Media and Treatment on Rosela Germination

No	Observation Parameter	Seed	Media	Treatment	B*M	B*P	M*P	B*M*P
1	Germination power	0.0103*	0.0279*	0.0000*	0.6493tn	0.0000*	0.0000*	0.9438tn
2	Simultaneous power	0.3353tn	0.0003*	0.0000*	0.7476tn	0.0000*	0.0000*	0.9626tn
3	Maximum growth potential	0.0003*	0.6072tn	0.0000*	0.6072tn	0.0000*	0.0135*	0.8995tn
4	The percentage of seeds does not grow	0.0005*	0.9206tn	0.0000*	0.3708tn	0.0065*	0.1393tn	0.2424tn
5	High Plumula	0.0000*	0.0000*	0.0000*	0.0017*	0.0000*	0.0017*	0.0593*
6	Radicular length	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	0.0001*	0.0001*
7	Fresh weight of sprouts	0.0000*	0.0000*	0.0000*	0.0293*	0.0000*	0.2061tn	0.1865tn
8	Dry weight of sprouts	0.0151*	0.0151*	0.0073*	0.3740tn	0.0215*	0.7707tn	0.3447tn

Description: * = Significant; ** = very significant; tn = not significant

The interaction between the media and the treatment gave a very significant effect on all parameters except the percentage of non-germination percentage parameters, the wet weight of sprouts and the dry weight of sprouts which gave no tangible results. The interaction between the types of seeds, media and treatment gave a very significant effect on the length of the radicle, a significant effect on the length of the plumula and did not give a significant effect on the observation parameters of germination, simultaneous growth, maximum growth potential, seed germination, wet weight and weight dried sprouts.

Rosela Seed Germination

Table 2 shows that the interaction between seeds and dormancy breakdown techniques had a significant effect on further DMRT tests for germination parameters, growth simultaneity, maximum growth potential, and percentage of seed not growing. Based on the results of further tests for the four parameters, it showed that the interaction between Madura Rosella 1641 seed using scarification treatment (B_2P_7) resulted in an average germination, simultaneous growth, maximum growth potential, and the best percentage of seed grown with a mean of 84%, 80%, 90 and 6 seeds. Unlike the case of Madura rosella seeds which used cold water for 12 hours (B_2P_4) each produced the lowest germination of 0.2%, simultaneous growth of 0%, maximum growth potential of only 0.5% and the percentage of seeds that did not grow to produce 100% which showed that seeds with the use of cold water have not been able to increase the seed germination of rosela.

Table 2. Rosela seed germination recapitulation

Benih (B)*Pematahan dormansi (P)	DB (%)	KST (%)	PTM	PBTT
B_1P_0	6.00 ^{cd}	6.00 ^{cde}	7.00 ^{cd}	93.00 ^{a}
B_1P_1	9.00 ^{c}	8.00 ^{c}	9.00 ^{c}	90.00 ^{a}
B_1P_2	3.00 ^{de}	3.00 ^{efg}	4.00 ^{def}	96.00 ^{a}
B_1P_3	5.00 ^{cd}	5.00 ^{cdef}	6.00 ^{cde}	92.00 ^{a}
B_1P_4	6.00 ^{cd}	5.00 ^{cdef}	7.00 ^{cd}	80.00 ^{b}
B_1P_5	8.00 ^{c}	7.00 ^{cd}	9.00 ^{c}	91.00 ^{a}
B_1P_6	8.00 ^{c}	7.00 ^{cd}	9.00 ^{c}	91.00 ^{a}
B_1P_7	65.00 ^{b}	55.00 ^{b}	75.00 ^{b}	13.00 ^{c}
B_2P_0	1.00 ^{e}	0.00 ^{g}	0.25 ^{f}	100.00 ^{a}

B_2P_1	6.00 ^{cd}	5.00 ^{cdef}	6.00 ^{cde}	94.00 ^{a}
B_2P_2	4.00 ^{de}	4.00 ^{defg}	4.00 ^{def}	95.00 ^{a}
B_2P_3	1.00 ^{e}	0.00 ^{g}	2.00 ^{ef}	98.00 ^{a}
B_2P_4	0.20 ^{e}	0.00 ^{g}	0.50 ^{f}	100.00 ^{a}
B_2P_5	0.50 ^{e}	0.50 ^{fg}	0.50 ^{f}	100.00 ^{a}
B_2P_6	1.00 ^{e}	1.00 ^{fg}	1.00 ^{f}	99.00 ^{a}
B_2P_7	84.00 ^{a}	80.00 ^{a}	90.00 ^{a}	6.00 ^{c}

Description: The numbers followed by the same letter are not significantly different in the 5% DMRT test. DB = Power of Germination; KST = Simultaneous Growing; PTM = Maximum Growing Potential; PBTT = Percentage of Seeds Not Growing

Plumula Height (cm) and Radicula Length (cm)

Based on variance recapitulation data (Table 1), shows that the interaction between seed, media and treatment were significantly different for height parameters of plumula and radicle length. Thus, further testing was carried out using the α 5% DMRT test as presented in Table 3. Based on the results of further tests, the treatment of $B_1M_1P_6$ and $B_1M_1P_7$ produced the best plumala height with an average of 11.00 cm while the $B_1M_1P_5$ treatment produced a high plumula 0.00 cm or in other words the seed does not germinate.

Unlike the case for the radicular length parameter, $B_2M_1P_7$ treatment produced the best radicle length with an average of 10.00 cm while, $B_2M_1P_4$, $B_2M_1P_5$, and $B_2M_1P_6$ treatment produced the lowest radicle length with an average of 0.00 cm. Similar to the radicular length parameters, the seeds used do not germinate so that radicular growth does not occur.

Table 3. Long recapitulation of plumula and radicle of Rosela seed sprouts

Treatment	Plumula Height (cm)				Radicular Length (cm)			
	B1		B2		B1		B2	
	M1	M2	M1	M2	M1	M2	M1	M2
P_0	9.00 *ab*	3.00 *defg*	1.00 *fg*	1.00 *fg*	7.00 *bc*	2.00 *def*	1.00 *def*	0.30 *ef*
P_1	10.00 *ab*	4.00 *def*	9.00 *ab*	2.00 *defg*	7.00 *bc*	2.00 *def*	6.00 *bc*	1.00 *def*
P_2	8.00 *bc*	1.00 *fg*	9.00 *ab*	2.00 *defg*	5.00 *c*	1.00 *def*	5.00 *c*	1.00 *def*
P_3	5.00 *cde*	3.00 *defg*	2.00 *efg*	1.00 *fg*	2.00 *def*	2.00 *def*	0.30 *ef*	1.00 *def*
P_4	11.00 *a*	4.00 *defg*	2.00 *defg*	0.00 *g*	8.00 *ab*	1.00 *def*	0.00 *f*	1.00 *def*
P_5	11.00 *a*	5.00 *cde*	0.00 *g*	1.00 *fg*	8.00 *ab*	2.00 *def*	0.00 *f*	0.30 *ef*
P_6	11.00 *a*	5.00 *cde*	4.00 *def*	0.00 *g*	8.00 *ab*	2.00 *def*	3.00 *de*	0.00 *f*
P_7	11.00 *a*	5.00 *cde*	10.00 *ab*	5.00 *cde*	5.00 *c*	2.00 *def*	10.00 *a*	3.00 *de*

Description : The numbers followed by the same letter are not significantly different in the 5% DMRT test.

Wet Weight and Dry Weight of Rosella Sprouts

Based on the results of the recapitulation of the analysis of the variety of roselle seeds, there was a significant effect on the interaction between seed and treatment on parameters of wet weight and dry weight of sprouts. Furthermore, 5% DMRT further testing was carried out. The B_1P_7 treatment produced the best wet weight of germination with an average of 0.40 g and was significantly different from the other treatments. Whereas, B_2P_5 treatment produced the lowest wet seed sprout weight with an average of 0.01 g but was not significantly different from treatment B_2P_0, B_2P_3, B_2P_4, B_2P_5, and B_2P_6.

Table 4. Recapitulation of wet weight and dry weight of Rosela seed sprouts

Treatment	Wet Weight of Sprouts (g)		Dry Weight of Sprouts (g)	
	B_1	B_2	B_1	B_2
P_0	0.300 ^{abcde}	0.030 ^{f}	0.200 ^{b}	0.004 ^{b}
P_1	0.310 ^{abcd}	0.230 ^{bcde}	0.200 ^{b}	0.020 ^{b}
P_2	0.200 ^{de}	0.210 ^{cde}	0.010 ^{b}	0.010 ^{b}
P_3	0.190 ^{e}	0.070 ^{f}	0.010 ^{b}	0.007 ^{b}
P_4	0.310 ^{abcd}	0.040 ^{f}	0.200 ^{b}	0.003 ^{b}
P_5	0.340 ^{ab}	0.010 ^{f}	0.200 ^{b}	0.001 ^{b}
P_6	0.320 ^{abc}	0.080 ^{f}	0.030 ^{b}	0.007 ^{b}
P_7	0.400 ^{a}	0.350 ^{ab}	0.020 ^{b}	0.600 ^{a}

Description : The numbers followed by the same letter are not significantly different in the 5% DMRT test.

The best sprouts dry weight parameters were shown in B_2P_7 treatment with an average of 0.600 g and different from other treatments. Meanwhile, B_2P_5 treatment resulted in the lowest seedling dry weight by an average of 0.001 g and was not significantly different from other treatments except perlekuan B_2P_7.

Rosella Seed Water Content (%)

Moisture is the amount of water contained in the ingredients expressed in percent. Water content is also one of the most important characteristics of food, because water can affect the appearance, texture, and taste of foodstuffs as shown in Table 5.

Table 5. Recapitulation of the water content of roselle seeds

Seed Type	Repeat	Water Content %	Mean
B1	1	8,287	8,537
	2	8,786	
B2	1	9,287	8,931
	2	8,576	

Table 5 shows the highest water content of B2 seed (Madura rosela seed 1614 of 2004) with an average of 8,931% and the lowest at B1 (AC 1301 2005 roselle seed with an average of 8,537%. The moisture content in B2 indicates that the seed is able to germinate maximally compared to B2 as shown in the germination data (Table 2).

Discussions

Rosela Seed Germination

Scarification techniques (P7) are performed with an opening on the skin of rosella. The high parameters of rosella seed germination are because water can easily enter the seeds and stimulate the imbimbition process and germination enzyme activation. Imbibisi causes the seeds to expand and break the wrapping skin and trigger metabolic changes in the embryo so that it can continue its growth. Enzymes will hydrolyze ingredients stored in cotyledons and nutrients in them.

Enzymes that play a role in the hydrolysis of food reserves are α-amylase, β-amylase and protease enzymes (Surya, 2010). According to Kamil (1986), seed skin and surrounding structures can affect the ability of seed germination through inhibition of water absorption, gas exchange, diffusion of endogenous inhibitors or embryo growth inhibition. Kuswanto (1996), who argues that seed germination that contains non-permeable seed shells can be stimulated by scarification, which is the conversion of seed coat to make it permeable to gases and water. This Impermeability can be

caused by deposition of various substances (eg cutin, suberin, lignin) on the membrane.

Scarification is a dormancy solving method aimed at reducing thickness, breaking or removing hard seed skin (Yuniarti and Djaman, 2015). Discarified seeds will produce a better imbibition process. Water and gas will enter the seeds faster because of the permeable seed skin. Water that enters the seeds causes the metabolic processes in the seeds to run faster as a result of the better germination.

Plumula Height (cm) and Radicula Length (cm)

The use of AC 1301 rosella seeds using cold water immersion treatment for 20 hours then germinated on red paper media and with saccharification use is able to produce the best high plumula. This can be caused by several factors including the age of seeds that are younger than the type B2 because B1 was harvested in 2005 and B2 was harvested in 2004, the germination conditions were also made as optimal as possible due to M1 treatment, germination in germinator, soaking time effect on skin permeability and imbibition. This is supported by the opinion of Sadjad (1975) who said that water is an environmental factor that influences the process of seed germination (Sadjad 1975).

The process of entering water into the seed is a physical process, there is no connection with the death of the seed. According to the dictionary of biology and seed technology of forest plants (2004), the process of absorption of water by seeds before germination is called imbibisi. The rate of imbibition,

besides being influenced by the permeability of the seed coat, is also influenced by the water content in the seed. Imbibisi occurs because the potential of the water in the seed is lower than its surroundings, so that the water will move into the seeds (Beneach and Sanchez 2004). Setyamidjaja (2006), in growth or germination, the embryo will come out through the hole in the shell by forming roots and stems.

The best radicular length parameters in the treatment using scarification compared with other fracture treatments. This is because the scarification treatment is able to inhibit the mechanical properties of the skin so that ir and oxygen can easily accumulate into the seeds for the germination process so as to encourage maximum growth of the radicle. Dharma et al., (2015) stated that the breakdown of dormancy through full scarification with sanding gave a mean length of plumula 12.35 cm and was significantly different from cracking and no scarification scarification treatment which only gave an average length of successive radicle length 11.38 cm and 8.64 cm. The results of Williyatno's (2007) study showed that plumula and radicle more than 2 cm long existed at intervals of 5-10 days after the seeds began to germinate.

Wet Weight and Dry Weight of Rosella Sprouts

Discarification of rosella seed germination can be proven by increasing basag weight and normal sprout dry weight (Table 4). This is because a good metabolic process will produce good germination. Seeds that germinate can utilize food reserves in seeds well, in the presence of water, oxygen will enter the seeds by

breaking down food reserves that are used as energy sources for normal sprout growth in a fast and simultaneous time.

The increase in germination can be seen in the germination variables observed, namely germination, speed of germination, simultaneous germination and dry weight of normal sprouts. Yuniarti (2002), for saga seeds that use the treatment, it is thought that then soaked in cold water for 24 hours, can produce a high germination value of 77.83%. Bewley and Black (2006) stated that water absorption starts from the imbibition process (phase I) to 24 hours which is a rapid absorption phase and is followed by (phase II) which is characterized by a constant water absorption which tends to be 68 hours. With a good rate of imbibition, the need for water for seeds is met so that the process of seed metabolism can run well.

Rosela Seed Water Content (%)

Seed germination is strongly influenced by seed moisture content. Seed treatments, namely B1 and B2, showed that the best moisture content of Madura rosela seed was 1614 in 2004 compared to AC rosela seed 1301 of 2005. This is because B1 experienced a decline in seeds during storage, which is characterized by the low water content produced. Balittas (2016) shows that the water content for kenaf seeds and the like ranges between 4% -13% so that it can increase the respiration rate and the CO2 produced is lower.

Purba (2013) states that seed decline during storage is caused by higher water content. This results in a faster rate of

respiration so that more CO2 and heat is produced. This physiological activity can be suppressed through ideal moisture content so that the germination of the seed is still maintained until the time for the seed to be germinated (Hidayat and Marjani, 2017).

Conclusions

The method of dormancy breaking technique using scarification was able to increase the germination for Roselle seed of Madura seed 1614 in 2004 and AC 1301 Year 2005 roselle seed which was germinated on paper medium.

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Of Roselle Seed Germination (*Hibiscus sabdariffa* L.) Through Various Dormancy Breaking Techniques

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Abstract

This study aimed to determine the response of dormancy breaking techniques in increasing roselle seed germination and obtaining effective dormancy breaking techniques to increase seed roselle germination. This research was conducted at the Research Institute for Industrial Crops and Fiber Highway Ngijo Karangploso, Kepuharjo, Karangploso, Malang, East Java, which took place in July and August 2017. This study uses a three-factor factorial design consisting of seed factor (B), the growing medium (M), and the technique of breaking dormancy (P). Seed factor consists of AC 1301 (B_1) roselle and Madura 1614 rosela (B_2), the media factor consists of medium paper (M_1) and sand media (M_2). While the dormancy breakdown technique consisted of 5 minutes of hot water immersion (P_1), 10 minutes (P_2), 15 minutes (P_3), 12 hours (P_4) cold water immersion, 16 hours (P_5), 20 hours (P_6), and scarification (P_7). The results showed that the interaction between seeds, media, and dormancy breaking techniques significantly affected the high character of plumula and radicular length. B_2P_7 treatment produces an average of germination, growing simultaneity, the maximum growth potential, and the percentage of the seeds do not grow best with their respective 84, 80, 90 and 6%. The treatment of $B_1M_1P_7$ produced the best plumula length with an average of 11 cm, $B_2M_1P_7$ treatment produced the best radicle length with an average of 10 cm, B_1P_7 treatment produced the best sprout wet weight character with an average of 0.40 g, B_2P_7 treatment produced the best sprout dry weight character with on average 0.06 g and seed treatment of Madura rosela 1614 (B_2) showed the best moisture content with an average of 8.93%.

Keywords: dormancy breaking technique, germination, medium, roselle (Hibiscus sabdariffa L.), seed

Background

Rosella (Hibiscus sabdariffa L.) is an outdoor ornamental plant from the hibiscus family from western India. Rosella ferrous secure consumed humans and have benefits including fruit rosella can be used as a salad, syrup, pickles., Extracts of petals rosella helpful for lowering high blood pressure, the leaves of roselle useful for treating wounds and seeds of roselle efficacious as a diuretic and tonic (Noor et al. 2010).

Rosella plants have been known by the Indonesian people since 1922 as ornamental plants, hedges and sklerenkim fiber-producing plants. Rosella is currently a plant that is in demand by the public because of various products that can be produced from flowers and sklerenkim fibers, so that it experiences a high increase in cultivation. There are 2 types of plant varieties belonging to the Malvaceae family, namely roselle with yellow flower petals (*Hibiscus sabdariffa* var. Altisima) and roselle with red flower petals (*Hibiscus sabdariffa* var. Sabdariffa). Rosella is usually used as a stem fiber as a material to make rope and burlap sacks. In terms of health, rosella has benefits for disease prevention. The high content of vitamin C can function as an antioxidant in the body. Antioxidants can inhibit the accumulation of free radicals of chronic diseases, such as kidney damage, diabetes, coronary heart disease and cancer (Maria and Sulastri, 2008).

Harvesting of the crops that are developed for seed usually depends on the maturity of the fruit or seed and is carried out in stages. f done in a non-gradual way, there may be risks including fruits that ripen first will be scattered, seed germination is still bound in the fruit, and vigor and viability decreases for the seeds (Kartasapoetra, 2003). In this case, based on the results of a study by Syarovy (2012), the maturity of roselle at the age of 33 days after anthesis. Productivity of roselle petals abroad far exceeds domestic productivity. For example, the production of flower petals in California reaches 1.3 kg per plant. n Puerto Rico around 1.8 kg per plant. Whereas in Indonesia, precisely on the island of Java, each new roselle tree can produce 0.2-1 kg per plant (Mardiah et al., 2009).

One of the factors causing the low productivity of crops is the low quality of seeds. Low quality seeds have low viability and vigor. Another thing that causes low seed quality is dormancy. Dormancy is defined as the status where the seeds do not germinate even under ideal environmental conditions for germination. Some dormancy mechanisms occur in both physical and physiological seeds, including primary and secondary dormancy. The intensity of dormancy is influenced by the environment during seed development. Duration (persistence) of dormancy and the mechanism of dormancy differ between species and between genotypes.

Seed dormancy is a state of seed that does not have the ability to germinate in a certain period of time even though it is in

an environment that meets the requirements for germination (Baskin & Baskin, 2004). Dormancy in certain species results in seeds not germinating in the soil for several years (Ilyas 2010). According to Razavi and Hajiboland (2009), some species have dormancy as a strategy to defend themselves and disseminate their adaptation areas. In addition, the germination is low or below 80% and the germination period reaches 5 days after planting (Baliitas, 2016) is thought to be caused by a hard seed skin structure because it is composed of dense sklerenkim tissue (Dianxiang & Hartley, 2008).

Solving dormancy is one of the important efforts in increasing germination so that it facilitates the technical implementation of planting. Solving dormancy can be done through seed immersion with water temperatures of 80 ° C and 60 ° C, as well as through mechanical scarification. Soaking seeds with 80 ° C water for 10 minutes can increase yute seed germination up to 77% (Velempini, 2003). Soaking seeds with water temperature of 80 ° C and continued with mechanical scarification can improve Sesbania sesban seed viability up to 94% (Wang & Hanson, 2008). Yute seed immersion in 2006 which was soaked in water temperature of 80 ° C until cold for 3 hours was able to increase the percentage of simultaneity to grow to 90.5%, germination power to 90.1%, reduce the number of hard seeds to 8.75%, produce the best plumula and radicle 3.88 cm and 3.89 cm (Hidayat & Marjani, 2017). Soaking the seeds with a temperature of 60 ° C and left to

cool for 24 hours is also a potential to increase germination and shorten andaliman seed germination time (Siregar, 2010).

Materials and Methods

This research was conducted at the Research Institute for Industrial Crops and Fiber at Jalan Raya Ngijo Karangploso, Kepuharjo, Karangploso, Malang, East Java, which was held on July to August 2017. Tools and materials used are paper, oven, germinator, analytic scales, envelopes, clear plastic, rubber, germination tanks, desiccators, rulers, scissors, press tools, aluminum containers, nail clippers, sand, AC rosel seeds 1301 2005 (B_1), the seed of the Madura rosela 1614 of 2004 (B_2). This study uses a completely randomized design with 3 (three) factorials. The first factor is seed (B), the second factor is media (M) which consists of medium of paper (M_1) and sand (M_2), and the third factor is the dormancy break (P) technique. Seed treatments used were control (P_0), soaking hot water for 5 minutes (P_1), soaking hot water for 10 minutes (P_2), soaking hot water for 15 minutes (P_3), soaking cold water for 12 hours (P_4), soaking cold water for 16 hours (P_5), soaking cold water for 20 hours (P_6), scarification (P_7).

AC 1301 (B_1) roselle seeds and madura 1614 rosela (B_2) were weighed as much as 500 grams then put into different containers. Furthermore, counted as many as 200 seeds for the treatment (four replications) in medium grain rice paper and 100 for a single treatment (four repetitions) in the sand media.

Each group of seeds is grouped into 8 according to the treatment to be carried out, starting from P_0, P_1, P_2, P_3, P_4, P_5, P_6 and P_7. Then testing the initial germination power, and testing the initial moisture content. Parameters observed and measured were germination (%), simultaneous growth (%), maximum growth potential (cm), percentage of seed not growing (%), height of plumula (cm), length of radicle (cm), wet weight of sprouts (g), dry weight of sprouts (g), and moisture content

Results

Investigation Analysis of Rosella Germination

Based on the variance fingerprint in table 1 shows that the type of seed gives a very real influence on all parameters of observation except for the parameters of simultaneous growth. The media also gave a very significant influence on the parameters of simultaneous growth, plumula height, radicle length, wet weight of sprouts and dry weight of sprouts, gave a significant effect on the germination and did not give effect to the observation parameters of maximum growth potential and the percentage of seeds did not grow.

The treatment of the seeds gives a very real influence on all observation parameters. The interaction between the seeds and the media gave a very significant effect on the height of the radicle and the length of the radicle, the real effect on the parameters of the sprout wet weight and no effect on germination, simultaneous growth, maximum growth potential, percentage of seeds not growing and dry weight of sprouts. The interaction between the

seeds and the treatment gave a very real influence on all observation parameters except the parameters of the dry weight of the sprouts which were significantly affected.

Table 1. Variety Analysis of the Effect of Seeds, Media and Treatment on Rosela Germination

No	Observation Parameter	Seed	Media	Treatment	B*M	B*P	M*P	B*M*P
1	Germination power	0.0103**	0.0279*	0.0000**	0.6493tn	0.0000**	0.0000**	0.9438tn
2	Simultaneous power	0.3353tn	0.0003**	0.0000**	0.7476tn	0.0000**	0.0000**	0.9626tn
3	Maximum growth potential	0.0003**	0.6072tn	0.0000**	0.6072tn	0.0000**	0.0135**	0.8995tn
4	The percentage of seeds does not grow	0.0005**	0.9206tn	0.0000**	0.3708tn	0.0065**	0.1393tn	0.2424tn
5	High Plumula	0.0000**	0.0000**	0.0000**	0.0017**	0.0000**	0.0017**	0.0593*
6	Radicular length	0.0000**	0.0000**	0.0000**	0.0000**	0.0000**	0.0001**	0.0001**
7	Fresh weight of sprouts	0.0000**	0.0000**	0.0000**	0.0293*	0.0000**	0.2061tn	0.1865tn
8	Dry weight of sprouts	0.0151**	0.0151**	0.0073**	0.3740tn	0.0215*	0.7707tn	0.3447tn

Description: * = Significant; ** = very significant; tn = not significant

The interaction between the media and the treatment gave a very significant effect on all parameters except the percentage of non-germination percentage parameters, the wet weight of sprouts and the dry weight of sprouts which gave no tangible results. The interaction between the types of seeds, media and treatment gave a very significant effect on the length of the radicle, a significant effect on the length of the plumula and did not give a significant effect on the observation parameters of germination, simultaneous

growth, maximum growth potential, seed germination, wet weight and weight dried sprouts.

Rosela Seed Germination

Table 2 shows that the interaction between seeds and dormancy breakdown techniques had a significant effect on further DMRT tests for germination parameters, growth simultaneity, maximum growth potential, and percentage of seed not growing. Based on the results of further tests for the four parameters, it showed that the interaction between Madura Rosella 1641 seed using scarification treatment (B_2P_7) resulted in an average germination, simultaneous growth, maximum growth potential, and the best percentage of seed grown with a mean of 84%, 80%, 90 and 6 seeds. Unlike the case of Madura rosella seeds which used cold water for 12 hours (B_2P_4) each produced the lowest germination of 0.2%, simultaneous growth of 0%, maximum growth potential of only 0.5% and the percentage of seeds that did not grow to produce 100% which showed that seeds with the use of cold water have not been able to increase the seed germination of rosela.

Table 2. Rosela seed germination recapitulation

Benih (B)*Pematahan dormansi (P)	DB (%)	KST (%)	PTM	PBTT
B_1P_0	6.00 *cd*	6.00 *cde*	7.00 *cd*	93.00 *a*
B_1P_1	9.00 *c*	8.00 *c*	9.00 *c*	90.00 *a*
B_1P_2	3.00 *de*	3.00 *efg*	4.00 *def*	96.00 *a*
B_1P_3	5.00 *cd*	5.00 *cdef*	6.00 *cde*	92.00 *a*
B_1P_4	6.00 *cd*	5.00 *cdef*	7.00 *cd*	80.00 *b*
B_1P_5	8.00 *c*	7.00 *cd*	9.00 *c*	91.00 *a*
B_1P_6	8.00 *c*	7.00 *cd*	9.00 *c*	91.00 *a*

B_1P_7	65.00 ^{b}	55.00 ^{b}	75.00 ^{b}	13.00 ^{c}
B_2P_0	1.00 ^{e}	0.00 ^{g}	0.25 ^{f}	100.00 ^{a}
B_2P_1	6.00 ^{cd}	5.00 ^{cdef}	6.00 ^{cde}	94.00 ^{a}
B_2P_2	4.00 ^{de}	4.00 ^{defg}	4.00 ^{def}	95.00 ^{a}
B_2P_3	1.00 ^{e}	0.00 ^{g}	2.00 ^{ef}	98.00 ^{a}
B_2P_4	0.20 ^{e}	0.00 ^{g}	0.50 ^{f}	100.00 ^{a}
B_2P_5	0.50 ^{e}	0.50 ^{fg}	0.50 ^{f}	100.00 ^{a}
B_2P_6	1.00 ^{e}	1.00 ^{fg}	1.00 ^{f}	99.00 ^{a}
B_2P_7	84.00 ^{a}	80.00 ^{a}	90.00 ^{a}	6.00 ^{c}

Description: The numbers followed by the same letter are not significantly different in the 5% DMRT test. DB = Power of Germination; KST = Simultaneous Growing; PTM = Maximum Growing Potential; PBTT = Percentage of Seeds Not Growing

Plumula Height (cm) and Radicula Length (cm)

Based on variance recapitulation data (Table 1), shows that the interaction between seed, media and treatment were significantly different for height parameters of plumula and radicle length. Thus, further testing was carried out using the α 5% DMRT test as presented in Table 3. Based on the results of further tests, the treatment of $B_1M_1P_6$ and $B_1M_1P_7$ produced the best plumala height with an average of 11.00 cm while the $B_1M_1P_5$ treatment produced a high plumula 0.00 cm or in other words the seed does not germinate.

Unlike the case for the radicular length parameter, $B_2M_1P_7$ treatment produced the best radicle length with an average of 10.00 cm while, $B_2M_1P_4$, $B_2M_1P_5$, and $B_2M_1P_6$ treatment produced the lowest radicle length with an average of 0.00 cm. Similar to the radicular length parameters, the seeds used do not germinate so that radicular growth does not occur.

Table 3. Long recapitulation of plumula and radicle of Rosela seed sprouts

Treatment	Plumula Height (cm)				Radicular Length (cm)			
	B1		B2		B1		B2	
	M1	M2	M1	M2	M1	M2	M1	M2
P_0	9.00 *ab*	3.00 *defg*	1.00 *fg*	1.00 *fg*	7.00 *bc*	2.00 *def*	1.00 *def*	0.30 *ef*
P_1	10.00 *ab*	4.00 *def*	9.00 *ab*	2.00 *defg*	7.00 *bc*	2.00 *def*	6.00 *bc*	1.00 *def*
P_2	8.00 *bc*	1.00 *fg*	9.00 *ab*	2.00 *defg*	5.00 *c*	1.00 *def*	5.00 *c*	1.00 *def*
P_3	5.00 *cde*	3.00 *defg*	2.00 *efg*	1.00 *fg*	2.00 *def*	2.00 *def*	0.30 *ef*	1.00 *def*
P_4	11.00 *a*	4.00 *defg*	2.00 *defg*	0.00 *g*	8.00 *ab*	1.00 *def*	0.00 *f*	1.00 *def*
P_5	11.00 *a*	5.00 *cde*	0.00 *g*	1.00 *fg*	8.00 *ab*	2.00 *def*	0.00 *f*	0.30 *ef*
P_6	11.00 *a*	5.00 *cde*	4.00 *def*	0.00 *g*	8.00 *ab*	2.00 *def*	3.00 *de*	0.00 *f*
P_7	11.00 *a*	5.00 *cde*	10.00 *ab*	5.00 *cde*	5.00 *c*	2.00 *def*	10.00 *a*	3.00 *de*

Description : The numbers followed by the same letter are not significantly different in the 5% DMRT test.

Wet Weight and Dry Weight of Rosella Sprouts

Based on the results of the recapitulation of the analysis of the variety of roselle seeds, there was a significant effect on the interaction between seed and treatment on parameters of wet weight and dry weight of sprouts. Furthermore, 5% DMRT further testing was carried out. The B_1P_7 treatment produced the best wet weight of germination with an average of 0.40 g and was significantly different from the other treatments. Whereas, B_2P_5

treatment produced the lowest wet seed sprout weight with an average of 0.01 g but was not significantly different from treatment B_2P_0, B_2P_3, B_2P_4, B_2P_5, and B_2P_6.

Table 4. Recapitulation of wet weight and dry weight of Rosela seed sprouts

Treatment	Wet Weight of Sprouts (g)		Dry Weight of Sprouts (g)	
	B1	B2	B1	B2
P0	0.300 ^{abcde}	0.030 ^{f}	0.200 ^{b}	0.004 ^{b}
P1	0.310 ^{abcd}	0.230 ^{bcde}	0.200 ^{b}	0.020 ^{b}
P2	0.200 ^{de}	0.210 ^{cde}	0.010 ^{b}	0.010 ^{b}
P3	0.190 ^{e}	0.070 ^{f}	0.010 ^{b}	0.007 ^{b}
P4	0.310 ^{abcd}	0.040 ^{f}	0.200 ^{b}	0.003 ^{b}
P5	0.340 ^{ab}	0.010 ^{f}	0.200 ^{b}	0.001 ^{b}
P6	0.320 ^{abc}	0.080 ^{f}	0.030 ^{b}	0.007 ^{b}
P7	0.400 ^{a}	0.350 ^{ab}	0.020 ^{b}	0.600 ^{a}

Description : The numbers followed by the same letter are not significantly different in the 5% DMRT test.

The best sprouts dry weight parameters were shown in B_2P_7 treatment with an average of 0.600 g and different from other treatments. Meanwhile, B_2P_5 treatment resulted in the lowest seedling dry weight by an average of 0.001 g and was not significantly different from other treatments except perlekuan B_2P_7.

Rosella Seed Water Content (%)

Moisture is the amount of water contained in the ingredients expressed in percent. Water content is also one of the most important characteristics of food, because water can affect the appearance, texture, and taste of foodstuffs as shown in Table 5.

Table 5. Recapitulation of the water content of roselle seeds

Seed Type	Repeat	Water Content %	Mean
B1	1	8,287	8,537
	2	8,786	
B2	1	9,287	8,931
	2	8,576	

Table 5 shows the highest water content of B2 seed (Madura rosela seed 1614 of 2004) with an average of 8,931% and the lowest at B1 (AC 1301 2005 roselle seed with an average of 8,537%. The moisture content in B2 indicates that the seed is able to germinate maximally compared to B2 as shown in the germination data (Table 2).

Discussions

Rosela Seed Germination

Scarification techniques (P7) are performed with an opening on the skin of rosella. The high parameters of rosella seed germination are because water can easily enter the seeds and stimulate the imbimbition process and germination enzyme activation. Imbibisi causes the seeds to expand and break the wrapping skin and trigger metabolic changes in the embryo so that it can continue its growth. Enzymes will hydrolyze ingredients stored in cotyledons and nutrients in them.

Enzymes that play a role in the hydrolysis of food reserves are α-amylase, β-amylase and protease enzymes (Surya, 2010). According to Kamil (1986), seed skin and surrounding structures

can affect the ability of seed germination through inhibition of water absorption, gas exchange, diffusion of endogenous inhibitors or embryo growth inhibition. Kuswanto (1996), who argues that seed germination that contains non-permeable seed shells can be stimulated by scarification, which is the conversion of seed coat to make it permeable to gases and water. This Impermeability can be caused by deposition of various substances (eg cutin, suberin, lignin) on the membrane.

Scarification is a dormancy solving method aimed at reducing thickness, breaking or removing hard seed skin (Yuniarti and Djaman, 2015). Discarified seeds will produce a better imbibition process. Water and gas will enter the seeds faster because of the permeable seed skin. Water that enters the seeds causes the metabolic processes in the seeds to run faster as a result of the better germination.

Plumula Height (cm) and Radicula Length (cm)

The use of AC 1301 rosella seeds using cold water immersion treatment for 20 hours then germinated on red paper media and with saccharification use is able to produce the best high plumula. This can be caused by several factors including the age of seeds that are younger than the type B2 because B1 was harvested in 2005 and B2 was harvested in 2004, the germination conditions were also made as optimal as possible due to M1 treatment, germination in germinator, soaking time effect on skin permeability and imbibition. This is supported by the opinion of Sadjad (1975)

who said that water is an environmental factor that influences the process of seed germination (Sadjad 1975).

The process of entering water into the seed is a physical process, there is no connection with the death of the seed. According to the dictionary of biology and seed technology of forest plants (2004), the process of absorption of water by seeds before germination is called imbibisi. The rate of imbibition, besides being influenced by the permeability of the seed coat, is also influenced by the water content in the seed. Imbibisi occurs because the potential of the water in the seed is lower than its surroundings, so that the water will move into the seeds (Beneach and Sanchez 2004). Setyamidjaja (2006), in growth or germination, the embryo will come out through the hole in the shell by forming roots and stems.

The best radicular length parameters in the treatment using scarification compared with other fracture treatments. This is because the scarification treatment is able to inhibit the mechanical properties of the skin so that ir and oxygen can easily accumulate into the seeds for the germination process so as to encourage maximum growth of the radicle. Dharma et al., (2015) stated that the breakdown of dormancy through full scarification with sanding gave a mean length of plumula 12.35 cm and was significantly different from cracking and no scarification scarification treatment which only gave an average length of successive radicle length 11.38 cm and 8.64 cm. The results of Williyatno's (2007) study

showed that plumula and radicle more than 2 cm long existed at intervals of 5-10 days after the seeds began to germinate.

Wet Weight and Dry Weight of Rosella Sprouts

Discarification of rosella seed germination can be proven by increasing basag weight and normal sprout dry weight (Table 4). This is because a good metabolic process will produce good germination. Seeds that germinate can utilize food reserves in seeds well, in the presence of water, oxygen will enter the seeds by breaking down food reserves that are used as energy sources for normal sprout growth in a fast and simultaneous time.

The increase in germination can be seen in the germination variables observed, namely germination, speed of germination, simultaneous germination and dry weight of normal sprouts. Yuniarti (2002), for saga seeds that use the treatment, it is thought that then soaked in cold water for 24 hours, can produce a high germination value of 77.83%. Bewley and Black (2006) stated that water absorption starts from the imbibition process (phase I) to 24 hours which is a rapid absorption phase and is followed by (phase II) which is characterized by a constant water absorption which tends to be 68 hours. With a good rate of imbibition, the need for water for seeds is met so that the process of seed metabolism can run well.

Rosela Seed Water Content (%)

Seed germination is strongly influenced by seed moisture content. Seed treatments, namely B1 and B2, showed that the best moisture content of Madura rosela seed was 1614 in 2004

compared to AC rosela seed 1301 of 2005. This is because B1 experienced a decline in seeds during storage, which is characterized by the low water content produced. Balittas (2016) shows that the water content for kenaf seeds and the like ranges between 4% -13% so that it can increase the respiration rate and the CO2 produced is lower.

Purba (2013) states that seed decline during storage is caused by higher water content. This results in a faster rate of respiration so that more CO2 and heat is produced. This physiological activity can be suppressed through ideal moisture content so that the germination of the seed is still maintained until the time for the seed to be germinated (Hidayat and Marjani, 2017).

Conclusions

The method of dormancy breaking technique using scarification was able to increase the germination for Roselle seed of Madura seed 1614 in 2004 and AC 1301 Year 2005 roselle seed which was germinated on paper medium.

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Sugarcane Callus Morphology On Multiple concentrations of 2,4 *Dichlorophenoxy Acetic Acid* and *Indole Acetic Acid* In-Vitro

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Abstract

This study aimed to obtain callus of sugarcane in large quantities in a short time and obtain the concentration of 2.4-D and IAA are effective against cell division. This research was carried out at the Plant Tissue Culture Laboratory, Faculty of Agriculture, University of Cokroaminoto Palopo, from March to June 2018. The method used in this study is a factorial experiment in a Completely Randomized Design (CRD) consisting of two factors. The first factor is 2.4-D with the level of concentration N_0 = 0 ppm/L, N_1 = 3 ppm/L, N_2 = 4 ppm/L, and N_3 = 5 ppm/L. Whereas, the second factor is IAA with a concentration level of I_0 = 0 ppm/L, I_1 = 5 ppm/L, I_2 = 6 ppm/L, and I_3 = 7 ppm/L, each of which is repeated 3 times. Observation parameters observed for quantitative characters are callous age (days after subculture/DAS), callus diameter (cm), and callus weight (g). Meanwhile, qualitative parameters are callus color, callus texture, and callus structure. The results showed that adition of 2,4-D and IAA was significantly different for the parameters of callous age (DAS) and callus diameter (cm). The treatment of I_3N_3 resulted in smooth age and best callus diameter with a mean of 38.00 DAS and 1.24 cm respectively. In addition, I_3N_3 produced callus with white colour, rough texture and a compact structure. Whereas, the best callus weight was produced by I_2N_1 treatment with an average of 0.64 g.

Keywords: callus formation, effectiveness, indole acetic acid (IAA), sugarcane, 2.4 dichlorophenoxy acetic acid (2.4-D)

Background

Sugarcane consumption and demand from 2013 to 2019 has increased, respectively 6,648 kg/capita and 1,654,196 tons, 7,504 kg/capita and 1,829,175 tons, 7,432 kg/capita and 1,898,665 tons, 7,361 kg/capita and 1,904,289 tons, 7,297 kg/capita and 1,911,023 tons, 7,241 kg/capita and 1,918,920 tons, and in 2019 it was assumed that the increase amounted to 7,185 kg/capita and 1,925,274. To overcome sugar consumption and increasing demand for sugar cane, it is necessary to develop or multiply sugarcane. One of them is through biotechnology, namely plant tissue culture (Pusat Data Sistem Informasi Pertanian, 2014).

Sugarcane (Saccharum offiinarum L.) is one of the agricultural commodities that occupies an important position, because 70% of the world's sugar production comes from sugar cane (Khan and Khatri, 2006). This commodity is included in seasonal crops and belongs to the Poaceae family or better known as grasses. This commodity comes from India and is widely cultivated in Irian. Sugar cane plants can be cultivated in the lowlands of the tropics and subtropics. The main benefit of sugar cane is as a raw material for making sugar. Bagasse or commonly called bagasse is a byproduct of the process of extraction of sugar cane from the stem of sugar cane. From one factory, bagasse is produced around 35-40% of the weight of milled sugarcane (Zultiniar et al., 2011).

Plant tissue culture is a technique of growing plant parts in the form of pieces of tissue or plant organs that are separated from

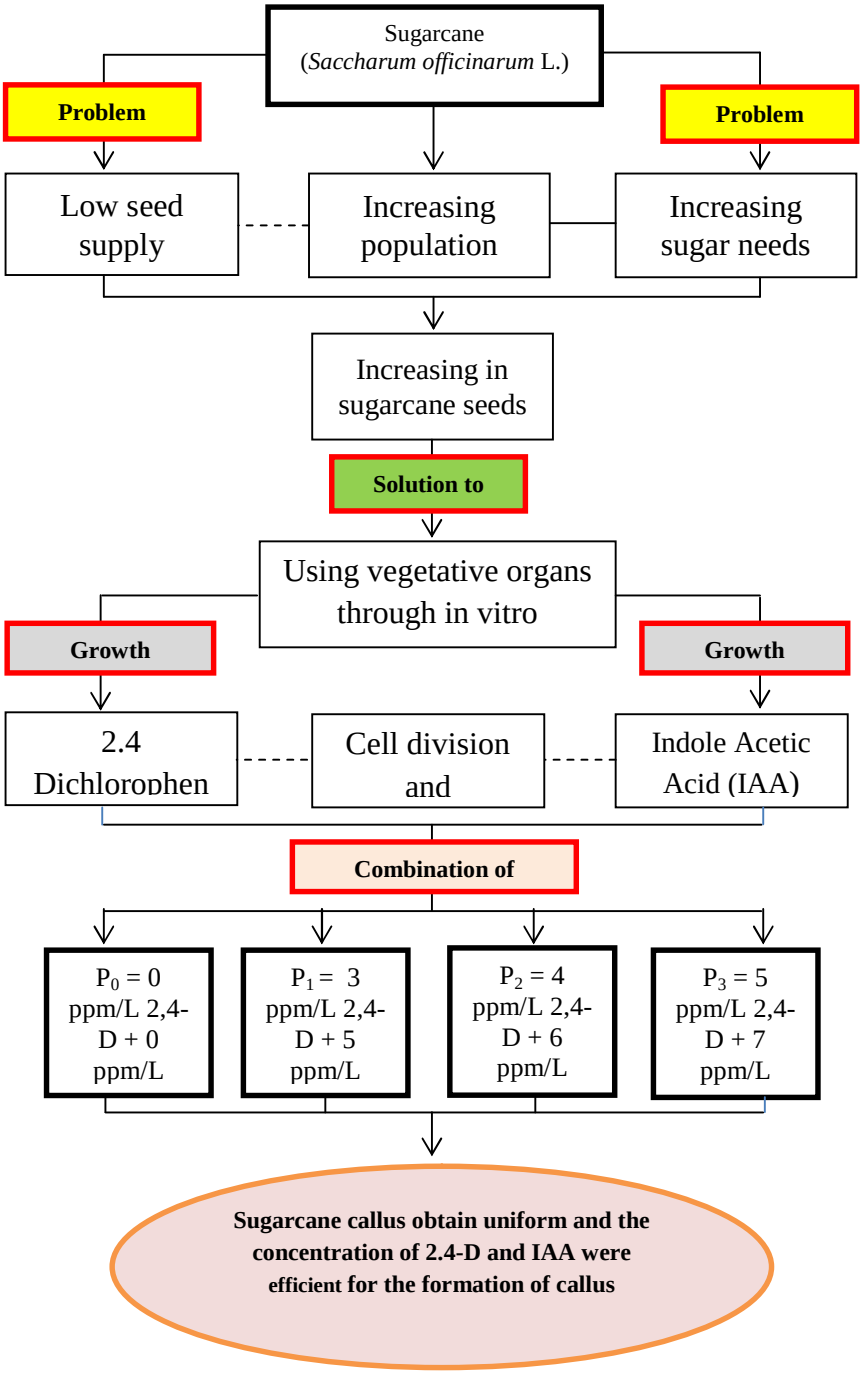
the natural environment in an artificial medium aseptically (Ayabe and Sumi 1998). Tissue culture has long been used as a method for the production of bioactive compounds from plants. The advantages of using tissue culture in the production of bioactive compounds compared to intact plants include lack of climate limitation, not requiring large areas of land, and bioactive compounds can be produced continuously under controlled conditions (Collin and Edward, 1998). To increase cell division, growth regulating agents are needed. Growth regulators are very necessary as a medium component for explant cell growth and differentiation. Without the addition of growth regulators in the medium, is very stunted growth, and even might not grow at all. Each explant originating from different organs and species will require different growth regulators (Narayanaswamy, 1994). In addition, Gunawan (1987) also explained that growth regulating agents affect growth and morphogenesis in cell, tissue or organ culture in vitro. The growth regulating agents used are auxin and those included in auxin groups include IAA (indole acetic acid), IBA (indole butiric acid), NAA (Napthalene acetic acid), and 2,4-D (Dichlorophenoxy acetic acid).

Auxin is a plant hormone that can support physiological processes such as growth, cell division and differentiation and protein synthesis. Auxin has the ability to encourage cell division by affecting the cell wall. IAA type auxin can cause both elongation at the shoots and at the roots and auxin, ZPT 2,4-D has an N content of 8,9 mg. Based on this, the ZPT 2,4-D can

potentially increase the density of Nannochloropsis oculata because there is an element of N to increase growth (Brotowidjoyo et al. 1995).

Purwitasari et al. (2012) stated that the concentration of growth regulators (dichlorophenoxyacetate-2.4 acid) can affect the growth of Nannochloropsis oculata, the administration of growth regulators (2,4-dichlorophenoxyacetacetic acid) with a concentration of 5 ml resulted in the highest Nannochloropsis oculata cell density. 4,800,000 cells / ml obtained on the 7th day after treatment. Alfian et al (2015) stated that the administration of MS media with the addition of 4 ppm 2,4-D produced a genetic embryo with a high percentage reaching 90% in inducing callus with a fast time which only required 12.67 days.

The purpose of this study was to obtain large amounts of sugarcane callus in a short time and obtain concentrations of 2.4-D and IAA which were efficient for sugarcane callus induction. Based on the description above, it is necessary to do research with the title Effectiveness of 2,4-D (Dichlorophenoxy Acetic Acid) and IAA (Indole Acetic Acid) on callus formation in sugarcane (Saccaharum officinarum L.) in vitro.



Research Thinking Framework

Materials and Methods

This research was carried out at the Plant Tissue Culture Laboratory, Faculty of Agriculture, University of Cokroaminoto Palopo, which took place from March to June 2018. The material used in this research was MS 1962 media, sugar cane, aquades, compactor (gelatin), sugar, spritus, alcohol, aluminum foil, pH paper, 70% alcohol, 96% alcohol, and klipwrap. Meanwhile, the tools used are culture bottles, erlemeyer, autoclaves, laminar air flow cabinets, analytical balance sheets, rulers, books, pens, petridishes, knives, aluminum foil, pingset, scalpel rods, scalpel eyes, bunsen, cup glasses, measuring cups, spoit with a capacity of 5 ml and a digital microscope.

His study uses a factorial design of two factors where the first factor uses several concentrations of 2.4-D namely N_0 = 0 ppm/L, N_1 = 3 ppm/L, N_2 = 4 ppm/L, and N_3 = 5 ppm/L. Whereas, the second factor uses several IAA concentrations, namely I_0 = 0 ppm/L, I_1 = 5 ppm/L, I_2 = 6 ppm/L, and I_3 = 7 ppm/L, each of which is repeated 3 times. The linear model of the design used is as follows:

$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$; i = 1,2,3,4; j = 1,2,3,4; and k = 1,2,3

Description:

Yijk : Observations on the Kth experimental unit that gets a combination of treatment at the i level of factor A and the jth level of factor B;

M : Average population value

α_i : The effect of the level of i from factor I

β_j : Effect of the jth level of factor N

$(\alpha\beta)_{ij}$: Effect of factor I and factor N interactions

ε_{ijk} : The random effect of the kth experimental unit that received the ij treatment combination. εij ~N (0, σ2)

Data obtained subsequently using ANOVA and if there is influence, then continue to use the Duncan's Multiple Range Test (DMRT) with an error rate of 5%.

The first step is the sterilization of tools, namely bottles of culture, petridish, pingset, and scalpel stems. The tools were washed thoroughly using laundry soap and then soaked using a backlin for ± 60 minutes. After that it is sterilized using wet sterilization with a temperature of 121 ° C and a pressure of 15-17 psi for 30 minutes. fter that, the MS stock solution was made by weighing chemicals, macro nutrients, micro nutrients, and ZPT according to the composition of MS media. Then the ingredients are dissolved in distilled water, placed in a dura bottle, and stored in a cooling container.

Making the media by taking and measuring each stock solution in accordance with the predetermined treatment and size then put it in erlemeyer. The manufacture of I0N0 media (control) was done by mixing 7 grams of agar + 30 grams of sugar + 100 ml of coconut water and adding distilled water to 1000 ml. After all

the ingredients are mixed, cook on medium heat until boiling, the cooked medium is poured in a culture bottle, each bottle of ± 10 ml. The culture bottle was closed using aluminum foil then sterilized using autoclave for 15 minutes and stored in a storage room (incubation). The procedure of making media I0N1 and so on is the same as the making of I0N0 media, but what distinguishes only the use of a combination of 2.4-D and IAA in accordance with their respective concentrations

Planting explants were carried out at LAFC and starting with explant sterilization using burn sterilization. Dyed sugar cane explants using 96% alcohol and burned. fter that, the segment is opened and re-dyed and burned until the leaves are rolled white. Furthermore, leaf explants were cut into pieces using a scalpel with a thickness of 0.1-0.2 cm. Each piece of leaf planted in a culture bottle is then covered using aluminum foil and tightened using klipwrap around the mouth of the bottle. After the young leaves are smooth, then subculture is carried out into two new inductions before the sub-culture is carried out into the treatment medium. Observations were made when explants began to smear or ranged 1-2 weeks after planting. The observation process is done every 2 days for the age parameter. As for the diameter, weight, and color of callus done at the end of the study. For qualitative characters, the structure and texture of callus were observed using a digital microscope.

The parameters used in this study are quantitative characters including callous age (DAS), callus diameter (cm), and callus weight (gr). Meanwhile, for qualitative characters include callus color, callus texture, and callus structure.

Results

The difference in the middle square value for the quantitative character of sugarcane callus for the combination of several IAA and 2.4-D concentrations can be seen in Table 1.

Table 1 Recapitulation of Variations in Quantitative Character of Cane Callus

Observation Characters	Middle Square				KK (%)
	Treatment	IAA (I)	2.4-D (N)	I*N	
Smooth Age (DAS)	45.643**	49.909*	62.576**	38.576*	56.6
Callus Diameter	0.038tn	0.024tn	0.722tn	0.048tn	16.32
Callus Weight	0.033*	0.034tn	0.028tn	0.034*	18.54

Description: * significant; ** = very significant; dan tn = not significant

The results of the variance in Table 1 show the treatment factors, IAA, 2.4-D have a significant effect on the age character of the callus. Unlike the case with callus weight characters that produce a significant effect only on treatment and interaction factors. However, for callus diameters both single factors and their interactions do not show any real influence.

Smooth Age (DAS)

Callus age is one indicator that the explants used have responded to the treatment used in this case, namely a combination of 2.4-D and IAA as presented in Table 2.

Table 2 Age of cane explant callus in several combinations of 2.4-D and IAA in vitro

IAA	2,4-D			
	N_0	N_1	N_2	N_3
I_0	47.33 ^{c}	42.00 ^{bc}	42.67 ^{bc}	42.00 ^{bc}
I_1	39.33 ^{b}	38.00 ^{b}	44.67 ^{bc}	38.67 ^{b}
I_2	38.67 ^{b}	38.00 ^{b}	39.33 ^{b}	41.33 ^{bc}
I_3	42.00 ^{bc}	38.67 ^{b}	45.33 ^{c}	30.33 ^{a}

Description: The numbers followed by the same letter are not significantly different from the 5% DMRT test.

Based on the table below, it shows that there are significant differences in interaction using the 5% DMRT test. The treatment of I3N3 produced the best callus age and was significantly different from the other treatments with an average of 30.33 DAS. Whereas the I0N0 treatment produced the lowest flowering age with an average of 47.33 DAS, but not significantly different from the I3N2 treatment with an average of 45.33 DAS.

Callus Diameter (cm)

The results of the analysis of variance showed no significant effect on the character of sugarcane callus diameter on the administration of 2,4-D and IAA to callus formation presented in Figure 1. The treatment of I_3N_3 produced the best callus diameter with an average of 1.24 cm while the lowest was shown in I_0N_0 treatment with average 0.90 cm.

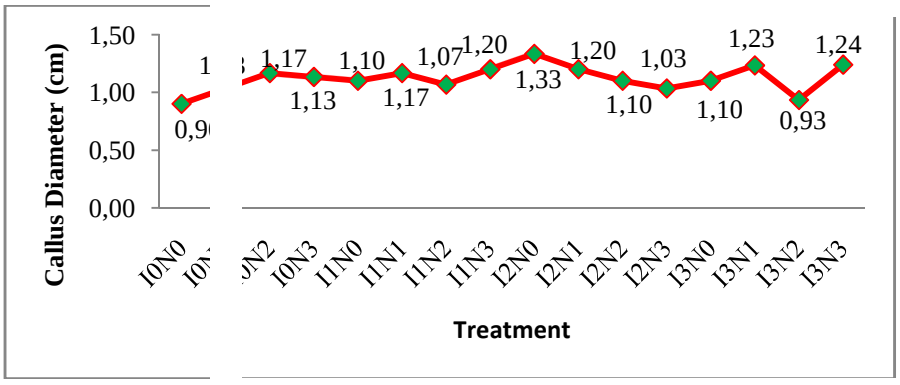


Figure 1 Average diameter of sugarcane callus in several combinations of 2.4-D and

Callus Weight (g)

The results of variance for sugarcane callus weight characters showed significant differences in the α 0.05 DMRT test which is presented in Table 3. The treatment of I2N1 produced the best callus weight with an average of 0.64 g and was not significantly different from the average I_3N_2 treatment 0.62 g.

Table 3 Cane callus weights in several combinations of 2.4-D and IAA in vitro

IAA	2,4-D			
	N0	N1	N2	N3
I_0	0.48 ^{abc}	0.34 ^{bc}	0.39 ^{bc}	0.33 ^{c}
I_1	0.44 ^{abc}	0.37 ^{bc}	0.40 ^{abc}	0.55 ^{ab}
I_2	0.52 ^{abc}	0.64 ^{a}	0.34 ^{bc}	0.56 ^{ab}
I_3	0.47 ^{abc}	0.62 ^{a}	0.41 ^{abc}	0.30 ^{c}

Description: The numbers followed by the same letter are not significantly different from the 5% DMRT test.

Callus Color

Callus color is used as a good indicator of callus quality. The results showed that giving 2,4-D and IAA had an effect on color, and can be seen in the figure below (Figure 2).

Figure 2 Callus color of sugar cane after addition of 2,4-D and IAA, A (I_3N_3), B (I_1N_I), C (I_0N_1).

Callus Texture

The results showed that administration of 2,4-D and IAA had an effect on callus texture and was presented in Figure 6. The treatment of I_3N_3 produced a rough texture while the slippery textured treatment was produced in I_1N_1 treatment.

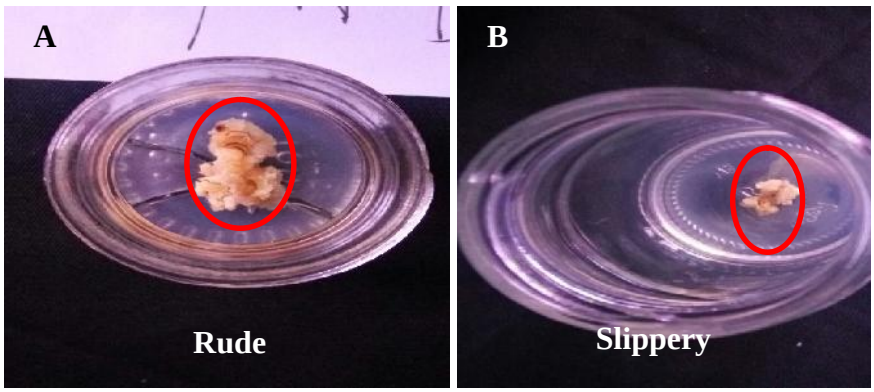


Figure 3 Callus texture of sugarcane plants after addition of 2,4-D and IAA, A (I_0N_0) and (I_3N_3), B (I_1N_I).

Callus Structure

The results showed that administration of 2,4-D and IAA had an effect on callus structure. Figure 4 shows the best structure found in I_3N_3 treatment with a compact structure while crumb structured treatment was found in I_1N_1 treatment.

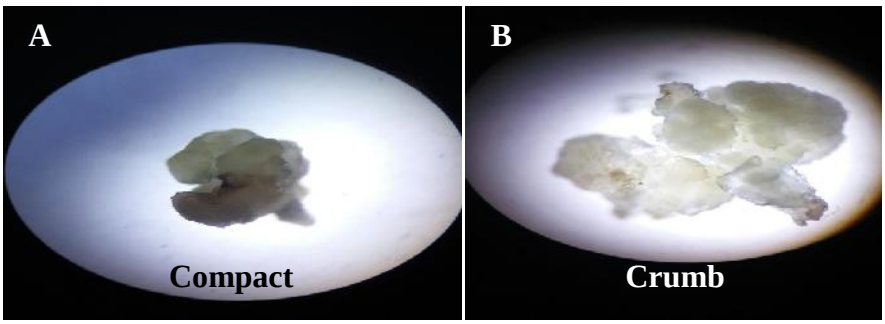


Figure 4 Sugarcane callus structure after the addition of 2,4-D and IAA on MS medium, A (I_0N_0) and (I_3N_3), B (I_1N_I).

Discussions

The difference in callus phenotype produced for each of the different observational characters caused by the influence of the environment in this case is the difference from each treatment combination given and the presence of genetic influences from the explants used. Yamin (2014) states that the appearance of plant phenotypes is influenced by the presence of environmental influences and gene expression and expression. Natawijaya (2012) stated that the phenotype differences for each genotype in the ahronomy character were caused by gene expression.

Smooth Age (DAS)

The use of 7 ppm / L IAA + 5 ppm / L 2.4-D can accelerate callus formation quickly. This is because IAA and 2.4-D are

auxiliary growth regulating substances that play a role in the formation, callus growth, and are able to increase the natural chemical compounds of flavonoids. 2.4-D are stable growth regulators and are not easily damaged by light or heating the time for sterilization takes place (Hendaryono and Wijayani, 1994).

Success in callus formation, in addition to being strongly influenced by growth regulating substances is also influenced by media factors that not all commodities are able to call MS media use. Developmental phase explant and genotype also affects the formation of callus quickly. if the genotype used has a slow development cycle, the callus produced will be slow too. The results in exploiting somaclonal variations are influenced by plant genotypes (Tripathy and Reddy, 2002; Shirin et al., 2007), medium (Abadi and Kaviani, 2010), growth regulating substances (Hoesen et al., 2008; Jahan et al., 2009).), and the phase of development of explants can also affect callus speed (Ibrahim et al., 2010; Reddy et al., 2011). Romeida and Ganefianti (2016) stated that young leaves, shoots, stems and root tips are ingredients that can improve in vitro success because they have high regeneration. Wu et al., (2012) stated that 99.67% of plantlet formation was obtained from callus derived from young leaves.

Callus Diameter (cm)

I_3N_3 produce the best treatment diameter compared with the treatment of other woods, it is due to their concentration levels

which affect the diameter of the resulting callus. This is consistent with the opinion of Ling et al (2013) which states that the smallest callus diameter is due to the high concentration of 2,4-D added to the explant so that it will inhibit callus growth.

Providing optimal concentration can get a larger diameter of callus. This is in line with the opinion of Azar and Kazemiani (2011) stating that the concentrations of 2,4-D and IAA that are given optimally for callus growth can increase the size of the callus to obtain a larger diameter of callus. Budisantoso et al., (2017) states that the addition of growth regulators is one of the determining factors in the success of in vitro culture. 2.4-D as a plant ZPT in the media can stimulate cell division, enlarge explants and increase callus formation and growth.

Callus Weight (g)

Giving 2.4-D with a low concentration is not able to form callus, it can even cause explants that are used do not develop, but there is a brownish color change. Noorrohmah et al., (2017) stated that the addition of media without the use of 2.4-D did not occur callus formation. Eksplan at week 2 was still green but then at week 4, 6, and 8 explants were brown and all explants were not developed.

Dewita (2015) stated that the effect of giving a high 2,4-D concentration to explants was able to increase the average callus weight and increase the effectiveness of 2,4-D which stimulated the

tissue to become stressed and cause continuous cell division in the tissue which ultimately affects callus size and callus weight.

Callus Color

Figure 2 can show that all treatments produce the best callus color, which is white in I_3N_3 treatment, then the treatment that produces brownish white color that is I_1N_1 while the treatment that produces brown color is I_0N_1. The difference in callus color produced is due to the provision of growth regulating substances that are synthetic and capable of producing optimal results so that the color of the average callus is white, as stated (Andaryani, 2010) that good callus quality has a green color. While bright or white colors can indicate that the callus condition is still good enough. The white callus is an embryonic tissue that does not contain chloroplasts, but has a high content of starch (Tsuro, 1998).

Widayanto (2004) states that changes in callus from white to brown indicate a decrease in growth in callus cells. These cells have very low cleavage activity so their regeneration power has been reduced and due to toxic phenol metabolism, which is often aroused due to explant sterilization process, which inhibits growth and even death in tissues.

Callus Texture

Coarse-textured callus shows that some callus experience a lignification formation (thickening of the cell wall) so that the callus has a rough and dense texture (Shofiah and Purnawanto,

2010). This is due to improper use of media and the ability of tissues in explants to absorb nutrients.

Pierik (1987) states that many factors that influence the texture of callus include the type of plant used, growth regulating substances, and environmental conditions. Kartika et al. (2014) stated that differences in growth rates were also influenced by the ability of the network to absorb nutrients available, this was much influenced by aeration and callus texture.

Callus Structure

Observation parameters of the best callus structure in I_0N_0 and I_3N_3 treatment produced a compact structure and in I_1N_1 treatment the structure was crumb. This is due to various factors. Pierik (1987) states that the structure in callus can vary from compact to crumb, depending on the type of plant used, the composition of media nutrients, growth regulating substances and environmental conditions of the culture.

Conclusion

Sugarcane is one of the sugar producing commodities that plays an important role in the country's economy. In order to fulfill the need for these commodities, efforts were made to develop and increase sugarcane seedlings through plant propagation using in vitro techniques. Through this technique can produce seeds in large quantities and quickly using growth regulating substances namely 2.4-D and IAA. Provision of 2,4-D and IAA significantly affected

callus age and weight parameters callus. The treatment of I3N3 resulted in smooth age and best callus diameter with a mean of 38.00 DAS and 1.24 cm respectively. Additionally, I3N3 produce callus white, coarse textured and has a compact structure. Whereas, the best callus weight was produced by I2N1 treatment with an average of 0.64 gr. Treatment of 7 ppm / L IAA + 5 ppm / L 2.4-D is a combination of effective concentrations to stimulate sugarcane callus formation. Whereas by giving 6 ppm / L IAA + 3 ppm / L 2.4-D can increase the weight of sugar cane callus.

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Effect of Characteristics of Cocoa Farmers on Farmers Group Dynamics in Pengkendekan Village, Sabbang District, North Luwu Regency

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Abstract

This study aims to determine the effect of the characteristics of cocoa farmers on the dynamics of farmer groups. This research was conducted in Pengkendekan Village, Sabbang District, North Luwu Regency. Determination of the location of the research was done intentionally (purposive). This research was conducted in March to April 2018 with a total sample of 36 respondents. Data collection is done in two ways, namely using primary data obtained directly from respondents, while secondary data is data or documents obtained from the office or agency associated with this research. The data analysis method used is multiple regression analysis using the SPSS program. The results showed that the variable number of family dependents significantly affected the dynamics of farmer groups with a significant value of 0.018. While the variables of age, level of education, experience of farming, and land area have no significant effect on group dynamics because the significant value is greater than 0.05.

Keywords: characteristics of cocoa farmers, farmer group dynamics, influence

Background

Farmer groups can grow and develop among Indonesian farmers because basically the Indonesian people are known as people who like to work together, help, work together and have a high concern for each other. One of the things that need to be considered in the development of farmer groups is the dynamics of farmer groups. Group dynamics can be interpreted as forces within the group that result in groups effectively achieving their goals.

Farmer groups should be a tool for its members to achieve goals, both personal goals and group goals (Damanik. 2013).

For this reason, it is imperative that the existing farmer groups must have movements or strengths that can determine and influence the behavior of the group and its members in achieving their goals effectively (Lestari. 2011). Group or organization can be said to be dynamic if the group or organization is effective in achieving its goals. This aspect of group dynamics provides the greatest opportunity for members to cooperate and participate in group activities (Tuyuwale in Makawekes, N., 2016). Therefore, to know the dynamic or not of a group can be done by analyzing group members through the behavior of the members and leaders.

According to Rusidi (1989), group dynamics will develop towards a more advanced direction determined by the characteristics of farmers as group members. This is in line with the results of Lestari research (2011) which shows that internal factors and external factors together directly affect group dynamics. The factors that influence the dynamics of farmer groups are individual or farmer characteristics such as age, level of education, number of family dependents, and experience of farming.

The establishment of farmer groups in the Pengkendekan Village, Sabbang District has a very important role in cocoa farming, especially in increasing the production of cocoa farmers. The aspects of the dynamics of farmer groups provide opportunities for members to collaborate and participate in group activities. The purpose of this study was to determine the effect of farmer characteristics on the dynamics of farmer groups in Pengkendekan Village, Sabbang District, North Luwu Regency.

Research Methods

The research method used in this study is descriptive quantitative research by finding information about the existing symptoms, clearly defined the objectives to be achieved.

Place and Time of Research

This research was carried out in Pengkendekan Village, Sabbang Subdistrict, North Luwu Regency. The location selection

was done intentionally (purposive sampling). The selection of research locations is based on the consideration that the village is one of the centers of cocoa production and has formed farmer groups and farmer groups. This research was conducted from March to April 2018.

Population and Sample

Sampling in this study using a simple random sampling (Simple Random sampling) is a sample taken in such a way that each research unit or elementary unit of the population has the same opportunity to be chosen as a sample. The population in the Pengkendekan Village is 125 farmers who are members of 5 farmer groups. Of the total population, the farmers who were sampled were 36 farmers consisting of 5 farmer groups.

Method of Collecting Data

The data used in this study consisted of primary data and secondary data. Primary data is data obtained from the results of direct observation (observation) and through interviews. Whereas secondary data is data obtained from agencies related to this research.

Data Analysis Method

Data analysis techniques were carried out in two ways, namely descriptive statistics and linear regression analysis. Descriptive statistics are used to see the dynamics of farmer groups. While multiple linear regression analysis is used to see the effect of independent variables on the dependent.

Results

Characteristics of Farmers

Respondents are cocoa farmers in the Pengkendekan Village who have diverse characteristics. In this case, the characteristics of farmers include gender, age, level of education, number of family dependents, farmer's land area and farming

experience. The following is a description of the characteristics of farmers in the Shortening Village as follows:

Table 1. Desc ription of Farmer Characteristics according to Gender, Age, Education Level, Number of Family Dependents, Farm Area and Farming Experience

No.	Characteristics	Amount	Percentage
1.	**Age**		
	20 – 29	5	13,8 %
	30 – 35	9	25 %
	36 – 45	14	38,9 %
	46 – 60	6	16,7 %
	61 – 80	2	5,6 %
	Amount	**36**	**100 %**
2.	**Level of Education**		
	SD	6	16,7 %
	SMP/SLTP	14	38,9 %
	SMA/SLTA	16	44,4 %
	Amount	**36**	100 %
3.	**Number of Family Dependents**	12	33,3 %
	2 – 3 orang	19	52,8 %
	4 – 5 orang	5	13,9 %
	6 – 7 orang	**36**	**100 %**
	Amount		
4.	**Experience of Farming**		
	5 – 10 Tahun	29	80,6 %
	11 – 20 Tahun	5	13,8 %
	21 – 40 Tahun	2	5,6 %
	Amount	**36**	**100 %**

Source : Primary data after processing, (2018)

The age of the farmer is one of the factors that influence the ability and skills in managing his farm. In addition, age is clearly related to its performance and productivity as well as one's way of thinking. Mulyasa (2002) suggests that the development of thinking

abilities occurs with age. In general, farmers whose age is older have decreased physical ability but have more experience and skills in managing their farming. While the younger farmers have good potential and ability, because of adequate physical ability to manage their farming. Based on table 1 data, generally shows that most of the age of the respondent farmers in the range of 36 - 45 years amounted to 14 people with a percentage of 38.9%, and at least 2 people with a percentage of 5.6% in the range of 61-80 years. While the average age of farmers is 39.39 years, this shows that the farmers who are sampled belong to productive age.

Education is one of the factors that play an important role in human life, both for themselves and for the surrounding environment. The level of education greatly influences the mindset and way of acting of farmers. This is because education provides certain values for someone, especially in opening horizons / thoughts and ways of thinking scientifically. Farmers who have a higher level of education or adequate will more quickly and easily accept new innovations, so that in applying and applying innovations is younger and more directed. Based on the data in table 1, it shows that most respondents have taken 16 high school / high school students with a percentage of 44.4%, while the least have taken up to 6 elementary schools with a percentage of 16.7%. In this case, if it is associated with farming activities carried out by the respondent farmers, it is certainly very influential in the development of farming that is carried out primarily in technology adoption.

Family dependents are people who live in one family and are directly dependent on the head of the family. The number of family dependents is one of the factors that influence the level of welfare of farmers. Farmers who have dependents who increasingly need to be able to make the right decisions, so that their farming can run well. The number of family dependents who are in productive age is one of the important human resources in their farming activities. Based on the data in table 1 shows that most of the respondents farmers have family coverage in the range of 4 - 5 people totaling 19 respondents with a percentage of 52.8%, while the least number of 5 people with a percentage of 13.9% in the

range of 6-7 people. If on average, the number of dependents of the farming family is 4 people.

In general, the activities and management of farming management are influenced by the experience of farming. Farmers in making decisions about the farms they manage are always considering the production risks that might be faced by farmers who are always based on the assumption that something new will have a positive or negative impact. Where each farmer has different abilities in accepting these risks, and these differences can be influenced by experience in doing farming. According to Hernanto (1993) states that farmers develop their farming capabilities from experiences gained from generation to generation. Farmers who are longer in their efforts will find it easier to implement innovations and manage their farms compared to novice farmers, so that the impact on the results obtained. Based on the data in table 1 shows that most farmers have experience farming in the range of 5 - 10 years totaling 29 people with a percentage of 80.6%, while those who have the least experience for 21-40 years amounted to 2 people with a percentage of 5.6%. The average farming experience is 9.6 years so that farmers can manage their farms well.

Discussions

Farmer Group Dynamics

Group dynamics are strengths found in groups that influence members and groups in achieving goals. To determine the dynamics of the group, an assessment is made of the elements of group dynamics. In this study the elements of group dynamics analyzed included: (1) group goals; (2) group structure; (3) task function; (4) group development and development; (5) group cohesiveness; (6) group atmosphere; (7) tension in the group; (8) group effectiveness and (9) covert intent.

Assessment of group dynamics element indicators in farmer groups in Pengkendekan Village, Sabbang Subdistrict can be seen in the table (attached table), where the total overall score is 6398 and 74.05% so that it is categorized as dynamic. This shows that

the average elements of the farmer group dynamics are going well. The dynamics of the farmer groups are shown by the interaction between members in the group is well established and the

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	180,458	5,912		30,522	,000
	Age	,020	,139	,033	,144	,886
	Number of family dependents	-2,897	1,156	-,493	-2,507	,018
	Experience of farming	,161	,280	,113	,576	,569
	Land area	1,596	,959	,310	1,664	,107

a. Dependent Variable: Dynamics

cooperation of the members in achieving group goals is very strong.

Effect of Farmer Characteristics on the Dynamics of Farmers' Groups

The characteristics observed in this study are age, formal education, experience in farming, and the number of family dependents. To determine the effect of cocoa farmers' characteristics on group dynamics, a regression test was carried out simultaneously (Test-F) and partially (t-test). From the results of the t-statistic test, it can be seen that the variable whose significance is less than 0.05, namely, the number of family dependents (Sig 0.018) so that it can partially affect the dynamics of the farmer group.

While the variables that do not significantly affect the dynamics of the farmer group are age (Sig 0.886), experience of farming (Sig 0.569), and land area (Sig 0.107), so that it can be said

to have no significant effect on the dynamics of farmer groups. Following are the results of statistical calculations with the SPSS tool as follows:

Table 2. Results of Multiple Linear Regression Characteristics of Cocoa Farmers

Coefficients[a]						
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	180,458	5,912		30,522	,000
	Age	,020	,139	,033	,144	,886
	Number of family dependents	-2,897	1,156	-,493	-2,507	,018
	Experience of farming	,161	,280	,113	,576	,569
	Land area	1,596	,959	,310	1,664	,107

a. Dependent Variable: Dynamics

Source: Analysis of primary data processed, 2018

Effect of Age on Farmer Group Dynamics

The age of a farmer will determine the work performance of the farmer, the older the age of a farmer, the absorptive capacity and understanding of new innovations will be difficult to accept. The results of the t test obtained the value of Sig 0.886 then this value indicates that the probability value t is greater than the value of the level of significance ($\alpha = 0.05$) so that the age variable does not significantly affect the dynamics of farmer groups.

Effect of Number of Family Dependents on Farmer Group Dynamics

The number of family dependents significantly affects the dynamics of the farmer groups in the Shortening Village because the value of Sig 0.018 is smaller than the level of significance value (α = 0.05). Basically the number of family dependents is one of the factors that can influence the motivation of farmers to be able to work harder or more diligently in managing their farms, because many or at least the number of family dependents will determine how much farmers should support. Farmers who have dependents who increasingly need to be able to make the right decisions, so that their farming can run well.

Effect of Experiences the Farmer Group Dynamics

In general, the activities and management of farming management are influenced by the experience of farming. Farmers in making decisions about the farms they manage are always considering the production risks that might be faced by farmers who are always based on the assumption that something new will have a positive or negative impact. Where each farmer has different abilities in accepting these risks, and these differences can be influenced by experience in doing farming. The results of the t test obtained a Sig 0.569 value greater than the value of the level of significance (α = 0.05) so that the experience of working variables did not significantly affect the dynamics of the farmer group.

Effect of Land Area on Farmer Group Dynamics

The land area is very supportive of farmers in improving their farm management so that it can improve the welfare of farmers, because the land area has a positive impact in improving their farming. Based on the results of the t test obtained the value of Sig 0.107 is greater than the level of significance value (α = 0.05) so that the land area variable does not significantly influence the dynamics of the farmer group.

Determination Coefficient Analysis (R2)

The results of the analysis of the coefficient of determination (R2) characteristics of farmers can be seen in the table below:

Model Summary				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	,479[a]	,019	-,073	7,27627

In general, the coefficient of determination (R2) can be interpreted as the ability of the independent variable to contribute to the fixed variable in one percentage. Based on the table above, it can be concluded that independent variables have an effect of 22.9% on the dynamics of farmer groups, while 77.1% are influenced by other variables not examined. Because R Square is smaller, it can be concluded that the ability of independent variables in explaining variable variations is very low.

Conclusions

Based on the results of the study it can be concluded that:

1. The dynamics of the farmer groups in the Pengkendekan Village, Sabbang Subdistrict are categorized as dynamic, where the total overall score is 6398 and the percentage of 74.05%. This shows that the average elements of the farmer group dynamics are going well. The dynamics of the farmer groups are shown by the interaction between members in the group is well established and the cooperation of the members in achieving group goals is very strong.
2. Partially from the results of the t-statistic test that the variable number of family dependents has a significant effect on group dynamics, while the variables of age, farming experience, and land area have no significant effect.
3. Based on the analysis of coefficient of determination (R2) that independent variables have an effect of 22.9% on the dynamics of farmer groups

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Combination of Sago Pulp and Fertilizer of Cow Manure on Nodule Root Effectiveness of Generative Phase Related to Peanut Yield on Acid Soil

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Abstract

The use of organic material as fertilizer for crop cultivation can restore soil fertility which has an impact on increasing plant growth and yield. Organic fertilizers for plants are not only sourced from livestock waste such as cow manure but also waste from sago plants can also be utilized. In this study trying to combine cow manure and sago pulp with different doses as fertilizer for peanut plants (*Arachishypogaea* L). The purpose of this study was to determine the exact effect and dosage of the administration of sago pulp and cow manure on generative phase root nodule formation in relation to the results of peanut (Arachishypogaea L.) on acid soil. This study was conducted from March to June 2018, in the experimental garden of the Faculty of Agriculture, University of CokroaminotoPalopo. This study uses a *Complete Randomized Block Design* (RAKL) consisting of 6 (six) treatments and 4 (four) replications. The treatment determined consisted of P0 (control), P1 (50 grams of cow manure + 250 grams of sago pulp), P2 (75 grams of cow manure + 225 grams of sago pulp), P3 (150 grams of cow manure + 150 grams of sago pulp), P4 (225 grams of cow manure + 75 grams of sago dregs), P5 (250 grams of cow manure + 50 grams of sago pulp). The results showed that the percentage increase in the number of active root nodules from the vegetative to generative phase reached an average of 50% for all treatments except controls. Application of 75 grams

of cow manure and 225 grams of sago pulp waste can significantly increase the number of active root nodules in the generative phase as well as the large number of pods, number of seeds per pod, fresh weight of planting pods and dry weight of pods per plant.

Keyword :*peanut, acid soil, cow manure, sago pulp, root nodule, yield*

Introduction

Peanuts are agribusiness commodities that have high economic value. Community needs for peanuts will certainly increase year by year along with increasing population. However, on the other hand the amount of peanut production over the last six years, namely 838,096 tons in 2006 (the highest production) to 709,063 tons in 2012. The land area of peanut crop also decreased from 706,753 ha in 2006 to 561,960 ha in the year 2012 (BPS, 2013). Meanwhile South Sulawesi province, in 2014 with an area of 24,459 Ha, experienced a decrease in land area in 2016, which was 20,302 Ha (BPS, 2017). This will certainly have an impact on the decline in production caused by the decreasing area of planting. In addition to the reduced area of planting land, other factors that can affect crop productivity are soil conditions.

Soil conditions in Indonesia generally react sourly, soils that react acidically are often the main cause of declining crop productivity. This condition generally occurs on dry land as a result of chemical changes, soil conditions with most nutrients becoming

less available for plants. Dry land is a acidic soil that can generally be found in various reliefs, ranging from lowlands to highlands in Indonesia (Prasetyo et al., 2006). Dry land in Indonesia is dominated by dry land reacting sourly and has undergone further weathering such as ultisol, oxisol, and inceptisol (Noor, 2006). Acidic soil has a pH ranging from 4.2 to 4.3 which is classified as very acidic soil (Sudaryono, 2009).

Peanut plants can grow well in conditions of soil pH between 5.0-6.3, in soils that are very acidic the efficiency of bacteria in binding N elements from the air will decrease, while in soils that are too alkaline, the elements of nutrients are less available (Suprapto, 1993). Plant growth in acid soils is affected by the availability of nutrients. The low availability of nutrients in the soil can cause low levels of soil fertility, this will be a limiting factor of crop yields (Tania et al., 2012). Infertile soil conditions will have an impact on growth and yields that are not optimal, so it is necessary to improve cultivation techniques in terms of fertilization so that nutrient availability can be used properly for plants. Nutrients that are essential for plant growth include phosphorus (P) and nitrogen (N) (Mehrvarz and Chaichi, 2008).

Nitrogen (N) element acquisition in legume crops such as peanuts, one of which is the contribution of nitrogen-fixing bacteria which is symbiotic with plant root nodules. Several studies related to the acquisition of nitrogen (N) elements for peanut plants are usually by inoculating nitrogen-fixing bacteria. The addition of

organic matter can spur the development of a population of nitrogen-fixing bacteria (BPN). This causes the amount of nitrogen tethered by bacteria to vary due to the ability of bacteria to compete with other microbes in the soil environment (Simanungkalit et al., 2006)

The main obstacle that is often encountered in acidic soils on wet climates is in addition to acidic soil reactions, also nutrient-poor, low organic matter content, high iron and aluminum content exceeding plant tolerance limits and sensitive to erosion so that the level of productivity is low (Hidayat et al. , 2000).Improved soil management that is needed to increase soil productivity during nutrient retention, especially the reaction of very acidic soil and very low base saturation, especially low P nutrient availability, and the danger of high aluminum poisoning, one of which is by adding soil organic matter (manure) to increase CEC land and nutrient availability N and P (Subardja, 2007).In an effort to improve soil conditions that have a low pH or classified as acid, can be done by adding organic matter both from livestock and plants. This study tried to combine cow manure and sago waste with different doses. The use of cow manure and sago waste is expected to improve soil conditions by providing organic matter to contribute to nutrient availability, especially phosphorus (P), potassium (K) and nitrogen (N) as essential macro nutrients so as to stimulate the effectiveness of root nodules and can improve plant growth and yield.

Matherials and Methods

This research was carried out in the Experimental Garden of the Faculty of Agriculture, CokroaminotoPalopo University, starting from March to June 2018. The materials used included peanut seeds, sago pulp and cow manure. Tools used are hoes, shovels, machetes, meters/rulers, labels, analytic scales, buckets, stationery and cameras. The study used a Randomized Block Design (RCBD) with 6 (six) treatments repeated 4 (four) times. So that in this study there were 24 experimental units. The treatment used in this study is as follows: P0: Control (without treatment), P1: cow manure 50 grams + sago pulp 250 grams, P2: cow manure 75 grams + sago pulp 225 grams, P3: 150 grams cow manure + sago pulp 150 grams, P4: cow manure 225 grams + sago pulp 75 grams, P5: cow manure 250 grams + sago pulp50 grams.Parameters observed in this study consisted of plant height (cm), number of root nodules in the vegetative and generative phases, number of pods per plant (pods), fresh weight of pods per plant (g), dry weight of pods per plant (g).

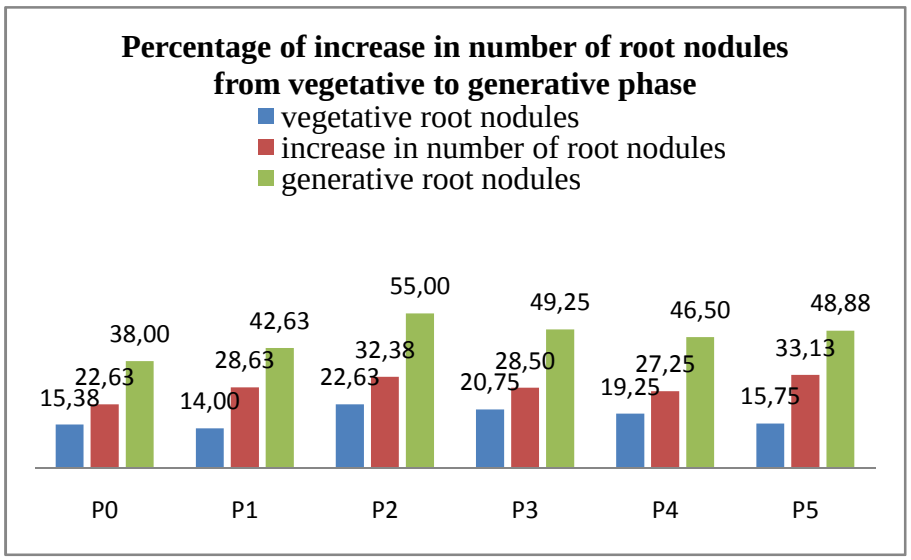
The research was carried out starting from land processing, making beds as high as ± 30 cm. Planting is done by making planting holes by dipping ± 3 cm deep, seeds planted as much as 2 grains in each planting hole with a spacing of 30 × 30 cm. Application of cow manure and sago pulp is done when the plants are 1 (one) week after planting (mst), the application is done with a

disk model by making a circular hole around the plant as deep as 2 cm. Plant maintenance includes watering done according to the needs of the plant and weeding is carried out if needed.

Data analysis was performed using analysis of variance (ANOVA) at the level of 5%. If the results of variance analysis show a significant difference between treatments, the analysis is continued by Duncan's multiple distance test (DMRT). The relationship between observation variables was determined by multiple linear regression correlation analysis.

Result

In the vegetative phase (25 days) showed the number of root nodules ranged from 14-22 root nodules and tended to differ among all treatments. Fertilization in the vegetative phase has not been able to increase the number of root nodules in the vegetative phase optimally, an increase occurs when the plant enters the generative phase (50 days). The percentage increase in the number of root nodules to the generative phase varies in each treatment.



Picture.Percentage of increasing number of root nodules from vegetative phase to generative peanut (Arachishypogaea L.) varTalam 1 on the combination of sago waste and cow manure.

The percentage increase in the number of root nodules from the vegetative to generative phase ranged from 50-60% of each number of root nodules in the vegetative phase of the plant applied to cow manure combined with sago pulp. In contrast to the control plants (without fertilizer application) which is lower with a percentage increase in the number of root nodules only 41% of the vegetative to generative phase, application of cow manure 250 grams + 50 grams of sago pulp (P5) has a percentage increase in the number of root nodules reaching 60 % with an average number of root nodules 48.88. However, when viewed from the number of root nodules in all treatments, application of 75 grams of cow manure + 225 grams of sago pulp (P2) had the most root nodules

compared to all treatments with an average of 55.00 with a percentage increase in the number of root nodules reaching 59%.

This shows that the administration of organic matter from cow manure and sago waste can increase the availability of nutrients in the soil and give effect to plant growth. In accordance with the opinion of Muliadi and Kartasaputro (1998) that cow manure contains macro nutrients such as N, P and K micro elements such as Mn, Fe and Zn. The usefulness of cow manure for the soil physically is to increase soil porosity, biologically increase the activity of organisms so that the process of organic matter changes faster in the soil. In accordance with the opinion of Lingga and Marsono (2003) that the addition of manure can improve soil chemical properties, it can improve physical properties and biological properties, so plants can grow well and can provide high production.

Table 1. The number of active root nodules in the vegetative and generative phases and the components of peanut yield (Arachishypogaea L) var. Talam 1 on the combination of sago waste and cow manure.

treatment	root nodules		Number of pods / plants	Number of seeds per pod /plants	Fresh weight of pods /plants (g)	Dry weight of pods/plants (g)
	vegetative	generative				
P0	15.38 a	38.0 b	27.1 b	1.5 c	39.5 b	28.81 b

		0	3	0	6	
P1	14.00 a	42.63 b	26.94 b	2.50 ab	63.13 ab	31.50 ab
P2	22.63 a	55.00 a	38.88 a	3.00 a	72.81 a	47.63 a
P3	20.75 a	49.25 b	21.19 b	3.00 a	56.25 ab	33.31 ab
P4	19.25 a	46.50 b	35.06 b	2.75 ab	66.56 a	36.56 ab
P5	15.75 a	48.88 b	28.88 b	1.75 c	54.38 ab	32.69 ab
mean	17.96	46.71	31.68	2.41	58.78	35.08
cv	33.50	17.32	22.37	26.17	24.89	28.61

Values followed by the same letters show no significant difference in Duncan's Multiple Distance Test (DMRT) at 5% real level.P0: Control (without treatment), P1: cow manure 50 grams + sago pulp 250 grams, P2: cow manure 75 grams + sago pulp 225 grams, P3: 150 grams cow manure + sago pulp 150 grams, P4: cow manure 225 grams + sago pulp 75 grams, P5: cow manure 250 grams + sago pulp 50 grams.

The results of analysis of variance showed that the administration of cow manure combined with sago pulp had no significant effect on plant root nodule formation in the vegetative

phase among all treatments (table 1). Fertilizers given only have a significant effect when the plant enters the generative growth phase. Administration of 75 grams of cow manure + 225 grams of sago pulp (P2) can increase the number of active root nodules that are higher in the generative phase than other treatments, significantly different from control plants (without fertilizer). This is also seen in the low number of pods, the number of seeds per pod, the fresh weight of pods and the dry weight of pods in control plants. This situation is thought to be closely related to the role of nutrient content in fertilizers, essential macro nutrients such as phosphorus (P), potassium (K) and nitrogen N which are available in appropriate doses can provide a good influence on plant growth.

Phosphorus and nitrogen will simultaneously affect plant growth in the formation of new cells in the meristematic tissue of plants, so that it can help the process of growth and development of plants (Tania et al., 2012).Fertilization is one effort that can be taken in maximizing crop yields. According to Wijaya (2008), fertilization is carried out as an effort to meet the needs of plant nutrients so that production objectives can be achieved. However, if the use of fertilizer that is not wise or excessive can cause problems for cultivated plants, such as poisoning, susceptibility to pests and diseases, low production quality and in addition high production costs and can cause pollution. Although the contribution of nutrients to organic matter is low, but organic matter and its

interaction can increase the uptake of P and K nutrients in plants (Ismon, 2006).

Table 2. Correlation of the effectiveness of root nodules on the components of peanut yield (Arachishypogaea L.) var. Talam 1 on the combination of sago waste and cow manure

r	BAv	BAg	JP	JBp	BSp	BKp
BAv	1					
BAg	*0.54**	*1*				
JP	0.34**	0.69*	1			
JBp	0.16**	0.10**	-0.08**	1		
BSp	0.33**	0.32**	0.58**	0.02**	1	
BKp	0.26**	0.55**	0.92ns	0.04**	0.53**	1

BAv: vegetative phase active root nodules, BAg: generative phase active root nodules, JP: number of pods, JBp: number of seeds per pod,BSp:Fresh weight of pods /plants, BKp:Dry weight of pods,): significantly different, **): very real difference, ns: (no significant / not significantly different)*

The results of the correlation analysis between active root nodule formation in the generative phase with several yield components including number of pods, number of seeds per pod, fresh pod weight and dry weight of pods showed a positive correlation with correlation values respectively (r = 0.05 *), (r = 0.01 **), (r = 0.01 **) ,, and (r = 0.01 **). This shows that the number of root nodules that are active until the generative phase can increase the yield components, namely the number of pods, the

number of seeds per pod, the fresh weight of pods and the dry weight of pods. Against the root nodule variable, interaction or significant relationship with the components of the production variable. The presence of active root nodules until the generative phase can supply the N plant needs during the generative phase. Nitrogen (N2) from the air fixed by root nodules can accumulate in the leaves for photosynthesis needs and translocated to the generative organs of plants to form pods, replenishment and maturation of seeds. Generative phase is a growth phase where the plant maximally uses more food or photosynthesis for the formation of reproductive organs (Amir *et al*, 2017)

Conclusions

Based on the research that has been done by looking at the results of how the components are observed, it can be concluded that the administration of organic matter as fertilizer with a combination of cow manure and sago waste can have a good influence on the number of root nodules from the vegetative phase to the generative phase compared with no fertilizer.Application of 75 grams of cow manure + 225 grams of sago pulp (P2) can increase the number of active root nodules that are higher in the generative phase than other treatments, significantly different from control plants (without fertilizer).

The increase in the number of active root nodules in the generative phase was positively correlated to the number of pods,

the number of seeds per pod, the fresh weight of pods and the dry weight of pods by providing organic matter for cow manure and sago waste on acid soils.

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OPTIMIZATION OF LAND USE AS WELL AS POC APPLICATIONS IN EMBANKMENT IMPROVEMENT OF GROWTH AND PRODUCTION OF RICE (*Oryza Sativa L.*) IN BONE

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Abstract

Special efforts increased the production of food crops that currently is encouraged, seems to be faced with some pretty difficult obstacles to be resolved. The decrease in the production of foodstuffs the perceived national currently more caused by the more extensive the narrowness of a productive agricultural land, as a result of control of functions such as conversion of paddy fields into non agricultural land. This research aims to minimize these problems is by the application of technology of cultivation by combining between agriculture and perikana in order to optimize the use of land for agriculture is increasingly limited, as well as utilization of liquid organic fertilizer in increasing growth and production of rice plant. This research was carried out in the village of Awangcenrana Sub-district Cenrana Bone Regency, which lasted from April until July 2018. This research was compiled by using the design of swath of Perpisah (RPT). The main plot was

farmed the land use with 2 treatments, namely: land of ponds to a depth of 120 cm (K1), farmed Land with into 100 cm (K2). Children hide is fertilizing with 4 treatments, namely: chemical fertilizers (control) (p0), a combination of Poc seaweed with cow urine Poc (p1), cow urine POC (p2), Poc seaweed (p3). The results showed that the combination of land farmed by into the 120 cm and the granting of POC from waste seaweed and cow urine shows the best response because it is able to produce production (7.9 tons/ha) from the ability of potential results (8.2 tons/ha). Farmed land use/UPS and downs to watch out for is water management. Water management can be defined by using the use of water appropriately to increase land productivity and pertanaman index

Key words: *optimization, land, Poc, production, rice*

Background

Food is one of the basic human needs including fishery products. Various actions have been undertaken to help the food needs of the population. The effort that needs to be done not only increased production, but also retain production have been achieved through improvements in production efficiency by conserving natural resources and the environment. The main purpose of food policy is to create and maintain a national food security system that is resilient and sustainable agricultural sector and through the fishery.

The increase of population from year to year increases that result in the need for food is also increasing on the other hand, the conversion of agricultural land to non-increasing agriculture. This

is compounded by widespread ownership of land farmers an average of 0.5 hectares under cultivation and systems that rely on one type of farming on the same land (monoculture). Extents and the monoculture pertanaman system is clearly difficult lifting the welfare of farmers and will just dwell on how to meet the needs of farmers living in limited (subsystem).

The various obstacles faced in creating and maintaining national food security i.e. a decrease in the rate of growth and productivity, there is a transfer of function of agricultural land into non agricultural land, as well as land use that has not been optimal. To address the matter needs to be done through the utilization of the existing land intensification. One way out to overcome the narrowness of getting tenure at once can increase revenue was by way of a reverse engineer the land with the appropriate technology (Supriadiputra and Setiawan, 1994). One of the ways that can be done is with Integrated Fish Farming System i.e. a mix of agriculture and fisheries, such as business system mina rice monoculture farming system is a change towards agricultural diversification.

Special efforts increased the production of food crops that currently is encouraged, seems to be faced with some pretty difficult obstacles to be resolved. The decrease in the production of foodstuffs the perceived national currently more caused by the more extensive the narrowness of a productive agricultural land, as a result of control of functions such as conversion of paddy fields

into non agricultural land. This is compounded by the existence of global issues about the increasing land degradation. One option that is expected to improve crop production potential in order to meet food needs is the utilization of wetlands

In addition to the land over the function because, internally, the cultivation of crops in the paddy fields are also faced with declining soil quality the more factors. It can be seen from the more destruction of the nature of the physical, chemical, and biological soil. One contributing factor is the large number of chemical fertilizer inputs dilahan rice fields without an offset racer, organic ingredients. Almost every time the harvest, whole plant biomass transported/moved from paddy fields.

According to Notohadiparwiro (1989), the use of chemical fertilizers with a high concentration and disproportionately on the wetland impact on nutrient status of the soil penimpangan. Meanwhile, another impact caused is eradicating the content of soil organic matter. Decrease in production of paddy fields resulted in decreased production of food crops. On the necessity of food continue to experience increased along with the increased number of people always tend to increase every year.

The use of organic fertilizer that can be used to help overcome the obstacles of agricultural production that is a liquid organic fertilizer. This organic fertilizer made from raw materials in the form of cattle dung, compost, natural, hormone waste plants and other natural ingredients that are processed naturally. Liquid

organic fertilizer in addition can fix the nature of the physical, chemical, and biological soil can also help improve crop production, improve the quality of plant products, reduce the use of inorganic fertilizers.

Materials and Methods

This research will be done in the village Awangcenrana, district Cenrana, Bone Regency. April to July 2018. Research related to the analysis of nutrient content of the waste and seaweed cow urine is done in the laboratory of BPTP Hall Ground Maros, Maros. This research was compiled by using the design of swath of Perpisah (RPT). The main plot was farmed the land use with 2 treatments, namely: land of ponds to a depth of 120 cm (K1), farmed Land with into 100 cm (K2). Children hide is fertilizing with 4 treatments, namely: chemical fertilizers (control) (P0), a combination of Poc seaweed with cow urine Poc (p1), cow urine POC (p2), and Poc seaweed (p3). There are 8 combination treatment repeated 3times by watching the 4 samples of plants per treatment plots. So there are 192 plants that will be observed. The seeds are seeds of rice inpari agritan 79 unsoed. Planting is done simultaneously with the use of the system moved to the planting, seeds are used was ± 12 days with the planting distance 20 cm x 20 cm by using two seeds per hole for planting.

Deployment of liquid organic fertilizer plant was carried out after 2 pm Mst with interval deployment once every 14 days. Thus,

the deployment of liquid organic fertilizer is done 5 times until the rice grain filling phases by means of menyeprotkan liquid fertilizer to plant leaves in the morning when the stomata of the leaves open with dilution of 1:10 fertilizer, organic fertilizer 1 L, 10 L water and spray volume of 10cc/ plant.

Observation of component organizations include: plant Height (cm), the height is calculated based on a sample of the plant. At the age of 25 in the HST, HST, HST 55 40. The number of chicks, counted the number of saplings on 7 sample perpetak plant clumps where done simultaneously observation of higher plants. Fresh extinct weights (g), whereas the heading plants in fresh State made on time after the harvest is done. Fresh root weight (g), weigh the plant roots in the fresh State is done after the harvest. Root length (cm), measure from the base up to the tip of the root on the measure before doing the harvest. Rice production (ton/ha GKP).

Results

Table 1. Average plant height (cm) rice at a water depth of treatment With various types of granting POC.

Water Depth	**Type of poc**			
	p0	**p1**	**p2**	**p3**
k1	83.33	89.14	83.38	82.99
k2	83.57	84.11	82.21	81.99
Average	83.45^{b}	86.62^{a}	83.24^{b}	82.49^{b}

Description: the figures who still followed the same letter on the line (abc) means no different with real on BNTα test = 0.05 with NP BNT i.e. 3.00.

Table 1 shows that the treatment of this type of Poc p1 (a combination of cow urine Poc with Poc seaweed) with an average of 86.62 cm high-the highest plant response and different types of treatment with other Poc.

Table 2. Average number of chicks and chicks productive (saplings. plant-1) rice crops in a water depth of treatment with different types of granting Poc.

Water	**Type of poc**			
Depth	**p0**	**p1**	**p2**	**p3**
The number of saplings of produktive				
k1	20.63	24.37	23.80	24.40
k2	21.00	22.37	21.97	21.97
Rata-rata	20.82[b]	23.37[a]	22.88[a]	23.18[a]
NP BNT$_m$				1.65
The number of saplings of produktive				
k1	16.53	21.10	20.47	20.73
k2	17.30	19.17	18.67	18.73
An average	16.92[b]	20.13[a]	19.57[a]	19.73[a]
NP BNT$_m$				1.82

Description: the figures who still followed the same letter on the line (abc) means no different with real on BNTα test = 0.05

Table 2 shows that the number of observations of the child type of Poc p1 (Poc urine Combination beef with Poc seaweed) with average 23.37 saplings. plant-1, p2 (cow urine) with an average of 22.88 saplings. plants-1dan m3 (Poc seaweed) with average 23.18 of plantlets. plants-1memberikan the most number of saplings and response not unlike real with p0 (control without treatment of the Poc). Observation of the number of saplings of

productive show that type of POC p1 (Poc urine combination beef with seaweed) with an average of 20.13 saplings. plant-1, p2 (cow urine) that is by average chicks 19.57. plant-1, p3 (Poc seaweed) with average 19.73 saplings. plant-1. give the number of the most prolific and saplings differ markedly with p0 (control without treatment of the Poc).

Table 3. The average weight of extinct fresh and root (g plant-1) rice crops in a water depth of treatment with different types of granting POC..

Water depth	**Type of poc**				**NP BNT$_s$**
	p0	**p1**	**p2**	**p3**	
Fresh weigth of extinct					
k1	$103.27^{\bar{d}}_{\underline{x}}$	$142.78^{\bar{c}}_{x}$	$183.33^{\bar{a}}_{\underline{x}}$	$162.22^{\bar{b}}_{\underline{x}}$	75.18
k2	$139.58^{\tilde{b}}_{\underline{x}}$	142.22^{b}_{x}	$170.56^{\underline{a}}_{\underline{x}}$	$144.44^{\tilde{b}}_{\underline{x}}$	
NP BNT$_m$				20.72	
Fresh root weight					
k1	$28.57^{\bar{b}}_{\underline{x}}$	$28.33^{\bar{b}}_{\underline{x}}$	$37.22^{\bar{a}}_{\underline{x}}$	$35.56^{\bar{a}}_{x}$	12.22
k2	$22.93^{\tilde{d}}_{\underline{x}}$	$34.44^{\tilde{b}c}_{x}$	$42.78^{\underline{a}}_{\underline{x}}$	35.00^{bc}_{x}	
Rata-rata				6.43	
NP BNT$_m$			5.14		

Description: the figures who still followed the same letter in a column (xyz) and line (abc) means no different with real on BNTα test = 0.05

Table 3 shows that in fresh weight of extinct observation treatment interaction between treatment water depth k1 (120 cm) with a type of POC p2 (cow urine) and between water depth of k2 (100 cm) and the type of Poc p2 (cow urine) and provide fresh weight of extinct response the heaviest and different interactions with other treatments. Fresh root weight treatment observation of interaction between water depth treatment of k1 (120 cm) with a type of Poc p2 (cow urine), p3 (seaweed), and between water depth of k2 (100 cm) and the type of Poc p2 (cow urine) gives fresh weight of the heaviest extinct response and does not real interaction with different treatment between water depth k1 (120 cm) with a water depth of k2 (100 cm).

Table 4 shows that the dry weight of observations on the extinct type of POC p1 (a combination of cow urine Poc with seaweed) with an average of 43.84 g. plant-1dan p2 (Poc cow urine) with an average of 43.30 g. plant-1 dry weight response extinct the heaviest and not unlike real with p0 (control without treatment Poc) and p3 (Poc seaweed). Dried root weights observations indicate the type of Poc p1 (a combination of cow urine Poc with seaweed) with an average of 8.52 g plant-1 dry weight response gives the heaviest roots and different real with other treatments.

Table 4. Average dry weight and root-extinct (e.g. plant-1) rice crops in a water depth of treatment with different types of granting POC.

Water depth	Type of poc			
	p0	**p1**	**p2**	**p3**
Bobot Pupus				
k1	42.10	47.49	45.86	37.63
k2	42.20	40.20	38.74	40.72
Average	42.15^{ab}	43.84^{a}	42.30^{a}	39.18^{abc}
NP BNTm				5.80
Bobot Akar				
k1	7.37	8.24	6.06	7.32
k2	7.14	8.80	7.46	7.09
Rata-rata	7.25^{b}	8.52^{a}	6.76^{bc}	7.21^{b}
NP BNTm				1.00

Description: the figures who still followed the same letter in a column (xyz) and line (abc) means no different with real on BNTα test = 0.05

Table 5. The average root length (cm) rice at a water depth of treatment with different types of granting POC.

Water depth	Type of poc			
	p0	**p1**	**p2**	**p3**
k1	21.00	24.94	24.11	25.37
k2	19.67	24.63	24.79	25.24
Rata-rata	20.33^{b}	24.79^{a}	24.45^{a}	25.31^{a}

Description: the figures who still followed the same letter on the line (abc) means no different with real on BNTα test = 0.05 with NP BNT i.e. 2.68.

Table 5 shows that the treatment of this type of POC m1 (a combination of cow urine Poc with Poc seaweed) with an average of 24.79 cm, m2 (urinsapi) with an average of 24.45 cm, m3 (Poc

seaweed) with average 25.31 cm, length response root the longest and different real with m0 (control without treatment of the Poc).

T Abel 6. The average weight of 1000 seeds (g) and the production of tons. ha-1 rice crops in cropping system of treatment with different types of granting POC.

Kedalaman Air	**JenisMol**				**NP BNT_s**
	p0	**p1**	**p2**	**p3**	
Bobot 1000 biji					
k1	25.17	28.93	27.70	27.60	11.18
k2	22.33	27.77	26.73	26.30	
NP BNT_m				1.38	
Produksi					
k1	7.33	10.45	9.93	8.77	
k2	6.73	9.69	9.62	8.55	
Rata-rata	7.03^{c}	10.07^{a}	9.78^{a}	8.66^{b}	
NP BNT_m				0.68	

Keterangan : Angka-angka yang masihdiikutihuruf yang sama pada baris$^{(abc)}$ berarti tidak berbeda nyata dengan pada uji $BNT_{\alpha=0.05}$

Table 6 shows that the weight of 1000 seeds observation on treatment of interactions between water depth k1 (120 cm) with a type of Poc p1 (a combination of cow urine Poc with Poc seaweed), p2 (Poc cow urine) and p3 (Poc seaweed) and between water depth of k2 (100 cm) with type of Poc p1 gives heaviest weight of 1000 seeds response and do not differ markedly with the interaction between the water depth of k2 (100 cm) and the type of Poc p2 (cow urine). Observation of production shows the type of POC p1 (a combination of cow urine Poc with Poc seaweed) with an

average of 7.9 tonnes. HA-1 and p2 (cow urine) with an average of 6.9 tons. HA-1 gives the highest production and response differs markedly with other treatments.

Discussions

The results of treatment with a combination of organic liquid seaweed fertilizer and cow urine (p1) paint the best response in the plant, it is allegedly due to the womb of the POC nutrient complement each other. For example cow urine from POC analysis results contain N 0.27%, P K 0.18%, 0.16%, organic-C 0.02% and the pH of 5.13. And the results of the analysis of the POC seaweed i.e. N 0.335, P 0.04% 0.50%, K, organic-C 0.02% and 3.33% pH. With the combination of the elements of content then the second of the POC nutrient increasingly increases, this is causing such a combination gives the best response of all treatment parameters.

Administering liquid seaweed fertilizer also needs to be done because of the granting of this organic fertilizer is more considering its effects on chemical properties because it has a very important role to prevent damage to the iron and aluminum on the lands of acting sour and can increase the availability of phosphate in the soil, increased levels of humus in the soil will improve the cation exchange capacity (CEC). This is in accordance with the literature Damanik et al (2010) stating that the granting of organic fertilizer is not aiming to increase nutrient elements, because the content of haranya is low, but if in terms of its effects on the

chemical properties of the soil, the organic fertilizer have an important role such as increased levels of humus in the soil will improve the cation exchange capacity (CEC), increasing the availability of phosphate in the soil and can prevent iron poisoning and aluminum on the lands of acting sour.

Farmed land use/tidal has great prospects in terms of the potential for broad or cruising espouses agronomist to serve as the area of production of rice. Indonesia's vast tidal area around 20.1 million hectares, is estimated at more than 9 million hectares of potential agricultural production acreage to be especially rice cultivation.

The characteristics of the land that became an issue in the development of farming in tidal lands include: fluctuation of water regime, various soil-chemical fisiko condition, the high kemasaman of soil and organic acids on the peat, the presence of toxic substances, water intrusion salt, natural soil fertility and low. Specifically for the farm include: sour kemasaman sulfate soil and water is very high; content of aluminum (Al), iron (Fe) and hydrogen sulfide (H2S) high; and the availability of nutrient elements P and K was especially low. As for peat include: soil and water kemasaman high, the availability of macro and micro nutrient especially P, K, Zn, Cu and Bo low, and very low ground power (Alihamsyah and Widjaya. 1998; Dakhyar, 2012). Meanwhile, according to Noor and Saragih (1993), the problem of tidal land is

characterized by a high level of kemasamannya (low pH), kahat hara N, P, and K to peat/bergambut kahat hara Cu and Zn. Solubility of Al, Fe, Mn and high SO4, so often result in the plant suffered a poisoning, structured base cations is low, and there is a layer of pyrites which when oxidized can increase the soil pH to kemasaman 2-3. The danger of a layer of pyrite commonly found due to its difficult grammar set.

The giving of liquid organic fertilizer from seaweed and cow urine can improve the physical or chemical properties of the soil, the land on which the research on tidal land generally have high levels of kemasaman soil or pH is low, the condition can affect nutrient availability especially phosphates. In addition, there are tidal land toxic substances for plants include iron (Fe2 +), aluminium (Al3 +), sulfate (SO4 3-), hydrogen sulfide (H2S), and sodium or salt water. Iron toxicity typically occurs in plants ferro rice cultivated in sulfate wry. Levels of pyrite (FeS2) above 200 ppm will poison plants, this can happen due to the oxidation of pyrite by various reasons, such as: land management, digging too deep channels, or exposed to drought-cekaman. While the aluminum poisoning usually occurs on dry soil conditions and accompanied by kaosfhat P, because P is tied into the insoluble aluminum phosphate (Ar-Riza, et al, 2005).

The combination of liquid seaweed organic fertilizer and cow urine is a good influence for the relation mutually supporting

synergies. This is in accordance with the opinion of Samosir (1994) stating that the addition of nitrogen can lower the value of the ratio C/N organic matter so quickly melapuk (breaks down). More (Gupta and Singh, 1981: Broder and Wagnes a.c.), stating that the organic materials that have a high c/n Ratio. The sooner organic materials undergo decomposition, then the faster anyway the main nutrient elements supply of N, P and K for the plant. Organic fertilizer providing nitrogen, phosphorus and sulfur to ppertumbuhan crops and improve soil structure.

The role of this fertilizer stimulates growth and development of plants, plants more resistant to stress, pests and diseases, increase yields and improve the quality of the crops (Verheyen, 2008). In addition the giving of liquid organic fertilizer to the plants that are applied by means of flushing to the ground and the leaves of the plant are also very helpful in the process of its growth. This is because either the macro or the micro nutrients required by plants directly can be absorbed and utilized by plants. Micro-nutrient is a nutrient that is usually only a few available in the soil and frequent competition with other plants or weeds to subjugate them. Then applied directly to the soil and the leaves will be very helpful in doing the plant growth.

Land use the tidal ponds/herus note is water management. Water management can be defined by using the use of water appropriately to increase land productivity and pertanaman index.

Water management in tidal land have significance because when there is an excess of water in the rice fields so it can be immediately removed and if the lack of water in the rice field will then be immediately added this way, plants will be awake from well water needs in the wet or dry.

Conclusions

Based on the results of research that has been carried out, then a conclusion can be drawn as follows:

1. Grant of liquid organic fertilizer from seaweed and cow urine can improve the physical or chemical properties of the soil, the land on which the research on tidal land generally have high levels of kemasaman soil or pH is low, the condition can affect nutrient availability especially phosphate
2. Kombination Treatment with seaweed Poc Poc cow urine (p1) with pond water depth 120 (k1) provides the best response, and delivers an average of 7.9 tonnes/ha of potential capability results (8.2 tons/ha).

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THE FACTORS INFLUENCING CONSUMERS' BEHAVIORS TOWARDS THEIR DECISION OF PURCHASING SAGOO-PROCESSED-PRODUCTS (KAPURUNG) IN THE TOWN OF PALOPO

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ABSTRAK

Consumer behavior was the things that underlie the consumer to make an informed decision in the purchase of goods and service also to meet his needs. This study focused on the behavior of consumers who consume sagoo-processed-products namely meal kapurung in the town of Palopo. The aims of the research to know The Factors Influencing Consumers' Behaviors Towards Their Decision Of Purchasing Sagoo-Processed-Products (Kapurung) In The Town Of Palopo. This research had for 2 months in February-March in the town of Palopo. As many as 30 people of the consumer respondents were interviewed. Purposive sampling was used as sampling method. The data were analyzed by using SPSS statistics data processing program. The results showed the factors influencing consumers' behaviors towards their decision of purchasing sagoo-processed-products (kapurung) i.e. cultural, social, personal, psychology has the positive influence of consumers in buying processed products sago (kapurung) in the town of Palopo, based on statistic results F > count table (7.995 > 2.76) and significant value 0.000 < 0.05.

Keywords: consumer, consumer behavior, purchasing decisions

Background

One of the most growing subsector in Palopo namely food crop subsector. Commodities included in subsector commodity food crops is rice and sago. Palopo is one of the cities with a level query sago are quite high. Demand for sago in Palopo is dominated by the processing industry which utilizes sago as the raw material in the manufacture of its products. One of the industry's many scattered in Palopo that utilizes the Sago in the manufacturing process that is processing industry of sago into be kapurung. Processing sago be kapurung not without reason, the communities surrounding Palopo has great interest in consuming kapurung.

Kapurung consumption of great interest so many established restaurants, namely Kapurung Mandiri, Lesehan Lela, Al Baroka, Kapurung Selatan, Penyingkul, N2 and Canteen of Arita. Consumers are satisfied not only back to buy but also spoke positively of product processed kapurung to others, as well as with bad service becomes one of the causes of the negative effects for the processing industry.

The consumer is one of the important factors in the success of a business. Consumer behavior is the important thing that should be noted by businessmen eating kapurung, by observing the behavior of consumers who come to buy home dining kapurung dining kapurung home will make the run can be survive and thrive. Based on explanation above, the authors are interested in doing research on "The Factors Influencing Consumers' Behaviors Towards Their Decision Of Purchasing Sagoo-Processed-Products (Kapurung) In The Town Of Palopo ".

Research Methods

The research is descriptive quantitative which this research focus towards the problems that existed at the time doing research or issues which are actual and describes the problem facts

investigated as is accompanied by an interpretation of the facts. Research carried out for 2 months in palopo.

The samples were taken in 5 restaurants kapurung that exist in the Palopo i.e. Kapurung Mandiri, Lesehan Lela, Al Baroka, Kapurung Selatan dan Lesehan Alisya. The sample is coming to buy and consume the product kapurung in the fifth House to eat. The sample of respondents is determined by the sampling technique is the technique whereby the determination of incidental samples based on coincidence, i.e., anyone who by chance or incidental meeting with researchers and considered appropriate criteria sample. The sample of respondents is taken as 6 people in the fifth House to eat, so that the total number of sampled respondents amounted to 30 people.

Linear regression analysis was used to study how the influence of the free variables, namely culture (X 1), social (X 2), personal (X 3), and Psychology (X 4) against the decision of purchase (Y).

Results

Factors consumer behaviour is one of the things that is very influential in making purchasing decisions by consumers. the factors influencing consumers' behaviors towards their decision of purchasing sagoo-processed-products (kapurung) in the town of Palopo which include cultural factors, social factors, personal factors and psychological factors. These research results are outlined as follows.

1. **The Responses of The Respondents Regarding The Factors that Affect The Behavior of Consumers Against Purchasing Decisions**

Table 1. The average Responses of respondents Regarding the factors PerilakuKonsumen

NO	Faktor-faktor Perilaku Konsumen	ALTERNATIF JAWABAN 5 SS	4 S	3 TS	2 STS
1.	cultural factors	44%	41,3 %	12%	4,6%
2.	social factors	20%	52%	19,3 %	8%
3.	Personal factors	22,6 %	42%	21,3 %	14%
4.	psychological factors	44,4	48,8 %	4,4%	2,2

Description: SS=Sangat Setuju/very agree; S=Setuju/Agree; TS==Tidak Setuju/Disagree; STS=Sangat Tidak Setuju/strongly disagree

Based on the table of the average responses respondents note that factors consumer behaviour is one of the things that influence on purchasing decisions of consumers against products of refined sago (kapurung) in the town of Palopo.

2. Data Analysis Factors Consumer Behaviour

a. Test Multikolinieritas

Multikoliniaritas test is carried out to find out the correlation between independent variables used in the study. To test for multicollinearity will use number Variance Inflation Factor (VIF) and tolerence. A

regression model would be free of multikolinieritas when the value of the VIF is smaller than 10 and have numbers greater than 0.10 tolerence. The result of the test of multikolinieritas can be seen in the table below.

Table 2. Results of Tes Multikolinieritas Factors Consumer Behaviour

Coefficients[a]					
Model		Unstandardized Coefficients		Collinearity Statistics	
		B	Std. Error	Tolerance	VIF
1	(Constant)	6.128	3.370		
	cultural factors	-.554	.195	.553	1.808
	social factors	.631	.175	.608	1.645
	Personal factors	.144	.136	.893	1.120
	psychological factors	1.048	.253	.951	1.051
a. Dependent Variable: purchasing decisions					

Multikolinieritas test results above, note that the value of the tolerance of each factor consumer behaviour greater than 0.10 and the great value of the VIF factor for each consumer behavior under 10.00 so that it can be concluded from not happening multikolinieritas against factors consumer behaviour so that the test results are said to be trusted or reliability.

b. Multiple Linear Regression

Multiple linear regression test results can be seen in the following table

.

Table 3. Result of Multiple Linear Regression Test of Factors Consumer Behaviour

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	T	Sig.
		B	Std. Error	Beta		
1	(Constant)	-.463	.587		-.788	.438
	Budaya	.276	.034	.425	8.130	.000
	Sosial	.230	.030	.377	7.564	.000
	Pribadi	.240	.024	.416	10.108	.000
	Psikologi	.262	.044	.237	5.958	.000

a. Dependent Variable: purchasing decisions

Model equation regression coefficient obtained from the constant and variable coefficients that are in a column of Unstandardized Coefficients B. Based on the above table of regression equations of the model is obtained as follows:

Y = -0.463 + 0,276 (X1) + 0,230 (X2) + 0,240 (X3) + 0.262 (X4)

Regression equations above can be described as follows:

1) b0 =-0463 suggests that cultural factors, social, personal and psychological influence the purchasing decisions of 46.3% this shows that influence consumer behavior factors effect negatively to the decision of purchasing sagoo-processed-products (kapurung) in the town of Palopo
2) b1 = coefficient variable culture amounted to 0.276 it showed positive figures so it can be inferred that the influential culture positive factors against the decision of purchasing sagoo-processed-products (kapurung) in the town of Palopo. If cultural factors have elevated

the then it will be followed by an increase in the purchasing decisions of 27.6%.

3) b2 = coefficient of social variables of 0.230 it showed positive figures approaching 1 so that it can be concluded that the positive effect of social factors towards the decision of purchasing sagoo-processed-products (kapurung) in the town of Palopo. If the social factor increased one unit so it can be followed by an increase in processed products purchase decision sago (kapurung) of 23%.
4) b3 = coefficient of private variables of 0.240 it showed positive figures approaching 1 so that it can be concluded that the positive effect of private product purchases of refined sago (kapurung) in the town of Palopo. If the personal factor increased one unit so it can be followed by an increase in the decision of purchasing sagoo-processed-products (kapurung) in the town of Palopo of 24%.
5) b4 = coefficient variable psychology of 0.262 it showed positive figures up to 1 so that it can be concluded that the positive effect of Psychology factor against processed products purchase decision sago (kapurung) in the town of Palopo. If psychology factor increased one unit so it can be followed by an increase in the decision of purchasing sagoo-processed-products (kapurung) in the town of Palopo amounted to 26.2%.

c. Analysis Of The Coefficient Of Determination (R^2)

The results of the analysis of the coefficient of determination (R^2) factors consumer behaviour can be seen in the table below.

Table 4. The Coefficient of Determination of FactorsConsumer Behaviour

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.981[a]	.962	.956	.298

a. Predictors: (Constant), cultural, social, personal and psychological factors.

1) From the results of the analysis of the processing of data between consumer behavior factors (cultural, social, personal and psychological) against the decision of purchasing sagoo-processed-products (kapurung) in the town of Palopo indicates that the magnitude of the value of R = 0.981. That is, the correlation of factors consumer behaviour (cultural, social, personal and psychological) of the processed products purchase sago (kapurung) has a very tight relationship and positive because the value of the coefficient of correlation is approaching + 1 and influence given the independent variables (X) against varieabel the dependent (Y) shown by the R square (R^2) in the table of 0.962.
6) Based on the above description can be summed up the relationship of consumer behavior factors (cultural, social, personal and psychological) shows the magnitude of the value R^2 i.e 0.962 or 96.2% and has a relationship very closely and positively against the decision of purchasing sagoo-processed-products (kapurung) in the town of Palopo, while the rest amounted to 3.8% dipengruhi by the other factors are not examined.

d. F test

In this study, the F-test is used to find out which significantly influence shopper behaviour factors (cultural, social, personal, psychology). The F-test is done by comparing the calculated F with F table. Test result F factors consumer behaviour can be seen in the table below.

Table 5. Test F Factors of Consumer Behavior

ANOVA[a]						
Model		Sum of Squares	Df	Mean Square	F	Sig.
1	Regression	56.579	4	14.145	159.187	.000[a]
	Residual	2.221	25	.089		
	Total	58.800	29			

a. Dependent Variable: purchasing decisions
b. Predictors: (Constant), cultural, social, personal and psychological factors.

1) Based on the results of a regression from the table above shows that the count of 159,187 F or F-value table 159.18 obtained was 2.76. Thus F F>count table (159.18>2.76) and significant value 0.000 < 0.05. Based on the above description it can be concluded that the free variables (cultural, social, personal and psychological) simultaneously positive effect against the decision of purchasing sagoo-processed-products (kapurung) in the town of Palopo.

e. T Test

T test known as the partial test, i.e., to examine how the influence of each free variable in singly against variables bound. This test can be done by comparing the t count with the t table. Test results q factors consumer behaviour can be seen in the table below.

Table 6. Test T Factors of Consumer Behaviour

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	T	Sig.
		B	Std. Error	Beta		
1	(Constant)	-.463	.587		-.788	.438
	Budaya	.276	.034	.425	8.130	.000
	Sosial	.230	.030	.377	7.564	.000
	Pribadi	.240	.024	.416	10.108	.000
	Psikologi	.262	.044	.237	5.958	.000

a. Dependent Variable: Purcashing Decisions

Based on the above table it can be seen the t test results of each factor in consumer behavior from the table it can be seen that the value t calculate each factor consumer behaviour (cultural, social, personal and psychological) larger comparison with value t tables namely 1.701 with significant value smaller than 0.05 so that it can be inferred that the consumer behavior factors (cultural, social, personal and psychological) each influential positively and significantly to decision of purchasing sago-processed-products (kapurung) in the town of Palopo.

Conclusions

Based on the results of the analysis and discussion of which has been described previously, then the conclusion can be drawn from the overall results of the study, the factors influencing consumers' behaviors towards their decision of purchasing sagoo-processed-products (kapurung) (cultural, social, personal, psychology) has the positive influence of consumers in buying sagoo-processed-products (kapurung) in the town of Palopo. Based on statistic results F > count table (7.995 > 2.76) and significant value 0.000 < 0.05.

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QUALITY OF EGG LOHMANN BROWN GAVES RATION FLOUR SKIN DRAGON FRUIT *(Hylocereus polyrhizus)* FERMENTATION

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Abstract

The objective of this study was to study the quality of egg Lohmann Brown received a fermented dragon fruit meal ration *(Hylocereus polyrhizus)* for weeks. The design used was *Completely Randomized Design* (CRD) with 4 treatments, 5 replications in which each replication consisted of 5 chickens so that the total chicken used was 100 heads.The treatment given were: R0: ration without fermentation dragon fruit skin flour, R1: ration with 5% fermentation dragon fruit skin flour, R2 : commercial ration , R3: ration with 50% commercial + 50% R1. Variabel observed : egg production, egg shell weight, egg shell thicknes , HU. The results showed treatment of R0,R1,R2 and R3 are not significantly different (P>0.05) for yolk colors , pH, Index, but % egg production, egg weight , HU, egg shell weight , egg shell thicknes R1 and R3 is significantly different (P<0.05) than R0, R2. Concluded this research that quality of egg Lohmann Brown gave ration fermentation flour skin dragon fruit (*(Hylocereus polyrhizus)* 5% (R1) and R3 (50% % commercial + 50% R1) increase the egg production , egg weight, HU, egg shell thicknes.

Keywords: egg weight, dragon fruit skin flour, HU, quality and yolk color

INTRODUCTION

Production of eggs and its quality may also be influenced by many other factors such as breed, mortality rate, culling age and season (North and Bell, 1990). However, egg weight, egg shell weight, shell thickness and egg shell membrane were higher in wet season than the dry season. Egg external and internal qualities are of major importance to egg industry worldwide.

Many factors interact affecting to optimal production of Lohmann Brown chicken. However, the maintenance of Lohmann Brown chickens faced with the variety of problems such as the increasing feed prices are enough sharply, because the feed is a primary need at a cost of approximately 60-70% . The high price of feed is indirectly require that farmers are looking for alternative feed ingredients so it can lower the feed costs and maximize revenues.

According Mustika (2014) dragon fruit peel is agricultural waste which has not been widely used by the community, especially in Indonesia. Dragon fruit is a key raw material in the manufacture of juices, jams, syrups, chips or other food ingredients by key material the dragon fruit. According Citramukti (2008) part of dragon fruit 30-35% is peel and still rarely or even not been fully utilized, although some studies have reported peel dragon fruit contains high antioxidant and contents phenolics in the dragon fruit peel amounted 28.16 mg/100 g, in addition to having antioxidant also contain anthocyanins (Nurliyana et al., 2010).

Sacharomyces cerevisiae yeast can increase fibrous fiber digestibility and can act as a probiotic in poultry (Ahmad, 2005 and Dewi et al., 2014). At the time of fermentation by yeast, the crude fiber content of ration can be degraded, so it can be utilized by poultry. Another benefit of fermentation products is to suppress the enzyme activity of *3-hydroxy-3-methylglutarylCo-A* reductase that serves to synthesize cholesterol in the liver (Tanaka et al., 1992).

And can decrease the amount of broiler fat (Katarin et al., 1999). However, Sacharomyces cerevisiae can increase the digestibility of high fiber feed into fatty acid products (acetate, propionate and butyrate) (Wallace and Newbold, 1993). Application of feed technology is absolutely must be applied in the optimization of waste utilization. Application of supplementation technology utilizing superior sacharomyces cerevisiae origin of yeast is very potential developed.

Research on dragon fruit peel for livestock feed is still rarely done according Mustika *et al*. (2014) dragon fruit peel can be given up to the level of 1% and Rosa *et al*. (2013) can be given up to the level of 4%, without have negative effects on the body of livestock. According Dewi et al.(2017a) that they were given the rations with used 5, 7% and 9% dragon fruit skin flour fermentation improved the process of digestion of feed in their digestive tract 2-8 of Kampung chicken and gave increase performans . While product fermentation dragon fruit peel for laying chickens the has been no research. From the description above, the researcher using dragon fruit peel meal without and fermented as a feed ingredients in diets for egg quality of Lohman Brown laying chicken

MATERIALS AND METHODS

Animal, ration and Feeding Treatment

This research conducted over 4 weeks and this research is located in Teaching Farm, Campus Bukit Animal Science, University of Udayana. A total 100 of 19 weeks with everage body 1360 ± 26.68 g were kept in individual cages of 40 x 40 x 45 cm .

Diets

Diets is used in this research was independently prepared by recommendation Scott *et al*. (1982)which consists of yellow corn, fish meal, soybean meal, rice bran, dragon fruit peel meal, dragon fruit peel meal fermented, coconut oil, premix and CaCO3. Diets given is iso energy (2.900 Kcal/kg) and iso protein (20%), Commercial ration.

Instrument

Instrument used in this research is a diet and drinking water, torch lighting cage, machine grinding feed, knife, bowl, spoons stirrer, scissors, paper labels, markers, plastic bags, oven, stove, pans, trays, thermometer, wood, bamboo, wire, plastic carpet, sprayer and digital scales.

Research Methods

In this research there are two stages making process meal dragon fruit peel, first making of dragon fruit peel meal is fresh dragon fruit peel chopped small, then dried and grinded up into flour. Second process namely the making of dragon fruit peel meal fermented with *Saccaromyces Sp.* In the process of fermentation, solution is ready for use. Fermentation process dragon fruit peel chopped small , be dried, inserted in plastic, then moistened with solution fermentation, closed tightly (3-5 days), after it is dried, ground into flour and ready for use.

Research Design

The design used was *Completely Randomized Design* (CRD) with 4 treatments, 5 replications in which each replication consisted of 5 chickens so that the total chicken used was 100 heads.

The treatment given were: R0: ration without fermentation dragon fruit skin flour, R1: ration with 3% fermentation dragon fruit skin flour, R2 : commercial ration , R3: ration with 50% commercial + 50% R1.

Variable Observed

Egg production and egg weight were recorded daily . Egg quality analysis was: was determined by egg multi terster, yolk color fan.

Data analysis

Data were analyzed statistic by ANOVA and when there are significant differences continued test Duncan (Steel and Torrie, 1993).

RESULTS AND DISCUSSION

The effects of treatmant for egg quality

The effects of ration for the external quality of egg Lohman Brown chickens aged 18- 23 weeks is summarized in Table 2. Table 2 shows the influence of diet on the external quality of egg Lohman Brown R0,R1 and R2 ,R3 there were significant ($p < 0.05$) different. The significant difference ($p < 0.05$) were exist in variable percentage eggs production , egg weight . The effect of treatment R0,R1, R2 and R3 did not significant difference ($p > 0.05$) in variable egg longth , egg widh and egg index (Table 1). But there was no significant ($p > 0.05$) compared with their fed R0,R1,R2 and R3 . According Dewi et al.(2017a) that they were given the rations with used 5, 7% and 9% % dragon fruit skin flour fermentation improved the process of digestion of feed in their digestive tract 2-8 of Kampung chicken and gave increase performans . and used for broiler chickens 1-5 weak aged due to fermentation dragon fruit skin flour containing various microbes that degrade fiber and probiotic microbes so it will be able to increase the ration digestibility and metabolism (Dewi et al. , 2017b).

Table 1. The effects of Treatmant for Exterior and Interior Egg quality.

Variabel	Tratment [1)] R0	R1	R2	R3	SEM[3)]
% Production	66.00[b]	70.00[a]	67.00[b]	72.00[a] [2)]	0.381
EXTERIOR					
Bobot Telur (g)	49.12[b]	50,35[a]	49.38[a]	51.39[a]	0.813
Egg longth (cm)	5.19[a]	5.13[a]	5.10[a]	5.26[a]	0.003
Egg width (cm)	4.35[a]	4.26[a]	4.67[a]	4.19[a]	0.005
Egg Index	80,84[a]	80.19[a]	79.80[a]	79.68[a]	2.345
INTERIOR					
Egg shell weight (g)	6.21[b]	6.82[a]	6.55[b]	6.81[a]	0.005
Egg shell thicknes (mm)	0.349[b]	0.376[a]	0.330[b]	0.397[a]	0.013
pH	6.71[a]	6.43[a]	6.43[a]	6.29[a]	0.012
Yolk Color	8.14[a]	8.49[a]	8.34[a]	8.99[a]	0.236
HU	89.89[b]	98.53[a]	92.5[b]	98.82[a]	3.204

Note:

1) R0: ration without fermentation dragon fruit skin flour, R1: ration with 3% fermentation dragon fruit skin flour, R2 : commercial ration , R3: ration with 50% commercial + 50% R1.

2) Superscript on the same line is not significantly different (P>0.05).

3) SEM: Standard Error of Means.

According Weiss and Hogan (2007) that material having the antioxidant content of livestock can reduce the effects of free radicals such as increasing feed consumption. Factor affecting egg weight are protein consumption (Tuleun and Adencola, 2013), according to Leeson and Summers (2005) protein and amino acids (methionine) are nutriens that have important role in controlling of eggs size. Feed with buah naga and combination of both could improve the performance and effect egg production. Antiocxidant (Bhat et al., 2014) which improve health condition and promote nutrient digestibility. In the present study, fermentation dragon fruit skin flour feed at the level of 3% (R1) and combination ration with 50% commercial + 50% R1 (R3) Table 1.

Table 1 showed that the addition of R1, and R3 in ration significantly effect (P>0.05) to eggshell weight and eggshell thickness. According to Cayan and Erener (2015) that was 6.82g (R1) and 6.81 g (R3) eggshell weight and eggshell thickness 0.376 mm(R1) and 0.397 cm (R3). The everage of eggshell weight in this research was higher than reported by Cayan and Erener (2015) that was 6.63 g. Ration with combination (R3) could be inhibit the activity of Ca2ATP-ase enzyme in transporting the calcium ions across the cell membrane that will decrease calcium. Inhibition of calcium absorption results in decreased eggshell quality , such as egg weight .

The average of eggshell thickness in this research was different from that reported by Park et al. (2015) that was 0.35 mm and 0.37 mm. The egg shell weight at early age was lower than older age, indicating that egg shell of the pullets was lighter than egg shell of spent layers. Suk and Park (2001) also observed that the egg shell was heavier in older birds. According to Kebreab et al. (2009), the higher the calcium intake the higher quality of the eggshell. The treatment R0,R1, R2 and R3 did not significantly (P> 0.05) different affect pH and yolk color (Table 1). Results showed that the egg produced in this experiment were 89.89 -

98.82 in grade AA egg with hough unit values > 72 , 60-72 , 31<60 and <31 are categorized as AA. According Olabatoke and Mulugeta (2015) who reported that haugh unit of laying hens supplemented with garlic powder was 95.90. But Nugraha et al.(2013) the absorption increased of amino acids could sustain ovomucin and lecithin thus enhanching the quality of eggs. Amino acids are used raise the viscosity of albumen and haugh unit will increase.

Conclusions

Concluded this research that quality of egg Lohmann Brown gaves ration fermentation flour skin dragon fruit (*(Hylocereus polyrhizus)* 5% (R1) and R3 (50% % commercial + 50% R1) increase the egg production , egg weight, HU, egg shell thickness.

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THE ROLE OF CABBAGE WASTE FILTRATE AS BICONTROL OF *Phytophthora palmivora* AND BIODECOMPOSER OF COCOA POD WASTE

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Abstract

Cocoa pod waste is still be a problem of the waste management program at the farmers level. It can be a medium for the growth of *P. palmivora*. Cabbage waste filtrate has a potential as biocontrol of *P. palmivora* and biodecomposer of the cocoa pod waste. This research aimed to determine the effectiveness of cabbage waste filtrate as biocontrol of P. palmivora and biodecomposer of cocoa pod waste. Cocoa pod waste were chopped by using a thrasher mechine. It was weighed as heavy as 1.5 kg, then put in a plastic box with a size of 15 x 15 x 20 cm. The suspension of cabbage waste was centrifuged for 10 minutes at 6000 rpm, then the supernatant was taken as the filtrate. The filtrate applied on chopped of cacao pod with treatment, i.e. control, 5%, 7.5%, 10%, and bioactivator EM4. Decomposition rate was analyzed by regression equation based on the level of decomposition and observation time. P. palmivora spores concentration were calculated using a haemocytometer and that was analyzed using a completely randomized design. The filtrate with a concentration of 10% had the highest decomposition rate than other treatments in the third week. The concentration of spores in the control treatment was significantly different from filtrate treatment of 5%, 7.5%, 100%, and EM4 (α 0.05).

Keywords: biological agent, cocoa, pod rot disease, spore consentration

Background

Cocoa pod waste is one of the problems of agricultural waste and that is an important problem of environmental sanitation. The cocoa production of Indonesia in 2014 was 651.6 ton. Cocoa production had agricultural wastes such as pod and pulp waste amounted to 73.77% of the total weight of the cocoa production and it could potentially contaminate the environment (CBS, 2015).

Agricultural waste management was still a constraint of farmer and government in the waste management program. Agricultural waste had not been widely used, even though it had the potential as a raw material for composting so that observation was needed to support the utilization program of potential waste. Cocoa pod waste was generally thrown away by farmers in the crop so that it could be a inoculum medium for the growth of pathogen *Phytophthora palmivora*.

Pathogen *P. palmivora* is one of the major pathogens that cause yield losses up to 90%, especially in the rainy season or dry season on a land with many ant population. This pathogen attacked all the phases of cocoa plant growth, i.e. caused leaf blight disease in the seedling phase and stem cancer and cocoa pod rot in the generative phase (Rosmana *et al*. 2010). Umrah, *et al*. (2009) reported that *P. palmivora* attack caused a yield loss of 32- 52%, and it increased in the areas that support the growth of the pathogen. One of the alternatives solution used in solving this problem is utilizing the cabbage waste filtrate that focused on two aspects, i.e. as a biological control of soil-borne pathogens especially *P. palmivora* and a biodecomposer of cocoa pod waste.

The cabbage waste was only used as the basic material for making organic fertilizers. The plant of the Brassicaceae family is also known to contain a glucosinolate which can be utilized as biofumigant for the control of some soil-borne pathogens. There has been much research into the effectiveness of biofumigants of Brassicaceae. Mirsam, et al. (2017) reported The dosage 2.5 kg of cabbage waste per polybag could be reducing the spore concentration of *P. palmivora* and suppressing the severity of *Phytophthora* up to 40%. This research aimed to determine

the effectiveness of cabbage waste filtrate as biocontrol of P. palmivora and biodecomposer of cocoa pod waste

Materials and Methods

Sampling

Sampling of cocoa pod was done on cocoa plantations in Village of Dorie, Sub-District of Bola, District of Wajo, South Sulawesi. Sampling was carried out by purposive method that based on specific criteria of sample. Samples were taken in the form of cocoa pod waste.

Chopping of cocoa pod waste

Cocoa pod waste chopped with a size of 0.5 x 0.5 cm using a shredder machine. Chopped of cocoa pod waste weighed 1.5 kg, then put in a plastic box with a size of 15 x 15 x 20 cm.

Preparation of filtrate

Cabbage waste chopped with a size of 0.5 x 0.5 cm using a shredder machine. Chopped of cabbage waste weighed 5 kg, then added 10 liters of clean water. Chopped of cabbage waste fermented for two weeks in an anaerobic condition. After two weeks, the fermented cabbage was filtered for suspension. The suspension was centrifuged for 10 minutes at 6000 rpm. The supernatant produced by the centrifugation was taken as a filtrate. A total of 20 mL of cabbage waste filtrate was added to the cocoa chopped. Cabbage waste filtrate was added to the chopped of cocoa pod with different treatment concentrations.

Application of cabbage filtrate on chopped of cocoa pod

The effectiveness of filtrate in the decomposition rate was known through filtrate concentration test with treatment of 5%, 7.5%, 10%, and EM4 100% (without dilution). In the control treatment, chopped of cacao pod was sprayed with distilled water.

Observation of the rate of decomposition

Observation of the rate of decomposition of cocoa pod waste was carried out every week until the cocoa pod waste decays perfectly by measuring temperature and compost changes such as color and texture of compost. The composting observation was calculated based on the following scale:

Table 1. Scale value of composting category

Scale value	Composting category
0	no weathering
1	weathering up to 25 %
2	weathering 26 % - 50 %
3	weathering 51% - 75 %
4	weathering >75 %

$$RD = \frac{\sum (ni \times vi)}{Z \times N} \times 100\%, \text{dengan}$$

RT, rate decomposition; ni, the number of observation units in the decomposition scale; vi, scale value of decomposition; Z, the highest decomposition scale value; N, the number of observation units.

Observation of spore concentration of *P. palmivora*

Calculation of spore concentration of *P. palmivora* was carried out by weighing 10 gr compost and inserted into the test tube, and then 20 ml of aquades was added. After that, it was shaken by using vortex. Observations were made weekly for three weeks. The concentration of *P. palmivora* spore was calculated using Haemocytometer, with the following formula: CP= t/(N x 25) x 10^6; CP, concentration of spores; N,

the number of haemocytometer quadrate; t, the average number of spores in the haemocytometer quadrate observed.

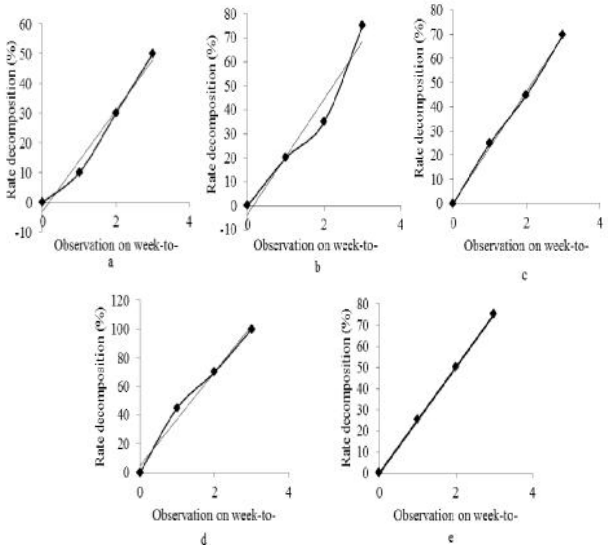
Analysis of data

This research was prepared by using a randomized block design that consisted of 5 treatments and 5 replications. The treatments were T1, 5% filtrate; T2, 7.5% filtrate; T3, 10% filtrate; T4, 100% EM4; and T0, control. Data were analyzed statistically continued by Tukey Test (LSD 5%) using SPSS application.

Results

The rate decomposition

The rate decomposition of cocoa pod waste in the third week showed the application of 10% filtrate concentration was able to decompose cocoa pod up to 100%, while in the control treatment, filtrate concentrations of 5%, 7.5%, and EM4 (without dilution) reached only 50%, 75%, 70%, and 75%, respectively. Regression analysis showed the higher the concentration of filtrate, the higher the rate of decomposition of cocoa pod waste into compost (figured 1).



Figured 1. Decomposition rate of cocoa pod waste after filtrate application at concentrations: a, control; b, 5% filtrate; c, 7.5% filtrate; d, 10% filtrate; and e, 100% EM4.

The concentration of *P. palmivora* spores

ANOVA analysis showed the lowest concentration of *P. palmivora* spores in the third week was in the EM4 treatment (without dilution), i.e. 0.50 x 10^6 / gr, and the highest was in the control treatment, i.e. 1.86 x 10^6 / gr. The growth of spore concentration in the control treatment was significantly different from filtrate treatment of 5%, 7.5%, 100%, and EM4, while the bioactivator treatment was not significantly different ($P \leq 0.05$) (Figured 2).

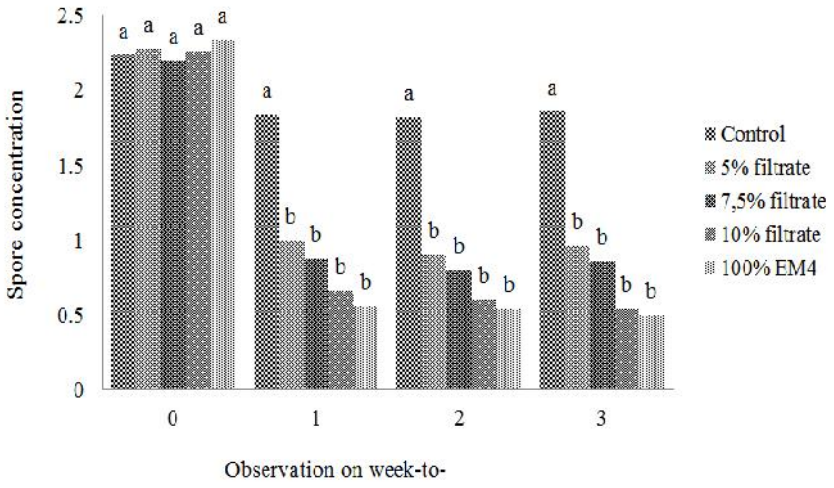


Figure 2. Growth of concentration spore of *P. palmiv*ora on compost per week (10^6 / gr). Means in the same column followed by same letter are not significantly different according to 5% LSD (α 0,05).

Discussions

(Cocoa pod waste is a potential raw material for making organic fertilizer because it is available throughout the year, easy to obtain, and high nutritional value for plants. Basically, cocoa pod waste can be used as a source of plant nutrients in the form of compost. The cocoa pod waste has a very potential nutrient and compound composition as a growing medium for plants. The results of the research by Soedarsono *et al.* (1997) showed that cocoa pod contained 86% water content and organic matter, pH 5.4, total N 1.30%, organic C 33.71%, P2O5 0.186%, K2O 5.5%, CaO 0.23%, and MgO 0.59%.

Filtrate concentration of 10% was more effective in decomposing cocoa pod waste than other treatments. This was allegedly caused by the type and concentration of fermented bacteria in each different treatment. Microbes contained in the filtrate each had a role, either as biodekomposer as well as biological agents of plant diseases. Howell and Stipanovic (1980) identified P. fluorescens strain Pf-5 as a biological agent that produced antibiotics and siderophores. They had a role as biological control of plant pathogen.

In addition to containing microbial fermentation, the filtrate of cabbage waste is also thought to contain biofumigant that known to decreased the number of *P. palmivora* spores. Mirsam et al (2017) reported cabbage waste in agriculture is not only used as organic fertilizer, but also can be used as biological control of soil-borne pathogens especially *P. palmivora*. Fahey *et. al.* (2001) reported that the *Brassicaceae* family produced isothiocyanate and benzyl isothiocyanate compounds which were able to act as antimicrobial compounds.

Conclusions

The concentration of cabbage waste filtrate which showed significantly effect to decomposed cocoa pod waste was 10%. The application of 10% filtrate concentration was able to decompose cocoa pod up to 100% in the third week. The lowest concentration of *P. palmivora* spores in the third week was in the EM4 treatment (without dilution), i.e. 0.50 x 10^6 / gr. Utilization of cabbage waste in agriculture is

not only used as organic fertilizer, but also can be used as biological control of soil-borne pathogens especially *P. palmivora* and biodecomposer of organic matterial

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LEAD HEAVY METAL CONTAMINATION IN SPINAL BONE MARROW OF BALI CATTLE SLAUGHTERED AT TRADITIONAL SLAUGHTERHOUSE

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Abstract

The lead heavy metal in exposed animal or human tissues is thought to be high in hematopoietic tissue. The main hematopoietic tissue in animals and human is the bone marrow. This study aim was to determine the levels of lead heavy metal in the spine bone marrow of Bali cattle slaughtered in traditional slaughter houses in Badung regency of Bali. As many as 20 samples of Bali cattle's spine bone marrow taken shortly after the cattle were slaughtered, then the tissue was stored in 0^{o}C. The measurement content of the lead heavy metal was done at Analytical Laboratory, Udayana University by using atomic absorption spectrophotometry (AAS) method. The results of the lead heavy metal contents were varied from 6,279 ppm to 18,025 ppm, while the average was 11,730 ± 3,468 ppm. These results indicate that lead heavy metal contamination in the spine bone marrow samples of the Bali cattle is higher compared to the maximal recommended level of consumption, which is 2.00 ppm. The lead heavy metal content that exceeds 2.00 ppm in food is really harmful to health when it is consumed. It can be concluded that the lead heavy metal contamination in the spine bone marrow of Bali cattle seems to be high. Therefore, beef consumer

communities need to be more careful in choosing and/or consuming Bali cattle's spine bone marrow as a food ingredient for consumption.

Keywords: lead heavy metal, bone marrow, AAS metho

Background

Food security is a meaningful unity in the adequacy of food quantity and quality. In fulfilling the quantity without regard to food quality, the effort to build a healthy society will turn into a disaster. An example is the availability of beef without regard to cattle raising properly, it can be a source of health problems for consumers. One of the pollutants of beef that endangers consumer health is heavy metal lead, as reported by Berata et al (2016). Heavy metal contamination of lead is very dangerous to health if consumed exceeding the maximum threshold. The main impact of heavy metal lead contamination in the body is anemia, because iron (Fe) is substituted by Pb elements (Jang et al 2011; Gillis et al, 2012). Lead heavy metals (Pb) are accumulative in the body (Brochin et al, 2008), so the disturbances will spread to other body tissues including the liver, kidneys, spleen and reproductive tissues (Jaishankar et al, 2014). Even heavy metal contamination of lead can cause brain disorders in the form of cognitive decline in children (Toscano and Guilarte, 2005).

Detecting heavy metal contamination of lead in various foodstuffs is very important, so that consumers get safe and healthy food. As a source of animal protein, it appears that beef is the most preferred meat of the community. Therefore the quality of beef must receive attention especially to ingredients that can pollute. Beef derived from cattle that are kept in urban waste disposal sites, the presence of heavy metal lead (Berata, et al 2017). It was further reported that hemopoietic tissues such as the liver and spleen are highest among body tissues exposed to heavy metal lead. There

have been no reports of heavy metal contamination of lead in the main hemopietik tissues namely the spinal cord of cattle. There is a trend of beef consumers who like to consume the bone marrow for various reasons. This study aims to measure the level of lead heavy metal contamination in the spinal cord.

Materials and Methods

Research Samples

The research was done using a total of 20 bali cattle which slaughter in the slaughterhouse. Bone marrow samples were taken from the border of the os atlas and cervical I, after the cattle was cut.

Measurement of lead heavy metal contamination

The samples were processed for the measurement level of lead heavy metals by using atomic absorption spectrophotometer (AAS) method (Sikiric, et al., 2003). The samples were divided into two parts, 0.5 ml for positive control and 0.5 ml as sample to be evaluated. Standard solution 0.25 ml of 1 mg/l was added as positive control. The control was evaporated on a hot plate at a temperature of 100ºC until it dried. Then, the spike and the samples were inserted into a furnace and covered half of their surface. In the process, the temperature furnace was raised gradually 100ºC every 30 minutes up to 450ºC and maintained for 18 hours. After that the spike was removed from the furnace and chilled at room temperature. Next, 1 ml HNO3 65% was added, before they were shaken carefully so that all the ash dissolved in acid and then they were evaporated on a hot plate at a temperature of 100ºC until they were dried. The samples and spike put them back into the ash furnace. Its temperature was raised gradually 100ºC every 30 minutes up to 450ºC and maintained for 3 hours. After they were formed white ash, the spike and samples were cooled at room temperature. A 5 ml of HCl 6 M solution was added

to each sample and spike then shaken carefully so that all the ashes were dissolved by acid. Then they were evaporated on a hot plate at a temperature of 100ºC until dried. A 10 ml of 0.1 M HNO3 was added and cooled at room temperature for 1 hour, the solution was transferred into a 50 ml flask poly propylene before they were added with matrix modifier solution, then added with 0.1 M HNO3 until it reached to the mark limit. Lead heavy metal working standard solution was prepared at least five points concentration. Working standard solution, samples, and spike were read on graphite fumace atomic absorption spectrophotometer at a wavelength of 288.3 nm for lead heavy metal.

Data Analysis

Data measurement on lead heavy metal in bone marrow was analyzed descriptively.

Results

Measurement of lead heavy metal levels in cattle bone marrow samples obtained results as presented in Table 1. The mean lead content of 20 samples was 11.730 ± 3.468 ppm. There was a variation of the lead heavy metal content from the highest 18.025 ppm and the lowest 6.279 ppm.

Table 1. Lead heavy metal content from 20 samples of cattle bone marrow

Code	Age of cattle (year)	Content of lead (ppm)
1	4	7,538
2	4	11,845
3	4	8,574

4	4	6,279
5	4	7,136
6	4	6,919
7	4	7,089
8	4	13,927
9	4	15,333
10	4	13,172
11	5	13,325
12	5	15,846
13	5	10,776
14	5	11,592
15	5	12,435
16	5	11,743
17	5	12,814
18	5	15,827
19	5	14,396
20	5	18,025
Mean		**11,730±3,468**

Discussions

Lead heavy metal in bone marrow be high (11,730 ± 3,468 ppm), showed that the bone marrow is one of the accumulation sites. As the main hemopoietic tissue for the body, accumulation of lead heavy metal in the bone marrow will interfere with the process of red blood cell formation. This is explained by

Jang et al (2011) that lead heavy metal can replace iron (Fe) elements in hemoglobin, which can cause anemia. Lead heavy metal circulating in the circulation can also cause oxidation because they are oxidant, which will gradually damage tissue cells (Sharma et al 2014). Other hemapoietic tissues including the liver are also sensitive to lead heavy metal contamination (Hegazy and Fouad, 2015) and the spleen will interfere with its role as an organ of defense (Turkay et al 2015). The brain can also cause interference with the occurrence of cognitive impairment, especially in children (Toscano and Guilarte. 2005).

Lead heavy metal in bone marrow exceeds the maximum threshold for consumption, which is 2.00 ppm in accordance with WHO (2016). The high levels of lead heavy metal in the bone marrow, it is very important to also check the bone marrow in every inspection of the quality of beef regularly. There needs to be supervision at every stage, starting from the farming system, cattle grazing, feed sources and cattle slaughterhouses.

Various sources can cause lead heavy metal to enter the body of the cattle, including through inhalation and drinking water with environmental pollution, feed and post-slaughter. The most lead heavy metal particles in water as a source of contamination of cattle (WHO, 2016). The cattle kept in urban landfills are potentially heavily contaminated with lead heavy metal, as reported by Berata et al (2016). Urban waste is generally a combination of organic and inorganic waste including lead heavy metals.

Plants as a source of animal feed can also be a source of lead heavy metal. Organically raised cattles are also reported to be contaminated with heavy metals lead to their milk (Malhat et al, 2012; Pilarczyk et al, 2013). Many plants contain lead heavy metals and can even be poisoned in high doses (Sharma and Dubey, 2005).

Conclusions

The lead heavy metal content of cattle bone marrow which was slaughter in traditional slaughterhouse was 11.730 ± 3.468 ppm. This level exceeds that recommended for consumption, so there needs to be periodic checks so that people avoid food contaminated with lead heavy metal.

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IN VITRO STUDY – ADDITION OF MOLADEF IN RATION TO RUMEN FERMENTATION, DRY MATTER AND ORGANIC MATTER DIGESTIBILITY

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Abstract

MOLADEF is a combination of molasses and saponin defaunation agents that aim to optimize the growth of selulolitic bacteria in the rumen. In this study to examined the addtion of MOLADEF in ration to in vitro rumen fermentation, dry matter digestibility and organic matter digestibility. This study used a complete randomized design with 4 treatments and 5 replications. The treatments were A: control (elephant grass + polard), B: control + *Hibiscus tiliaceus* moladef, C: control + *Hibiscus rosasinensis* moladef, D: control + *Aloe vera* moladef. Parameters observed were pH, NH3, partial VFA, ratio of acetic acid and propionic acid, methane, dry matter digestibility and organic matter digestibility. Parameters of rumen fermentation were analyzed descriptively, while dry matter digestibility and organic matter digestibility used analysis of variance (ANOVA) and significantly different data were analyzed used Duncan's test. The addition of moladef B in the ration has dry matter digestibility and organic matter higher than other treatments ($P < 0.05$). Descriptively the use of moladef in the ration does not negatively affect the rumen fermentation. The addition of moladef B has a higher NH_3 value than the control.

However, the use of moladef in ration has a lower total VFA than controls. In the parameter of CH_4, the moladef treatment gave a positive effect on the decrease of methane production. The use of moladef B has a positive impact on the digestibility of dry matter and organic matter, total NH_3 and the amount of methane.

Keywords : *Dry matter digestibility, Moladef, Organic matter digestibility, Rumen fermentation*

Background

Based on livestock and animal health statistic (2007), the beef cattle population in Bali province was 562.325 head, this data increased 2.9% from previous year. The increased beef cattle population need to be balanced with capacity feed production. Population of beef cattle are increasing along with methane gas production. Bali is producer of Bali cattle which is dominated by small scale farmers with low productivity. This is because feeding is dominated by forage.

In the tropic, forage have different nutritional quality, but in generally contain high crude fiber and low crude protein. The high crude fiber content in forage result low digestibility. One of the way to increase feed digestibility is adding saponin. In plants, saponin have function as antimicrobials and counteract insect attacks (Francis *et al.*2002). In the rumen fermentative digestion, saponin have function as defaunation agents. Defaunation is an effort to reduce the presence of rumen protozoa (Puastuti 2009). Saponins contentinhibit the growth of protozoa (Putra, 2006;

Susanti and Marhaeniyanto, 2014), hence at the same time increases bacterial population in rumen (Goel *et al*., 2008).In general, protozoa prey on bacteria to maintainance. Ruminants digestion is very dependent on enzymes produced by rumen microbes, on of which is cellulolytic bacteria. Therefore, the feed digestion in ruminants is strongly influenced by total rumen bacteria.

Moladef is a combination of molasses and defaunation agents which aims to optimize growth of selulolytic bacteria by providing an energy source for bacterial growth in the form molasses and defaunation agents in the form of saponins that reduce protozoa population. Some plants have high saponin content and are potentially used as defaunation agents such as *Hibiscus tiliceus*, *Hibiscus rosasinensis*, and *Aloe vera*. The purpose of this study was to examine the combination of several types defaunation agents with molasses on the characteristics of rumen fermentation and feed digestibility.

Material and Method

Material

The materials used are molasses, *Hibiscus tiliaceus* meal, *Hibiscus rosasinensis* meal and *Aloe vera* meal, then elephant grass and pollard. In addition, other materials used the rumen fluid of cattle from slaughterhouses in Denpasar. The tools used consisted

of fermenter tubes, Conway dishes, porcelain cups, gas chromatography (GC) and shaker water bath. Other supporting equipment used are centrifuges, pH meters and micro pipettes.

Method

This study used a completely randomized design with 4 treatments and 5 replication, namely treatments A : Napier grass 85% + pollard 15% (control), B : A + *Hibiscus tiliaceus* Moladef, C : A + *Hibiscus rosasinensis* Moladef, D : A + *Aloe vera* Moladef. The observed parameters consisted of pH, NH3, partial VFA, acetate and propionate ratio, methane gas production, dry matter digestibility and organic matter digestibility.

The observation of fermentative digestion was carried out by methods of Tilley and Terry (1963). From each treatment 0.5 grams of sample were taken and put into a fermenter tube. The fermenter tube which has been filled with a sample of 40 ml of McDougall solution and 10 ml of rumen liquid added. The CO_2 gas is inserted into fermenter tube for 30 seconds the closed using a ventilated rubber. The fermenter tube was put in a shaker water bath for 4 hours incubation. After 4 hours incubation, 1 ml of rumen fluid is taken into the ependor and 1 drop of H_2SO_4 is dripped, followed by partial VFA measurement using a gas chromatography device.

The rumen liquid remaining in the fermenter tube is dripped with $HgCl_2$ to stop fermentation process, then the separation of supernatant and solids using centrifuge is carried out. Supernatant is used for analysis of NH3 concentration while the solid are used to analyze digestibility of dry matter and organic matter. Analysis of N-NH3 concentration was carried out using Conway Microdifusion method (General Laboratory Procedure 1966). The measurement of dry matter digestibility and organic matter digestibility using gradual drying was oven 60^oC, 105^oC and furnace.

Result

Data of characteristic rumen fermentation are presented descriptively in Table 1 consisting of pH, NH3 concentration, total VFA concentration, partial VFA, ratio of acetic acid to propionic acid and methane gas production

Table 1. Rumen fermentation characteristic

Parameters	**Treatments**			
	A	B	C	D
pH	8.46	8.24	8.66	8.62
NH3 (mM)	15.66	16.53	15.84	14.97
C2 (Mmol)	17.56 (63,74%)	12.8 (62,81%)	13.14 (71,41%)	14.91 (71,89%)

C3 (Mmol)	4.93 (17,89%)	4.45 (21,84%)	1.29 (7,01%)	1.54 (7,43%)
C4 (Mmol)	0.74 (2,69%)	0.91 (4,47%)	0.64 (3,48%)	0.63 (3,04%)
Total VFA (Mmol)	27.55	20.38	18.4	20.74
C2:C3	3,56:1	2,88:1	10,19:1	9,68:1
CH4 (ml/g)	0,43	0,30	0,36	0,40

*C2 : acetate, C3 : propionat, C4 : butirat

While the digestibility data of dry matter and organic matter are presented in table 2.

Table 2. Dry matter digestibility and organic matter digestibility

Parameters	**Treatments**			
	A	**B**	**C**	**D**
DMD*	44.41±0.01a	56.20±0.03d	52.55±0.04b	52.76±0.04c
OMD**	47.75±0.12a	63.80±0.09d	58.62±0.10b	59.01±0.10c

*DMD : Dry matter digestibility; **OMD : organic matter digestibility

Discussion

Rumen fermentation characteristic

Descriptively, the effect of moladef addition on rumen fermentation characteristic is presented in table 1. The pH value is more alkaline than study Santoso and Hariadi (2007) which states

that the pH value of rumen fermentation ranges from 6.79 – 6.95 with supplementation of *Acacia mangium* as a source of saponins. This is indicated as a result of higher forages percentages compared to concentrates. It's similar with Arora (1989) which states that the use high forage in ration will resulth in alkaline pH. The high content of crude fiber in the forage results in alkaline pH of the rumen fluid because the amount of easily fermented carbohydrates is limited. The rumen inside, carbohydrates will be fermented anaerobic into acid compounds that can reduce rumen pH.

Parameters of NH3 concentration in rumen showed the level of ration protein degradation by rumen microbes. In this study NH3 production ranged from 14.97 -16.53 mM. The NH3 concentration in this study was lower than Santoso and Hariadi (2007) which ranged between 16,6 – 30,7 (mM) but still in the normal ranged according to McDonald et al. (2002) which states that the optimal NH3 concentration to maintain normal rumen function is 6 mM to 21 mM. Concentration of NH3 in this study is still low because the composition of feed ingredients in ration is more predominantly forage derived from elephant grass with low PK and high fiber. NH3 production is influenced by the type of feed, time and frequency of feeding, livestock and other factors (Hristov and Jouany 2005). Descriptively, the addition of Moladef B has a higher NH3 total than other treatments. This is consistent with the digestibility of dry matter and organic matter which also higher in treatment B (Table 2).

However, the digestibility level of dry matter and organic matter is not in harmony with the total VFA and partial VFA concentration. Acetic acid in this study ranged from 12.8-17.56 Mmol. This value is higher than the study of Santoso and Hariadi (2007) which states that the addition of *Acacia mangium* saponin sources leveled produces acetic acid ranging from 8.2-10.6 mM. Acetic acid is the largest proportion of fatty acids in VFA. Descriptively, acetic acid in treatment A was higher than the treatment with moladef addition (Table 1). This is not consistent with the lowest level of dry matter and organic matter digestibility (Table 2). In treatment A also has a higher total VFA which is not consistent with the digestibility of feed (Table 2). The inverse comparison between total VFA and digestibility in this study is indicated as a result of the role of bacteria to synthesize VFA inhibited by saponin content in the ration. Wang et al. (2000) stated that saponins can inhibit the activity of cellulolytic and amylolytic bacteria.

Saponins are also known to reduce methane gas concentration. Decrease in methane gas as an effect of anti-bacterial properties on saponins (Santoso and Hariadi, 2007). However, this study has not seen the effect of saponins on the reduction of methane gas production. Methane gas production in this study ranged from 0.30 to 0.43 (ml / g). This value is still high compared to the studies of Santoso and Hariadi (2007) which are 0.02-0.04 (ml / g). Methane gas production can also be affected by

the production of propionic acid and butyric acid. Moss et al. (2000) stated that the production of acetic acid and butyrate will increase methane gas production, while the production of propionic acid can reduce methane gas production. This shows that the use of saponins in the ration to reduce methane gas needs to be balanced with carbohydrate levels.

Dry Matter Digestibility and Organic Matter Digestibility

Digestion of dry matter and organic matter shows the amount of nutrient intake that can be used for livestock productivity. Digestibility of dry matter and organic matter is presented in Table 2. Dry matter digestibility is influenced by the addition of moladef in the ration ($P < 0.05$). Moladef addition can increase the digestibility of dry matter rations (table 2). At moladef B treatment (*Hibiscus tiliaceus*) has the highest dry matter digestibility compared to other treatments. This is also consistent with the digestibility of organic matter. Digestion of organic matter in moladef B treatment was higher than other treatments ($P < 0.05$). This shows that the use of moladef is able to support the digestibility of feed, but it needs to be reassessed when viewed from VFA synthesis which is still low compared to controls. This is indicated as saponin activity which inhibits the development of amylolytic bacteria so that the resulting VFA is lower than the control. The digestibility of dry matter and organic matter in this study was higher than that of Santoso and Hariadi (2007). This

shows that the use of defaunation agents and molasses in research has not caused a negative effect on the digestion of livestock.

Conclusion

The addition of moladef C and D has a higher ratio of acetic acid. The use of moladef B produces better digestibility of dry matter, organic matter, NH3 concentration and methane production.

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ISOLATION TYPE OF CONTAMINATION PARASITES IN SOIL

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Abstract

Research on soil around the cage and grazing areas has been done. The objective of the study was to isolate the type of parasitic contamination in the soil around cages and grazing areas. Each soil sample of 100 comes from Jimbaran and Mengwi Badung areas. Flotation method with MgSO4 (English salt) modified as a method for determining the type of parasite pollutant in soil environment. The results obtained 45% (90/200) soil polluted by parasites. The types of contaminated parasites found are coccidia oocyst, protozoan cysts, trematoda and nematode worm eggs, nematode worm larvae and eggs from mites. Conclusions from soil research results obtained 45% of the land is parasitic contamination in the form of protozoa, worm eggs, larvae and mite eggs from Bukit Jimbaran area (29%) and Mengwi (61%). Keyword : parasite, soil, egg worm, protozoa cyst, MgSO4.

Background

Intestinal parasitic infection in cattle is still a factor causing considerable economic losses, especially for smallholder livestock. Intestinal parasitic infections such as Fasciolosis can cause a loss of up to 20 trillion / year (Asian liver Fluke, 2007). Similarly Coccidiosis causes economic losses in cattle due to medical costs that reach 2-3 million cows become sick every year (Kvasnicka, 2001). One in five cows reported symptoms of coccidiosis to die (Mel Pence, 2001). According to estimates by Maas (2007) the loss due to coccidiosis in cattle reaches 100 million dollars per year. Parasitic diseases in cattle economically result in very high losses in livestock, let alone these parasites are zoonotic which will cause losses to farmers and at the same time endanger the health of farmers as well (Medicastore, 2011).

Intestinal parasite infection in cattle comes from food / beverages contaminated with oocysts or parasitic infective stages (Soulsby, 1982; Levine, 1994; Zajac and Conboy, 2012), which come from the surrounding environment, namely soil. Cows as definitive parasitic hosts, excrete oocysts or infectious stages of parasites through feces and contaminate soil and water, so that animals in polluted soil can be infected with parasitic diseases. Soil is a source of infection / L3 infestation (larval stage 3), infective eggs, oocysts and ticks (Brotowidjoyo, 1989).

Based on the survey results in several animal markets in Indonesia 90% of cattle and buffaloes have worm diseases such as leaf worms (Fasciola), roundworms (Neoascaris vitulorum) and gastric worms (Haemonchus contortus). Cattle become infected due to consuming forage contaminated by infective stages of soil (Abidin, 2002). Research on gastrointestinal parasites by Sugama and Suyasa (2007) in Balinese cattle kept in the Simantri cage model obtained 23.37% positive for parasites, while in cattle outside the cage Simantri model 66.67%. The study of Juliet et al. (2013) in cows from 569 samples obtained detected 50.79% positively infected with gastrointestinal worm parasites. Coccidiosis infection in female cows in 9 provinces in Indonesia is quite high ranging from 70-100% with mild infections, while 10% with severe infections (Fitriastuti et al., 2011).

The high percentage of parasitic intestinal infections (coccidiosis and helminthiosis) in cattle need to be researched on the source of infection. How big is the role of the source of infection from the soil contaminated by oocysts or infectious stages of the parasite, so that it will be observed how much infection by intestinal parasites occurs in bali cattle. Research on the types of parasitic soil environmental contamination around cages and pasture of Balinese cattle until now no one has conducted research, especially in Bali.

Parasitic intestinal disease, transmission directly through the mouth will consequently be very easy for parasites to enter if the surrounding environment supports. The occurrence of infection according to the epidemic of diseases such as host factors, disease agents (parasites) and the environment, which interact with each other affecting the occurrence of disease (Brotowijoyo, 1987). The ease of cows being infected by intestinal parasites, because of their transmission directly through the mouth. Forage food provided will somehow contact the soil around the cage during meals, as well as when cattle graze in the pasture. Soils contaminated by oocysts or infective stages of parasites that are in the vicinity of the cage or in the pasture are sources of transmission in cattle. The existence of such conditions inspires researchers to conduct research in order to find out the type of parasitic contamination of the soil environment in the vicinity of cows and cattle grazing fields. The results of this study will be able to illustrate that the land is very potential as a transmitting source for cattle or other animals that are often around the soil environment.

Materials and Methods

The material used is soil collected from the area around the cage and land from the cattle grazing area. Soil samples of 100 from each dryland and wetland region. Collection of soil samples. Sources of sampling are around the cage and around cattle grazing. The sample origin is the calcareous dry land area (Bukit Jimbaran)

and wetland area (Mengwi) in Badung Bali. Soil samples were taken ± 50 grams, a slightly moist soil (4% water content) was chosen. The number of samples collected 100 from each wet and dry land area. The land taken, placed in a plastic bag and closed tightly, is stored until the time is checked.

Oocyst examination and parasitic infective stage by flotation method using MgSO4 was modified by precipitation as a modification of the Matsuo *et al* method (2004). Oocyst examination and parasitic infective stage by flotation method using MgSO4 was modified by precipitation as a modification of the method of Matsuo *et al*., 2004.

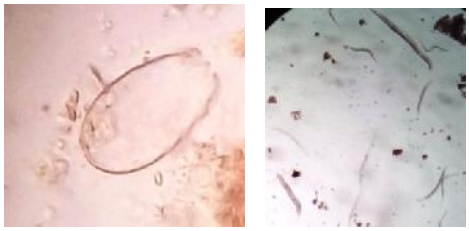
The sample soil is ± 30 grams, filtered with a 150 µm mesh filter. The filtered soil was added with 50 ml of 0.1% Tween 80, then centrifuged with 1100 g for 10 minutes. The supernatant is discarded. Sediment was added with 5 ml of saturated MgSO4 float solution (BJ 1.12), then centrifuged. Supernatant is taken, move it to a new tube. Add the aquadest with a ratio of 10: 1 with the volume of the supernatant, the centrifuge back. The supernatant is discarded. Sediment as material for further inspection

Results

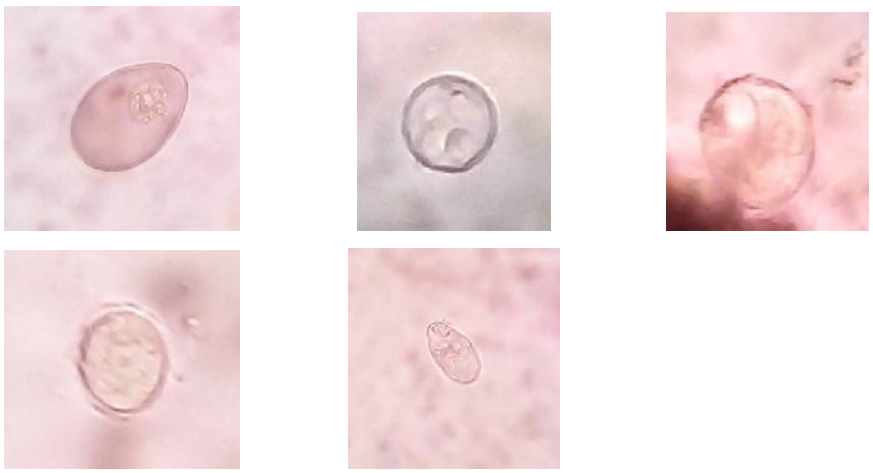
One hundred samples from each region (Bukit Jimbaran and Mengwi Badung) had positive parasitic contamination of 45%. Soil samples from Bukit Jimbaran 29% and Mengwi Badung 61%

parasitic contamination. The results of soil samples were found types of contaminants as follows : Protozoa (Coccidia oocyst, Balantidium Cyst, Amoeba Cyst, Giardia cyst), Helminth (Nematode and Trematode worm eggs, and larvae), Arachnida (mite eggs, tick eggs).

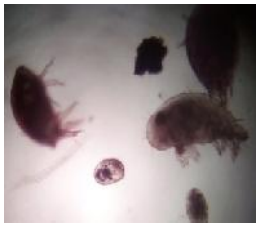
Pigure 1. Nematode and trematode eggs and larva nematode



Pigure 2. Coccidia oocyst, Eimeria oocyst, Balantidium cyst, Entamoeba cyst and Giardia cyst



Pigure 3. Mite larvae



Discussions

The average soils in the environment around the cage and pasture is 45% parasitic contamination. This parasite contamination is quite high as a source of infectious infection in cattle in the cage. Cows in South Sulawesi were detected as infected by parasites by 75% (Purwanto *et al*., 2009). Whereas cattle in Bima were identified by 16 species as 81.1% infected by internal parasites (Astiti *et al.,* 2011). Cows in the East Bali region were infected by Toxocara parasites by 42.5% (Agustina *et al*., 2013). These varied

differences illustrate that such transmitting sources vary depending on the region's local geography.

Different geographic conditions related to the condition of the land in the local area such as the Bukit Jimbaran region were detected 29% of parasitic contamination, while the Mengwi region was detected 61%. This difference is significant because the Bukit region includes the chalky dryland areas and Mengwi wetland areas. Rainfall varies according to the month and location of an area. Regional humidity is influenced by rainfall which results in soil conditions so that the disease agent becomes optimum (Lab Pengelolaan DAS dan Konservasi Sumberdaya Hutan, Tanah dan Air, 2009).

According to Brotowidjoyo (1987), epidemiologically, the source of transmission of parasitic diseases is: soil, water, plants (leaves, fruits and vegetables), sufferers and reservoirs. Land is the main and most important source of contagion. Parasitic diseases that are transmitted through the soil are called soil-borne parasitosis. Most infectious stages of parasites are found on the ground as eggs contain infective larvae (L2 Ascaris, Neoascaris, Parascaris, Ascaridia, Heterakis, Toxocara), infective larvae of various filariform nematodes (L3 strongyle worms) or L3 hookworms, coccidia protozoa oocysts (Eimeria , Isospora, Cryptosporidium, Toxoplasma) and cysts from Entamoeba (Brotowidjoyo, 1987). Parasitic disease in humans is transmitted

widely through the soil called soil transmitted helminth, especially in children under five. The results of examination of soil samples from several places in Semarang district showed 22% positive worm eggs, especially Ascaris, hookworm eggs and larvae (Health Lab Center Semarang, 1995).

Weather / climate can affect the chemical and physical properties of the soil, as well as the organisms in it (Lab Pengelolaan DAS dan Konservasi Sumberdaya Hutan, Tanah dan Air, 2009). Alluvial soil types in the lowlands, andosols in mountainous areas, litosol in hilly areas and podsolik in choppy areas. This type of soil also influences the soil chemical properties associated with soil acidity, thus impacting the life of microorganisms in it.

Conclusions

The types of soil contamination found in the soil around the cage and cattle grazing environments in the Bukit Jimbaran and Mengwi regions are protozoa (Coccidia oocysts, balantidium cysts, Entamoeba cysts and Giardia cysts), Nematode and Trematoda worm eggs and worm larvae, Mite larvae and eggs and tick. Prevalence Soil samples from Bukit Jimbaran 29% and Mengwi Badung 61% parasitic contamination.

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SOIL-TRANSMITTED HELMINTH INFECTION ON FREE-ROAM DOGS IN BALI

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Abstract

In the public area of Bali, there are many free-roam dogs that easily found. They go to find food, friends and defecate everywhere. These dog groups generally do not get good health care from their owners, some disease agents are threatening them, especially helminth. These study purposes were to identify and measure the prevalence of soil-transmitted helminth causes infection of free-roam dogs in Bali. As many as 1.611 fresh dogs fecal samples collected from all area of Bali province. All of them examined by the qualitative fecal examination using flotation techniques to find soil-transmitted helminth eggs.The results found that the prevalence of soil-transmitted helminth on free roam dogs in Bali was 38.36%. We identified three types of soil-transmitted helminth, that were *Ancylostoma sp, Ascaris sp,* and *Trichuris sp.* The *Ancylostoma sp.* was the highest prevalence with 37.8%, followed by *Ascaris sp.* and *Trichuris sp.* with 6.02 and 0.87% respectively. This study also recorded the combined infection of the worms. The combination of *Ancylostoma sp* and *Ascaris sp.* was 3.85%; followed by the combination of *Ancylostoma sp.* and *Trichurissp.* was 0.5%;*Ascaris sp*. and *Trichuris sp.* was 0.06%. The prevalence of the combination of all worms was 0.12%. Based on the sample location, lowland was more parasite prevalence than highland. It concluded taht the prevalence of soil-transmitted helminth causes infection of free-roam dogs in Bali was high.

Keywords:Bali, free-roam dogs, prevalence, soil-transmitted helminth

Background

Dogs can not be separated from human life, so they are often referred to as the Best Friend of Man. Not just a hobby, maintaining a dog can also provide many benefits for the owner. According to some studies, dog maintenance can have a positive impact on the psychology and health of the owners (Walsh, 2009). Especially for the Balinese, dogs are part of their lives. Dogs are used as friends as well as guardians of their homes and territories. Dog upkeep system in Bali is to be released in order to maintain its territory, considering the dog is a territorial animal (Hiby *et al.*, 2018). The population of free-roaming dogs in Bali is quite high, in rural areas reported as much as 69.71% (Agustina *et al.*, 2017b), while urbanized at 19.4% (Hiby *et al.,* 2018).

Contrary to the benefits provided by dogs, maintenance patterns by allowing dogs to roam turn out to have an adverse effect on the health of the community as well as dogs and other animals (Medina-Pinto *et al.,* 2018). There have been many reported cases in the community due to the presence of dogs that roam freely in an area, especially in Bali (Dalem *et al.*, 2012). Dog-borne dangerous diseases can be caused by viruses, bacteria or parasites (Pedersen, 1999). Specific diseases caused by parasites, generally do not get serious attention to the community. Due to the disease rarely cause clinical symptoms (Agustina *et al.,* 2017a).

Parasitic worms that commonly attack dogs are of the nematode species (Yacob *et al.,* 2007). Types of common nematode worms that attack dogs are *Ancylostoma sp., Ascaris sp.*, and *Trichuris sp.* These three types of worms are commonly classified into soil-transmitted helminth groups (Jia-Chi *et al.*, 2016). Some types of STH worms that attack dogs are reported as zoonotic agents namely *A. ceylanicum, A. caninum* and *Toxocara canis* (Crompton, 2000; Sariego *et al.*, 2012).

Several reports suggest the prevalence of worms in dogs in Bali is available. T. canis worms were reported to infect kintamani dogs in Sukawana Village area by 22.22% (Evayana *et al.*, 2017). While the prevalence of Ancylostoma sp in tourist areas in Bali was reported at 34% (Dharma *et al.,* 2017). However, there is no

reported prevalence of worms that covers all areas in Bali. So it is necessary to do research to know the prevalence and type of STH worms in dogs that freely roam throughout the province of Bali.

Materials and Methods

The sample used in this research is fresh dog feces. Fresh stools are taken in the morning around the residential alley, street or other public places. The stool is stored in 10% formalin before being examined at the Laboratory. As many as 1611 fresh dog stool samples have been collected from 9 regencies of Bali province. Samples also categorized based on location height.

This research use observational design with cross sectional method. The object of the study is the free-range dog feces in the province of Bali. Feces are grouped by regency in Bali Province: Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, BulelengRegency, and Denpasar City.

The stool examination is carried out by floating concentration method, using a saturated NaCl substance. Approximately ±3 grams of feces insert into the beaker glass, added with a little aquades, stirred until homogeneous. Strain, then put in centrifuge tube until ¾ tube, rotate with speed 1500 rpm for 5 minutes. The supernatant is removed, add saturated NaCl until the volume is ¾ tube and again stirred until homogeneous. Rotate at 1500 rpm for 5 minutes. The tube is placed on the tube rack perpendicularly, add a saturated NaCl solution by dropping it using a pipette until the surface becomes convex and left for 3 minutes. The cover glass is placed over the surface of the convex fluid carefully, then attach to the object glass and examined under a microscope with an objective 100x magnification to see the presence of the worm egg STH (Evayana *et al.*, 2017).

To measure the prevalence of STH can be calculated by the formula below, while the prevalence data obtained is presented descriptively.

$$Prevalence = \frac{Cases\ number}{Total\ samples} x100\%$$

Results

A total of 1.611fresh free-roam dogs feces have been examined. The results present in the table below.

Table 1 The prevalence of STH infection in free roam dogs in each regency of Bali Provonce

Regency	TotalSample	STH	%
Denpasar	168	74	44.05
Badung	139	41	29.5
Gianyar	199	84	42.21
Tabanan	290	129	44.48
Bangli	192	97	50.52
Klungkung	163	43	26.38
Karangasem	175	63	36
Buleleng	149	30	20.13
Negara	136	57	41.91
Total	**1611**	**618**	**38.36**

Table 2. The prevalence ofidentified wormsinfection in free roam dogs in each regency of Bali Provonce

Regency		Prevalence					
	TotalSample	*Ancylostomiasis*	%	*Toxocariasis*	%	*Trichuriasis*	%
Bangli	192	95	49.48	8	4.17	0	0.00
Badung	139	64	46.04	7	5.04	3	2.16
Denpasar	168	74	44.05	13	7.74	1	0.60
Tabanan	290	125	43.10	11	3.79	2	0.69
Gianyar	199	84	42.21	10	5.03	1	0.50
Negara	136	52	38.24	9	6.62	0	0.00
Karangasem	175	57	32.57	16	9.14	1	0.57
Klungkung	163	33	20.25	13	7.98	4	2.45
Buleleng	149	25	16.78	10	6.71	2	1.34

Total	**1611**	**609**	**37.80**	**97**	**6.02**	**14**	**0.87**

Table 3. The combine infection by STH in free roam dogs in each regency of Bali Provonce

Regency	Total Sample	A+ B	%	A+ C	%	B + C	%	A + B + C	%	Total	%
Bangli	192	10	5.95	1	0.60	0	0.00	0	0.00	11	6.55
Badung	139	6	4.32	3	2.16	0	0.00	0	0.00	9	6.47
Karangasem	175	11	6.29	0	0.00	0	0.00	0	0.00	11	6.29
Denpasar	168	10	5.03	1	0.50	0	0.00	0	0.00	11	5.53
Klungkung	163	6	4.03	0	0.00	1	0.67	0	0.00	7	4.70
Negara	136	3	1.84	2	1.23	0	0.00	1	0.61	6	3.68
Gianyar	199	6	3.13	0	0.00	0	0.00	0	0.00	6	3.13
Buleleng	149	4	2.94	0	0.00	0	0.00	0	0.00	4	2.94
Tabanan	290	6	2.07	1	0.34	0	0.00	1	0.34	8	2.76
Total	**1611**	**62**	**3.85**	**8**	**0.50**	**1**	**0.06**	**2**	**0.12**	**73**	**4.53**

Legend: A: *Ancylostoma sp.*; B: *Toxocara sp.*; C: *Trichuris sp.*

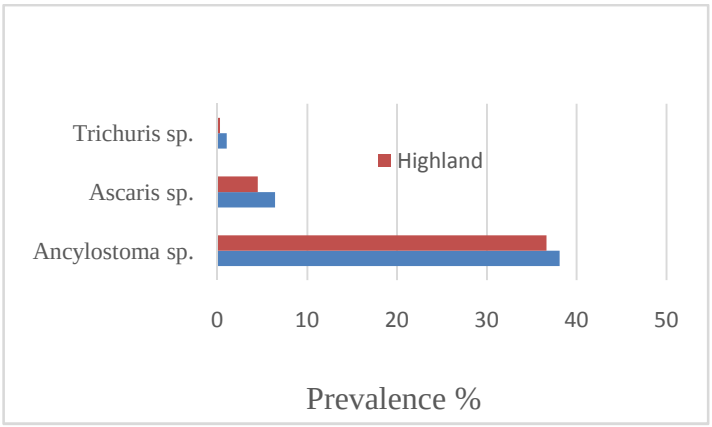

Figure 1. The prevalence of STH infection in free roam dogs in Bali based on sample location.

Discussion

Soil-transmitted helminth is a group of parasitic nematode that infect both humans and animals through the ingestion of infective eggs or through contacting with its larvae. It has also been suggested that domestic cats and dogs play a crucial role in parasitic transmissions to humans and other animals through an environment that has been contaminated with the infected animal'sfeces (WHO, 2016). There are three main species of STHs, which include hookworms, *Toxocarasp*. and *Trichurissp*., that are known to be the cause of major health problems among animalsand human (Jia-Chi*et al.,* 2016).

We found that STH infection in free roam dogs in Bali was high (Tab 1). Hookworm infection leads the prevalence with 37.8% followed by *Ascarissp.* with 6.02% and low prevalence of *Trichuriasis* that was 0.87% (Tab 2). We also found that there were a combined-infections among that worms. The combination between *Ancylostoma sp*.and*Toxocara sp.* was the most common combined-infection. The combined-infection of all three Soil-transmitted helminth also found in two cases (Tab 3).

In the Tab 3 we found that hookworm infection in lowland and highland was slightly different. Hookworm is a parasite that is known to inhabit the small intestines of humans and animals, in dog hookworm species, such as *A. ceylanicum, A. braziliense*and *A. caninum* are known as the agents to cause zoonotic disease in humans (Hotez,2016;Ngui*et al.,* 2014). The eggs of these parasites that are shed in the feces can eventually contaminate the ground where the animal defecates. People become infected when the hookworm larvae penetrate unprotected skin, especially when walking barefoot or sitting on contaminated soil or sand. This can result in a disease called cutaneous larva migrans, where the larvae migrate through the skin and cause inflammation (CDC, 2014). The symptoms caused by these zoonotic hookworms include eosinophilic enteritis, abdominal pain, diarrhea, and less frequent symptoms such as localized myositis and erythema multiforme, and ophthalmological manifestations may occur (Traub *et al.,* 2008; CDC, 2014b). Among the variant species, *A.ceylanicum* is the only species of animal hookworm known to produce patent infections in

humans and it is the second most common hookworm species infecting humans in Asian countries, such as Cambodia, Thailand, Laos, Malaysia, China and the Philippines (Traub *et al.,* 2008; Liu *et al.,* 2014).*A. caninum,* which is the canine hookworm, remainsthe leading cause of human eosinophilic enteritis (Bahgat*et al.,* 1999; Traub *et al.,* 2008).

Toxocariasis results from the zoonotic transmission of roundworms, *T. canis* are related to dog infection. Infection occurs when humans accidentally ingest the embryonated eggs that shed in dog and cat feces via hand to mouth contact. Children are particularly prone to infection because they are exposed to the eggs in sandboxes and on playgrounds contaminated with dog and cat feces (Traversa, 2012; Overgaauw and van Knapen,2013).

Zoonotic *Trichuriasis* is an infection caused by whipworm. A few clinical cases that were triggered by *T. vulpis* originating from dogs were reported in Thailand, the USA and Mexico (Areekul*et al.,* 2010). In comparison to *T. vulpis, T. serrata* and *T. campanula* are the two species of whipworms that can infect cats. There was a low prevalence of parasites in cats reported in previous studies. As a result, whipworm infection in cats is not a primary differential diagnosis for cats with diarrhea (Ketzis*et al.,* 2015).

According to the location of the sample, *Ancylostoma* infection mostly found in lowland by comparison with highland was 2:1. In contrary, *Ascaris* and *Trichuris* infections are more common in highland than lowland, but with only a slight difference. This data demonstrates that the location height affects the prevalence of STH infection.

Conclusion

To conclude, the prevalence of soil-transmitted helminth caused infection of free-roam dogs in Bali was high (38.36%). Soil-transmitted helminth caused infection in dogs in Bali were *Ancylostoma sp*(37.8%), *Ascaris sp.* (6.02%) and *Trichuris sp.* (0.87%).

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STUDY OF MORPHOLOGY AND MORFOMETRY OF KINTAMANI DOG'S DUODENUM

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ABSTRACT

The Kintamani dog is a native dog from Bali that has an attractive and a gorgeous appearance. The aim of this research was to know the morphology and morphometry of kintamani dog's duodenum. This study was used five females kintamani dog. The observation of histologic morphology was used a binocular light microscope with 100x, 200x, and 400x magnification. The results showed that the length of duodenum are 16.2 ± 1.3 cm, and the width of duodenum are 3.1 ± 0.1 cm. The duodenal histological structure is composed of four layers namely tunica mucosa, submucosa, muscularis, and serosa, respectively. The thickness of the mucosal tunica is 1364.584 ± 255,504 µm, the submucosal tunica is 360.136 ± 188.283 µm, the muscularis tunica is 689.178 ± 267.228 µm, and the serous tunica: 25.888 ± 11.93 µm.

Keywords: kintamani dog; histology; morphology; morphometry; duodenum

INTRODUCTION

Dogs are one of the animals that can life side by side with humans. Nowadays, dogs have been different from their wild ancestors, becoming animal figures with various features, especially on sight, hearing, and smell (Budiana, 2006). Indonesia has a race dog that became the only dog of the original race of Indonesia that is the kintamani dog. This dog is a local highland dog, living in the vicinity of Sukawana Village, Kintamani District, Bangli Regency, Bali Province, and in history, this dog is called gembrong dog (Puja, 2007). Kintamani dogs are now increasingly interested because dogs kintamani is the only original dog Indonesia that has an attractive appearance (Gunawan et al., 2012).

The number of dog kintamani enthusiasts is not matched by the many studies of dog kintamani. Where each dog race has different characteristics. Studies on the structure of anatomy and duodenal morphometry in kintamani dog have not been reported at this time. Therefore, it is necessary to do research about it. Preliminary data or information on the structure of duodenal morphologycan be used as a reference for subsequent research such as pathology, immunology, physiology, preclinics, and Subsequent clinics

MATERIALS AND METHODS

This study used a sample of duodenum from kintamani dog. Samples were taken from female kintamani dog during the depopulation program in Sukawana Village. Samples taken from 5 kintamani dogs..

Observation of the anatomical structure was done by direct observation of the duodenum in the abdominal cavity. The observed variables are weight, length, lumen diameter. Measurements were made using the fabric meter and the digital thrust term based on the variables to be observed. Histology preparation was made by referring to the method used by Luna (1968). The staining procedure of HE refers to the Kiernan method (2010).Observations of histological structures were performed in five field of view using a light microscope with 100X, 200X and 400X

objective lens enlargement (Suwiti *et al*., 2015). Histologic image variables observed included duodenal components and layers.Measurements were performed under a microscope using 100X, 200X and 400X objective lens enlargements using a *Calzeiss teaching micro*scope.

Data Collecting Method

The data was collected from the observation of anatomy and histology. Anatomical data obtained from morphological observations and measurements of length and width by opening the duodenum and then measured using a cloth meter. Histologic data were obtained from morphological observation and measurement of mucosal, submucosal, muscularis, and serousine tunica thickness using calzeiss teaching microscope.The data were obtained first, then analyzed. The observations of the morphometry of the anatomical and histological structures were analyzed descriptively qualitatively, while the length, width, and thickness were descriptively quantitative.

RESULTS

Morphology of the Duodenum

The results of morphological observations made on the duodenum of female dog kintamani anatomically obtained the result that the duodenum runs caudally and transversely through the coxae tuber, forming a U-shaped spin.

The morphological observations performed on the female duodenum of female kintamani dog histologically contain 4 layers in the duodenum, respectively the tunica layer of mucosa, submucosa, muscularis and serosa. Then some components are found such as villi, epithelium, goblet cell, crypt lieberkuhn, mucosal muscularis, lamina propria, brunerry gland, circular muscular, longitudinal muscularis, and nerves.

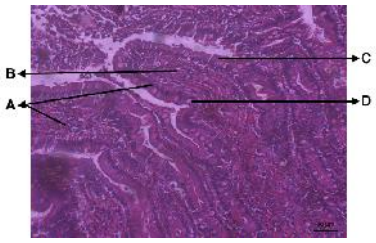
Duodenal morphomet

Table.1 Length and Wide Mean of the Kintamani Dog Duodenum

	Means ï Standard Deviation (cm)
length	16.2 ± 1.3
Wide	3.1 ± 0.1

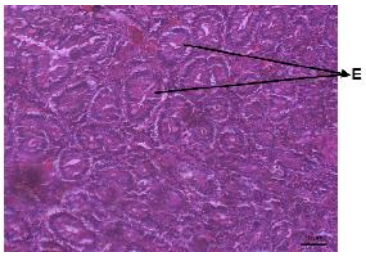
Table.2 Mean of Thickness of Mucosal Layer, Submucosa, Muscularis, and Serosa

	Rata-rata ï Standard Deviation («m)
Mucosa	1364.584 ± 255.504
Sub mucosa	360.136 ± 188.283
Muscularis	689.178 ± 267.228
Serosa	25.888 ± 11.93

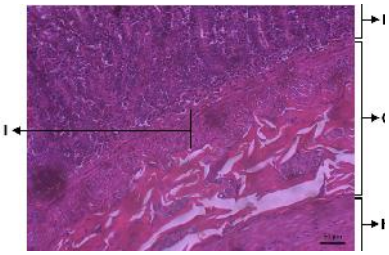


Picture 1. Tunica Mucousa (HE) (200X)

A: Villi, B: Lamina Propria, C: Epitel, D: Goblet cell

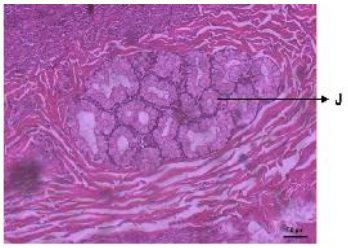


Picture 2. Tunica Mucousa (HE) (200X)
E: Crypt Lieberkuhn

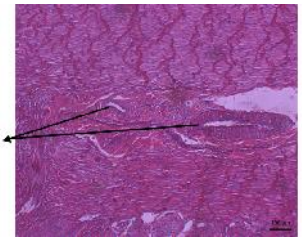


Picture 3. Histologi Duodenum (HE) (200X) F: Tunica Mucousa, G: Tunica Submucousa, H: Tunica Muscularis

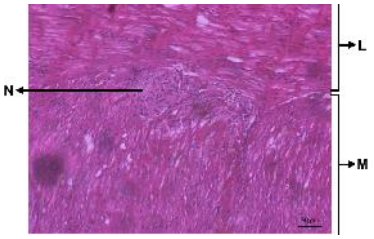
I : Lamina Muscularis



Picture 4. Tunica Submucousa (HE) (200X), J: Kelenjar Sekresi



Picture 5. Tunica Muscoularis (HE) (200X), K: Blood vessel



Picture 6.a Tunica Muscoularis (HE) (200X)
L: Muscularis sirculair, M: Muscularis Longitudinal, N: Nerve.

DISCUSSION

The results showed that the morphology of the female duodenal anatomy of female kintamani dogs runs caudally and transversely through the coxae tuber, forming a U-shaped spin, as well as that of Evans (1993). The cranial part of the duodenum is on the right dorsal, and opposite to the ninth intercoste space, then the duodenum extends along the right abdominal wall to the fourth and sixth lumbar. In this section the dorsal duodenal wall corresponds to the pancreas, the ventral is associated with the jejunum, and medial corresponds to the colon and cecum, and the duodenum extends cranial to the midline of the body between the colon and the mesenteric root (Dyce, 2010).

The results showed that the histology structure of female dog duodenum kintamani composed by four layers, tunika mukosa, submucosa, muscularis and serosa. The tunica mucosa is the innermost part of the duodenum layer. Many of the glands that secrete mucus into the lumen to lubricate the intestinal wall protect against frictional cyme. The mucosal tunica is composed of the three layers, the lamina mucosa, propria, and muscularis as reported by William *et al*. (2012) and Althnaian *et al*. (2013). The epithelium found in the duodenum of the kintamani dog is the simplek columner epithelium. Lamina mucosa is composed from simplex epidermis epithelium. In the mucosal tunica there are villi in the duodenum. Villi serves to expand the absorption surface. The cells found in the epithelium are the "absorptive cell" column cells. Goblet cells are scattered among villian and crypto columnar cells. Lamina propria occupies most of the mucosal tunica. Lamina propria is expanded through the villi core, consisting of loose connective tissue forming the tunic skeleton of mucosa, blood vessels, lymph vessels and smooth muscle (Althnaian *et al*., 2013). Lamina propria contains elastic fibers, leucocytes, and smooth muscle of solitary walking from the mucosal muscularis to the ends of the villus (Suwiti, 2012). The cells found in the lamina propria are goblet cells, crypt lieberkuhn, as well as those reported by William *et al*. (2012). Goblet cells or usually called bowl cells are located between absorbent cells and attached to the juxta-luminal

junctional complex, the basal polar region narrows and buffers the basophilic core and cytoplasm (Suwiti, 2012).

Lamina muscularis is a layer of smooth muscle that separates the tunica mukosa with submucosal tunica (Althnaian *et al*., 2013). In the submucosal tunica layer is found bramner gland and secretion unit. In the muscularis tunica layer there are two layers of longitudinal and circular muscularis, then there are blood vessels and nerves, the muscularis tunica layer which contains smooth muscle tissue. Muscles will contract in the presence of chyme and encourage it, this is reinforced by William *et al*. (2012). Serosa is the outermost layer of the duodenum that acts as the outer skin of the intestine. Serous membranes made of simple squamous epithelium provide smooth and smooth surfaces to prevent friction between the duodenum and surrounding organs. Serousos also secrete serous fluids to further reduce friction and keep the duodenum. The serous layer of tunica consists of a loose connective tissue that continues with mesentery (Dellman and Brown, 1987).

Duodenal morphometry

Based on the results of the length of the female kintamani duodenum length is 16.2 ± 1.3 cm is fairly short compared to the length of dog duodenum reported by Evans. (1993) which has a length of 25 cm. The width of kintamani dog duodenum is 3.1 ± 0.1 cm.

Based on the measurement of the duodenal layer of dog kintamani the thickness of the tunica mucosa is 1.364.5 ± 255.5 mm where the thickness of the mucosal tunica in female kintamani dog is thinner than the tunica mukuosa in dogs reported by Roux. (2015) which has a thickness of 3.54 ± 0.74 mm (3.540 mm). The thickness of the beagle's mucosal tunica tunnels is 3.613.0 ± 170.6 mm (Conto et al., 2014), indicating that the mucosal tunic of the kintamani dog is thinner than that of a beagle. The submissive submissive film thickness of the kintamani dog is 360.1 ± 188.2 mm where the submissive tunica thickness of the

kintamani dog is thicker than the submucosal tunica in dogs reported by Roux (2015) having a thickness of 0.26 ± 0.08 mm (260 mm).

The thickness of the submucosal tunic of the beagle is 227.0 ± 16.8 mm (Conto et al., 2014), indicating that the thickness of the submucosal tunica of the kintamani dog is thicker than that of the beagle. The thickness of the muskularis tunnel of kintamani dogs is 689.1 ± 267.2 mm where the thickness of muskularis tunica in female kintamani dog is thinner than that of the dog muscularis reported by Roux (2015) which has a thickness of 1.04 ± 0.35 mm (1.004 mm). The thickness of the beagle muskularis tunica's tunic is 1,168.3 ± 100.1mm (Conto et al., 2014), it indicates that the thickness of the muskularis of the kintamani dog is thinner than that of the beagle. The thickness of the serous tunic is 25.8 ± 11.9 mm where the thickness of the serous tunica in female kintamni dog is thicker than that of serosa tunica in dogs reported by Roux (2015) having a thickness of 0.02 ± 0.01 mm (20 mm).

CONCLUSION

The duodenum of kintamani dog morphologically anatomically run caudally and transversally through tuber coxae, forming a U-shaped spin. Histological morphology is composed by 4 layers of the tunica layer mukosa, submucosa, muskularis and serosa respectively. Morphometry anatomy of duodenum length is 16.2 ± 1.3 cm and the width is 3.1 ± 0.1 cm. The histological morphometry of the tunica mukosa is 1364.584 ± 255,504 mm, tunica submucosa is 360.136 ± 188.283 mm, tunica muscularis 6is 89.178 ± 267.228 mm, and serosa tunica is 25.888 ± 11.93 mm.

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QUALITY OF EGGS DUCK TO STORE AT ROOM TEMPERATURE

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ABSTRAC

The aim of this research is to know the influence of to store at room temperature eggs of duct that kept for 35 days. The method used in this study was Completely Randomized Design (CRD) with treatment 6 (0,7,14,21,28 and 35 day) with 5 replications and each replication consisted of 10 eggs. Variables observed both exterior and interior, Haugh Unit and egg nutrient content. The result showed that all treatment had no significant effect (P> 0,05) on egg exterior quality, while treatment with H21, H28 and 35 significantly effect (P <0,05) to yolk colour, pH, Haugh Unit. From the research results can be concluded the influence of to store at room temperature eggs of duct that kept for 35 days does not affect the exterior quality of the egg but affect to the interior quality of pH, Haugh Unit stored for 35 days.
Keywords: Duct, eggs, exterior quality, Haugh Unit,interior quality,

INTRODUCTION

The government intensified the livestock sector both from ruminants and non ruminants, especially poultry (laying hens, broilers, native chickens and ducks), to increase meat and egg production as a source of protein. The availability of livestock products from poultry for consumption eggs and eggs for salted egg processing and ceremonial facilities on the market in Bali is very dependent on the supply of eggs from Bali and from outside Bali. Duck eggs in the market in Bali are

supplied from intensive farming systems. Eggs that are offered after being marketed often contain broken eggs if they are broken and do not last long. As food ingredients eggs are one food that is easily contaminated with microbes either directly or indirectly. Direct contamination eggs generally come from spawning grounds, soil, air and poultry droppings. Raji et al. (2009) stated that, the faster the eggs are removed from the cage, the better the effect is to prevent pollution by bacteria.

Food safety is a condition and effort made to prevent food from the possibility of physical, chemical, biological and other foreign contamination that can harm and endanger human health (Schmidt et al., 2009). Egg consumption in the community is now fulfilled not only to fulfill basic living needs but towards functional food-based food (health). Eggs rich in DHA (Docosa Hexanoic Acid) are one of the livestock products of poultry that are currently developing. Fresh eggs, which are eggs that have just been laid by the mother hen, are stored for 10-14 days. After the age of 10-14 days the eggs undergo changes towards damage such as the evaporation of water content through the egg shell pores resulting in reduced egg weight, changes in chemical composition and the occurrence of dilution of egg contents (Melia et al., 2009, Dewi et al., 2017). Eggs are also susceptible to degradation due to microbial contamination, physical damage, and evaporation of water and gases such as carbon dioxide, ammonia, nitrogen and hydrogen sulfide from the eggs (Romanoff and Romanoff, 1963).

According to Yuwanta (2010), in addition to storage time, evaporation of egg contents is also influenced by temperature, relative humidity, eggshell quality and egg health. One of the things that effects egg quality is the health condition of livestock. Generally to maintain the health of livestock, farmers use management to prevent disease. Duck eggs have better quality when compared to chicken eggs because they contain higher protein, calories and fat (Sultoni, 2004). In addition to these advantages, duck eggs also have easy properties. damage. The damage is caused by contamination on eggshells by microorganisms that

come from the mother's stool and those in the cage (Kautsar, 2004). Different duck farming systems also cause differences in the quality of eggs produced.

In intensive farming systems, ducks are caged with all their needs fulfilled and served by farmers (Rasyaf, 1993). Programmed feeding coupled with the provision of vitamins and supplements will greatly affect the quality of the eggs produced. Whereas in semi-intensive farms, ducks when released in the rice fields will find their own food without being regulated by the farmers. The source of feed they get from the rice field environment in the form of insects, snails, small frogs and so on (Susilorini et al., 2008). The difference between duck farming systems will certainly produce different egg quality. But until now, research on egg quality on intensive and traditional farms has never been disclosed. The long time factor for egg storage is a problem that is closely related to aspects of distribution ranging from the level of farmers until the eggs are consumed by consumers. To get the number of consumption eggs in accordance with the number of needs, duck farmers generally store eggs in large quantities for 2-3 days in open space before being marketed to distributors and consumers. Eggs at the distributor level are generally stored for 3 - 5 days at temperatures space, so that there are not a few eggs that have undergone changes in the condition of egg contents in the form of decreasing viscosity of yellow and egg white, increasing pH and enlarging the air cavity in the egg. This happens because a lot of evaporation of liquid and gas from inside the egg causes a lot of internal quality.

Eggs that have decreased when consumed by the community. The longer storage time, the greater the evaporation of liquids and gases in the egg so that the air cavity will be larger which causes thick egg whites to become dilute (Sudaryani, 2003). Wirapartha et al. (2015) obtaining eggs stored at room temperature up to 21 days still yielded a quality score / grade B. Observing the results obtained by Wirapartha et al. (2016) eggs obtained from a farm, marketed in the Badung market in Denpasar City to fulfill aspects health. Wirapartha et al (2017) the effect

of various kinds of broiler egg storage material sold in Badung Market using cardboard material better than the physical and microbiological quality than using wire and wood for 28 days.

Until now, information about the quality of duck eggs at a certain storage period that is stored from the level of farmers to consumers has not been fully revealed. Therefore, it is important to conduct research on the quality of eggs duck to store at room temperature 35 days.

RESEARCH METHODOLOGY

The material used in this study are duck egg samples taken in the Intensive Farm of the Kediri Tabanan area, Infrastructure in this study are tools, egg Yolk Color Fan, Hough Unit, Micrometer made by AMES - USA , Electric Scales, pH meters located at the Poultry, Animal Nutrition and Food Laboratory as well as Microbiology and Animal Product Technology.

The study design used in this study was a Completely Randomized Design (CRD) with 6 treatments with 3 replications and each replication consisted of 10 duck eggs into 180 eggs.

Sampling and Sample Preparation

A sample of duck eggs taken simultaneously from in the Intensive Farm of the Kediri Tabanan area. After 35 days stored at room temperature, eggs are brought to the laboratory for analysis of both interior maun exterior. The tool used in this research ruler, micrometer, pH meter, egg Multi Tester EMT 7300.

Variables: Variables observed both exterior and interior, Haugh Unit and egg grade.

Data Analysis

The resulting data were analyzed by Anova using Completely Randomized Design (RAL) and if there was a significant different between treatments followed by Duncan't test (Sastrosupadi, 2000).

RESULT AND DISCUSSION

Effect of Treatment on Exterior of Storage Duck **Egg for 35 Days**

Influence of duck egg storage can be seen in Table 1. The result of observation of exterior variables on the visible egg weight from each time storage respectively of 66.96 g; 66.90 g.67,04g, 67,25g, 67,05 and 67.04 g stored for 35 days, were statistically not significant (P> 0.05). Result showed that the mean egg width and egg length were not significantly different (P> 0.05) between the treatment of storage time at room temperature.

Result showed that the mean egg index, respectively 74.95, 74.19, 74,06, 74,50,74,59 and 74.54 were statistically not significant (P> 0.05) (Table 1).

Table 1. Effect of Treatment on Exterior, Interior and of Duck Eggs

Variabel	PERLAKUAN 1)						SEM
	H0	H7	H14	H21	H28	H35	
EXSTERIOR							
Egg Weight (g)	66.96[a]	66.90[a]	67.04[a]	67.25[a]	67.05[a]	67.04[a]	0.125
Egg Width (cm)	4.50[a]	4.45[a]	4.47[a]	4.45[a]	4.44[a]	4.48[a]	0.123
Eggs Length(cm)	6.05[a]	6.00[a]	6.04[a]	6.02[a]	6.00[a]	6.00[a]	0.236
Eggs Index	74.95[a]	74.19[a]	74.06[a]	74.50[a]	74.59[a]	74.54[a]	0.189

INTERIOR							
Egg Shell Thickness (mm)	0.429[a]	0.434[a]	0.414[a]	0.404[a]	0,.413[a]	0.411[a]	0.097
Egg Shell Weight (g)	8.70[a]	8.52[a]	8.78[a]	8.49[a]	8.30[a]	8.49[a]	0.002
Egg yolk colour	13.42[a]	13.17[a]	13.23[a]	12.72[b]	11.94[b]	12.28[b]2)	0.14
pH	7.50[c]	7.80[b]	7,84[b]	7,90[a]	7,95[a]	8,07[a]	0.116
HoughUnit	78.21[a]	7703[a]	71,87[a]	69,59[a]	58,98[b]	46,83[c]	0.236
Grade	AA	AA	AA	A	B	B	

Description: 1) H0: 0 day storage H7: 7 day storage H14: 14 day storage H21: 7 day storage H28: 0 day storage

H35: 35 day storage.

2) Same superscript on the same line is not significantly different (P> 0.05).

3) SEM: *Standard Error of Means*

Effect of Treatment on Interior of Storage Duck Egg for 35 Days

The interior of the eggs observed after receiving storage treatment can be seen in Table 1. Duck Eggs stored for 35 days have consecutive egg sell thicknesses of 0,435 mm, 0,438 mm and 0,436 mm statistically not significant different (P> 0 , 05).

The weight of egg shell was obtained higher - smaller from treatment H0 –H35 was not significantly different (P> 0.05) Table 1.

The mean pH of eggs treated by storage time H0,H7 and H14 vs H21, H28 and H35.was significantly different (P <0.05). The longer time eggs are stored causing the pH of the egg to increase. Due to the increase in pH resulting in egg whites getting thinner, because of the loss of CO_2 through the egg shell causes the ion

concentration bicarbonate in the egg white decreases and damages the buffer system. So that the pH of the egg rises and egg white become base (Jazil, 2013) and Cornelia (2014) are also fibers ovomusin (gives a thick texture).

The effect of storage treatment on yolk color measured using *Yolk Color Fan* ranged from 11.94 to 13.42 statistically significant effect (P> 0.05). The highest yolk color was obtained in storage with treatment 13.42 (H0), 13.17(H7), 13.23(H14) significant effect (P> 0.05) than 12.72 (H21), 11.94(H28) and 12.28 (H35). The color of the egg yolk is determined by many factors such as environment (storage time, temperature, hen age, disease, strain of bird, and genetic (Ahmadi and Rahmini, 2011). The most dominant factor influencing is feed. As Sudaryani (2003) notes that good egg yolks range from 9 to 12. Mean the egg yolk color this research ranged from 11.94 – 13.42 is higher than suggested by Yuanta (2010).

CONCLUSION

From the research results can be concluded the influence of to store at room temperature eggs of duct that kept for 35 days does not affect the exterior quality of the egg affect the interior quality of pH, Haugh Unit, stored for 35 days.

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COMPARISON OF HEAD INDEX AND BODY MORPHOMETRIC BETWEEN WHITE, BLACK, BROWN AND MIXED COLORED FUR MALE KINTAMANI DOGS

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ABSTRACT

Determination of breed standards in dog kintamani is needed as a breeding reference. Dogs are animals that have the size and shape of the skull that is different from each breed. This study aims to determine the kinship relationship among adult white, black, brown, and mixed adult kintamani dogs through the head index and morphometric approach of the body. This research is an observational analytic study with cross sectional study design. This study used 15 black, black, brown and mixedcolored adult male kintamani dogs with the age of 1 year and over. Samples were obtained in Denpasar, Gianyar, Bangli, Klungkung, Tabanan, Karangasem, State and Sukawana villages as their native habitat. These dogs were measured in normal condition and clinically the dog is healthy and no deformed. Morphometric index data for head

and body were analyzed by one-way analysis of variance. The results showed that the index of the head and body morphometric among white, black, brown and mixed colored adult male kintamani dogs were no significant differences (P> 0.05).From these results it can be concluded that among white dog, black, brown and mixedcolored adult male kintamani dogscan be considered as the same breed. However, further research is needed to concerning kinship on the level of the gene.
Keywords: Kintamani Dog; morphometric body; head index

INTRODUCTION

Kintamani dogs are famous in dog lovers because they have notable characteristics, including moderate size, long fur, white coloured with cream coloured fur on the ears, shining sharp eyes, erect ears, darkly pigmented nose, red tongue, long fur around their neck and standing tail like a squirrel's tail. According to the observation of the outside parts, kintamani dogs have various fur colours, including white, black, brown, and mixed. However, white kintamani dogs dominate or most often seen; as a result, they are mostly known as the kintamani dog (anon, 1993).

Wayne and Gittleman (1995) stated that two groups of species is different and will grow separately may be caused by different characteristics of each group such as the size of the skull. According to Evan (1993), there is a considerable difference in dog skulls, which may be due to significant differences in the facial

region of every breed. To prevent mistakes in determining head size, head index measurement is performed. This index will give relative size of the head and profile of two-dimensional relationship of the head.

Mayr (1981) state, until now, morphological sizes such as body height, length, thigh width, chest width, hip width and hip height is still being used to determine the kinship in animals other than other different parts such as chromosomes, enzymes, blood proteins, and genetic DNA.

METHODS

Research Design

This research is an analytical observational study with a cross-sectional design with the consideration that no intervention was performed, only observation of phenomena and the study was conducted in particular times (Babbie, 1983).

Research Sample

The population was adult male kintamani dogs (aged 12 months or more) with healthy physical condition (no defects or limping). These samples were obtained from the region of Denpasar, Gianyar Regency, Bangli Regency, Klungkung Regency, Tabanan Regency, Karangasem Regency, Negara Regency and from their native habitat, which is Sukawana village, Kintamani District. These dogs were divided according to their fur colour.

Research Procedure

This study was conducted by measuring the parts of the dogs' body using measuring tape. These dogs were measured while standing with four legs with forelegs slightly leaned forward, and hind legs were slightly pulled backwards. These dogs were measured in normal condition, which means that they were clinically healthy without any abnormalities. The parts of the body measured were:

(a) Head width:The distance between the outermost parietal bone measured horizontally

(b) Head length: The distance from inion to nasion

(c) Head index: $\frac{\text{Head Width}}{\text{Head Length}} \times 100$

(d) Body height:The distance from the highest point to the ground along the front legs

(e) Chest width: The distance between the widest part of each side of costae

(f) Body length: The distance between *crista humeri* and *tuber ischia*

(g) Hip height: The distance from the end of glutes area to the ground along hind legs

(h) Hip width: The widest part between outer *tuber coxae*

(i) Thigh width: The widest part between lateral sides of the thigh

Data Analysis

Data obtained from the head index and body morphometric were analyzed by one-way analysis of variance. If any differences were found, then BNT 5% test was conducted (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Measurement results of the average head index, body length, hip height, chest width, thigh width, hip width, and measurement of body weight of white, black, brown, and mixed colored kintamani dogs can be seen in Table 1.

Table 1. Mean of Head Index and Body Morphometric between White, Black, Brown, and Mixed Colored Adult Male Kintamani Dogs (cm).

Parameter	Fur Color			
	White	Black	Brown	Mixed
Head Index	77.9553[a]	77.8935[a]	77.9487[a]	78.0347[a]
Body Length	55.7667[a]	52.4667[a]	53.2333[a]	53.0333[a]
Body Height	50.7667[a]	50.2000[a]	51.5667[a]	50.5333[a]
Chest Width	19.1333[a]	18.0000[a]	19.3667[a]	17.8000[a]
Thigh Width	16.8000[a]	17.4333[a]	17.7333[a]	17.6000[a]
Hip Width	12.7000[a]	12.0667[a]	12.9667[a]	11.7500[a]
Hip Height	49.4333[a]	48.7667[a]	48.3000[a]	49.3667[a]

The numbers followed by the same letters in the same row showed insignificant difference ($p > 0.05$).

Mean head index and body morphometric did not show any differences between white, black, brown, and mixed colored kintamani dogs. After analyzed statistically using one-way variance test, the results showed that head index and body morphometric differences between white, black, brown, and mixed colored kintamani dogs were not significant ($p> 0.05$).

From above data, distribution of measurements from one group was found overlapping with other groups, which means that those groups might be considered as the same breed. These resultswere supported by Evan (1993) who stated that breeds are the group of animals originated from the same ancestor and had characteristics that can be differentiated between one another. In determining a breed, especially in dogs, head size is often used as consideration. The skull size and shape between dog breeds are different. Surjoatmodjo (1992) also stated that in cow breeds in Indonesia, body morphometric analysis could also be used to determine the kinship of cows in Indonesia.

CONCLUSION

This study concluded that no differences were found in the measurement of the head index and body morphometric between white, black, brown, and mixed colored adult male kintamani dogs.

SUGGESTION

Further studies should be conducted involving the correlation of kinship between white, brown, black, and mixed colored adult male kintamani dogs in higher level, such as genetic level.

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SUBSTITUTION OF WOOD POWDER MEDIA WITH CARDBOARD ON THE GROWTH AND RESULT OF WHITE OYSTER MUSHROOM

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ABSTRACT

Oyster mushrooms is a consumption mushrooms that grow on moldy wood orwood powder. However, oyster mushrooms can also be cultivated using cardboard media. The study aimed to determine the effect of substitution of wood powder planting media with cardboard and coconut water and get the best media for substituting the planted media of wood powder with cardboard and coconut water on the growth of white oyster mushrooms. The main variables measured are: 1) Growth time and mycelium length (cm / day), growth time of mycelium (after planting) until evenly distributed in the log. 2) growth time of fruit body (days), starting the baglog ring is opened until the fruit body appears. 3) Number of hoods per log (fruit) at harvest. Harvesting is carried out up to 3

harvest periods with the criteria of a cover> 2 cm in diameter and then averaged. 4) Fruit body weight per log (grams) at harvest (up to 3 harvest periods). Based on the results of the study, we concluded that: there was an effect of substitution of wood powder planting media with cardboard on the growth and yield of white oyster mushrooms. It is 100% of sawdust media had the fastest time and length of mycelium distribution (6 weeks) as well as the fastest time of fruit body (15.17 days or ± 57.17 days) and on media 100% cardboard gave the highest fruit body weight (215.17 grams) and a combination of 40% wood powder and 60% cardboard gave the highest number of caps (12.89 strands).

Keywords: Oyster mushrooms; plant media; sawdust, cardboard and substitution; growth; result.

1. Introduction

Oyster mushroom is one type of consumption mushroom that grows on moldy wood or wood powder. The preferred medium for the mushroom is carbon contained in the form of polysaccharides, nitrogen, ammonium or nitrate. The growth of oyster mushrooms is dependent on the growing media, the availability of seeds, maintenance ways and the environmental conditions of the area (Djarijah, 2001).A common growing medium for the cultivation of oyster mushrooms is wood powder. Wood powder as a main ingredient used as a growing medium in the form of baglog (Sumiati, 2005). Suriawiria (2004) explained that wood sawdust is a place for growing oyster mushrooms which are

classified as fungi that use cellulose, hemicellulose and lignin which can break down and utilize wood components as a source of carbon. Along with its development, oyster mushrooms can also be cultivated using other media, such as cardboard (Agromedia, 2009).

Cardboard media comes from wood powder or wood pulp containing high cellulose. The fact indicates that cardboard can be used as a medium for the growth of oyster mushrooms and various other types of consumption mushrooms. Suharjo (2008) explained that harvested mushroom from cardboard media proved to be superior, namely whiter, chewier and harvested mushrooms had a longer shelf life than using ordinary wood powder media. In addition cardboard has other advantages that are easy to obtain, able to reduce costs or capital for procurement of planting media, does not contain oil or sap or heavy metals which are generally found in wood powder media (Suharjo, 2015). But the use of cardboard as a medium for oyster mushrooms is not widely known. Therefore, a study entitled "Substitution of Wood Powder Planting Media with Cardboard on the Growth and Yield of White Oyster Mushrooms" was conducted.

Formulation of problems

Based on the description above, the question of the problems in the study are:

1. Are there any effects of substitution of planting media for wood powder with cardboard and coconut water to the growth of white oyster mushrooms?

2. Which media shows the best influence on the substitution of wood powder media with cardboard and coconut water to the growth of white oyster mushrooms?

Objectives

This research aimed to :

1. Know the effect of planting media on wood powder with cardboard and coconut water to the growth of white oyster mushrooms.

2. Obtain the best media for substituting wood powder with cardboard and coconut water to the growth of white oyster mushrooms.

RESEARCH METHODS

Experimental design

The study used a single-factor of Completed Randomized Design(Gaspersz, 1991), which had 6 (six) treatments and replied six times.

The treatment is:

A0 = 100% wood powder

A1 = 80% wood powder and 20% cardboard

A2 = 60% wood powder and 40% cardboard

A3 = 40% wood powder and 60% cardboard

A4 = 20% wood powder and 80% cardboard

A5 = 100% cardboard

Thus, there are 36 experimental units. Placement of treatment into each test is done randomly.

Research Implementation

The research implementation includes:

1. Preparation

The activities carried out include a) Cleaned and sterilised thearea using sprinkling lime on the floor. Alcohol used with handsprayer to all parts of the area both on the roof, walls, floors and planting racks. b) media preparations such as sawdust, used cardboard, gypsum powder, agricultural lime, bran, sugar, coconut water and water. Wood powder mediasifted so that it is clean and prepared cardboard media before use or subsidized from dirt, plastic and duct tape. Put into lime water for a day and a night, then boil for 3 hours. Let stand for ± 60 minutes, drained and dried for a day and a night until there is no water dripping, but the humidity level is still sufficient.

2. Mixing media

Mixing treatment media A0, 100% total material consists of: 100% powder, 1% gypsum, 1% lime and 20% bran then all ingredients are mixed until evenly distributed. Then made 0.02% sugar solution, 15% coconut water and 85% water then stirred until homogeneous. The solution is mixed in a wood powder material (100% = 60 kg) to the material if it is still packed (the

water does not drip from the material). The material is filled into a plastic log. Plastic logs are closed using paralon and rice paper which is then tied using rubber then sterilized. Other treatments has the same way to mix the difference is the composition of the media.The treatment media are A1- 80% sawdust and 20% cardboard, 1% gypsum, 1% lime and 20% bran then all ingredients are mixed evenly; A2- sawdust 60% and cardboard 40%, gypsum 1%, lime 1% and bran 20% then all ingredients mixed until evenly distributed; A3- sawdust 40% and cardboard 60%, gypsum 1%, lime 1% and bran 10% then all ingredients are mixed evenly; A4 - sawdust 20% and cardboard 80%, gypsum 1%, lime 1% and bran 10% then all ingredients are mixed evenly; and A5- 100% cardboard, 1% gypsum, 1% lime and 10% bran then all ingredients are mixed evenly.

3. Pasteurization of baglog

Baglog pasteurilizationis done by heating baglog on a sterilization drum using a stove that is fueled by 5 liters of kerosene for ± 8 hours (until the kerosene runs out).

4. Planting

Planting is done by filling the log ± 2 tablespoons (1 g) of mushroom seeds. After planting, then the logs are arranged on a shelf that has been prepared according to the treatment.

5. Incubation period

The incubation period is the period of mycelium growth in the log that has been planted with fungus seedlings starting

from the seedlings planted until the mycelium grows to fill the media. Mycelium is grown at room temperature 23-280C.

6. Baglog ring opening

After the mycelium has filled the baglog, the lid is opened and the log ring is removed. Mycelium gets oxygen so that the growth of the oyster mushroom can be maximized and then ready to maintain

7. Maintenance

Maintenance carried out is:

a. Control of temperature and humidity

The temperature and humidity of the kumbung are intensively controlled. The temperature required is 25-280C while humidity is 70-90%. If the air temperature in the kumbung is too high, it is lowered by watering the kumbung floor.

b. Irrigation

Watering on the log or floor of the kumbung with a fogging system that aims to maintain temperature and humidity.

c. Pest control

Pests found during the cultivation process are spider pests.

Although this pest does not have an effect or adverse impact on the growth of oyster mushrooms, it makes the kumbung less attractive to look at so it needs to be cleaned up.

8. Harvest

Harvesting is generally on the 33rd day to 45 days or two days after the growth of the fruit body. Harvesting can be done manually, which is picked by hand.

Observation Variable

Supporting parameters are the temperature measured using a thermometer and humidity which is measured using a hygrometer two times a dayin the morning and eveningthen averaged.

The main variables measured are:

1. Mycelium growth time (cm / day) starts after planting until evenly distributed in the log.

2. When the fruit body grows (days), the baglog ring is opened until the fruit body appears.

3. Number of hoods per log (fruit) and fruit weight per log (grams) at harvest. Harvesting is done up to 3 harvest periods. The hood that is counted is a hood that has a diameter more than 2 cm.

RESULTS AND DISCUSSION

Temperature and humidity

Room temperature observed from January to April 2017 was measured using a room temperature thermometer. Temperature measurements are carried out in the morning and evening. The mean temperature data in the morning and evening can be seen in Table 4.1.

Table 4.1. Average Room Temperature of White Oyster Mushroom

Observing Time	**Temperature (^{0}C)**	
	ning	**Evening**
January	27,46	27,68
February	26,90	27,61
March	26,46	25,46
April	27,00	26,35
Average	**26,99**	**26,77**

Moisture measurement is done using a hygrometer in the morning and evening. The mean data of morning and evening humidity can be seen in Table 4.2.

Table 4.2. Average Moisture of the Room for White Oyster Mushrooms

Observing Time	Humidity (%)	
	Morning	**Evening**
January	92,77	92,85
February	92,50	93,60
March	93,69	91,85
April	91,70	89,30
	Average	**91,90**

Tables 4.1 and 4.2 show the average growth temperature of oyster mushrooms, in the morning of 26.99 ^{0}C and at night which is 26.77 ^{0}C. While the average humidity in the morning was 92.67% and at night it was 91.90%. Temperature and humidity significantly affected the growth of mycelium. If high temperature and humidity will affect the growth of mycelium because it can cause growth to become obstructed and cause contaminated media. Likewise if temperature and humidity are low the growth of mycelium becomes obstructed. This was in accordance with Suharjo's (2015) opinion which states that in general the optimal temperature for the growth of oyster mushrooms in the incubation phase is the air temperature ranges from 22-280C with room humidity 70-90% while the oxygen gas content is relatively low but the need for carbon dioxide gas was relatively high especially in the growth of mycelium. In the research conducted the average temperature

produced was 26.77-26.99 ^{0}C and humidity 91.90-92.67%. This shows that the temperature produced is suitable, but the resulting average humidity is higher so that it affects the growth of oyster mushrooms, especially in the process of spreading mycelium in baglog media.

Growth Time of Oyster Mycelium

Table 4.3. Average time of Growth and Length of white oyster mycelium (cm/day)

Treatment	Weeks											
	I				III		IV		V		VI	
100% Wood powder	0,28	A	5,03	ab	9,24	B	12,39	b	15,23	c	16,00	c
80% powder+20% cardboard	1,02	A	4,07	a	6,57	Ab	7,75	a	8,68	ab	10,29	ab
60% powder+40% cardboard	2,43	B	7,50	b	7,92	B	6,81	a	11,41	b	11,79	ab
40% powder+60% cardboard	0,55	A	7,22	b	8,05	B	11,82	b	10,57	b	12,25	b
20% powder+80% cardboard	0,40	A	2,82	a	4,35	A	5,80	a	7,18	a	8,98	a
100% cardboard	0,58	A	6,78	b	8,39	B	11,33	b	9,15	ab	11,28	ab
BNJ 5%	**0,88**		**2,65**		**3,35**		**3,27**		**3,04**		**2,94**	

Treatment	Weeks									
	VII		VIII		IX		X		XI	
100% wood powder	16,00	b	16,00	b	16,00	a	16,00	a	16,00	a
80% powder+20% cardboard	12,35	a	14,38	a b	15,13	a	15,67	a	16,00	a
60% powder+40% cardboard	13,59	a b	13,56	a b	14,79	a	16,00	a	16,00	a
40% serbuk+60% cardboard	13,57	a b	13,53	a b	15,43	a	16,00	a	16,00	a
20% powder+80% cardboard	11,13	a	12,88	a	14,47	a	15,95	a	16,00	a
100% cardboard	12,00	a	14,95	a b	15,76	a	16,00	a	16,00	a
BNJ 5%	**2,89**		**2,52**		**tn**		**tn**		**tn**	

Number followed by the same alphabet in every coloum showed unreal different to BNJ 5% test.

The results of the analysis of variance showed that the substitution of wood powder with cardboard and coconut water in the calculation of age 1-11 MST showed that ages 9, 10 and 11 MST had no significant effect on the growth time of white oyster mushroom mycelium while at age 1 MST to 8 MST gives a very real effect on the growth time of white oyster mushroom mycelium (Appendices 2.1 to 2.11). The results of the BNJ test of 5% level on the time of growing white oyster mushroom mycelium are presented in Table 4.3.

Table 4.3 shows that the mean growth time of white oyster mushroom mycelium at the ages of 9, 10 and 11 MST was not found to have a significant effect on the mycelium length. This is because the length of the mycelium has fulfilled the baglog of mushrooms (16.00 cm) from the age of 6 (100% wood powder), 9 and 10 MST. During the mycelium growth process from 1 MST to 8 MST showed a better influence on the growth time of white oyster mushroom mycelium.

The fastest growth of mycelium is in the 100% treatment of wood powder where the growth time needed for mycelium to meet baglog is 6 MST. This is because the wood powder media is a medium that is easily passed by the mycelium and has good porosity so that it can accelerate the growth of mycelium because the nutrients needed for the growth process are available. The results of this study are supported by Muffarihah's (2009) statement that the growth of mycelium is influenced by decomposed media so that the mycelium grows rapidly evenly. The decomposed media has many nutrients such as C, N, P and K which can be absorbed by the fungus, so that the mycelium will grow faster. Mycelium growing time is also affected by temperature and humidity. Optimal temperature and humidity for mycelium growth ranged from 22-280C and humidity of 70-90%, during the study the room temperature in the morning was 26.990C and nighttime was 26.770C, while in the morning 92.67% and nighttime 91.90% . Although the temperature during the study is optimal, the humidity tends to be high so that it can become an inhibitor in the growth of mycelium.

Unlike the combined treatment where 20% of wood powder and 80% of cardboard treatment showed rapid mycelium growth, especially at 1 MST with a mycelium length of 0.40 cm but the

time of mycelial fulfillment in baglog tended to be slow to 11 MST. The next treatment was the treatment of 40% wood powder and 60% cardboard which also showed rapid mycelium growth especially at 1 MST which was 0.50 cm but the time of mycelium fulfillment in baglog tended to be slow to 10 MST. Sawdust media is a medium that is easily traversed by the mycelium compared to cardboard media because it has a larger cavity than a dense cardboard media so that the mycelium meets baglog faster. In addition, according to Indriyani (2014) the growth of mycelium is supported by several factors, namely media composting factors, nutrition and environment, as well as the level of media density which affect the duration of mycelium growth. Cardboard media is a solid medium so that the media becomes difficult to decompose which affects the availability of nutrients which causes slow growth of mycelium.

Growing Time of Fruit Body and Body Weight of Fruit

The results of the analysis of variance showed that the substitution of wood powder planting media with cardboard and coconut water had a very real influence on the time of growing the body of the white oyster mushroom fruit. The results of the BNJ further test at 5% level on the body growth time of white oyster mushroom fruit are listed in Table 4.4.

Tabel 4.4. Average time of fruit body growth (days)

Treatment	Time growth (days)	
A1 = 100% Wood powder	15,17	a
A2 = 80% powder+20% cardboard	27,89	b
A3 = 60% powder+40% cardboard	26,17	b
A4 = 40% powder+60% cardboard	16,50	a
A5 = 20% powder+80% cardboard	29,72	b
A6 = 100% cardboard	25,67	b
BNJ 5%	**5,20**	

Number followed by the same alphabet in every coloum showed unreal different to BNJ 5% test.

Table 4.4 shows that the fastest average fruit body growth time is 15.17 days obtained in 100% wood powder treatment but not significantly different with 40% wood powder and 60% cardboard media which is 16.50 days. Whereas for the slowest fruit growing time, 20% of wood powder and 80% of cardboard were treated at 29.72 days but not significantly different with 80% wood powder and 20% cardboard, 60% wood powder and 40% cardboard and 100% cardboard with the average of each media that is 27.89 days, 26.17 days and 25.67 days.The time to grow mycelium will affect the growth time of the fruit body. According to Sumiati et al., (2005) the faster the spread of mycelium, the faster it will be in the formation of the fruit body in the fungus. This is in line with research that has been carried out in which the treatment of 100% wood powder is the fastest treatment for the growth time of mycelium and the time of appearance of the fruiting body. Likewise with other treatments that are 100% cardboard, 40% wood powder and 60% cardboard, 60%

wood powder and 40% cardboard, 20% wood powder and 80% cardboard and 80% wood powder and 20% cardboard which give the same results on the growth time of mycelium and the time to grow the body of the white oyster mushroom fruit.

The Number of Oyster Mushroom Hoods

The results of the analysis of variance showed that the substitution of wood powder planting media with cardboard and coconut water had a very real effect on the number of hoods of white oyster mushrooms. The results of further testing BNJ level of 5% to the number of hoods White oyster mushrooms can be seen in Table 4.5.

Tabel 4.5. The number of white oyster mushroomshoods(Strands)

Perlakuan	**Jumlah Tudung (Helai)**	
100% wood powder	7,39	a
80% wood powder + 20% cardboard	8,00	ab
60% wood powder + 40% cardboard	9,56	bc
40% wood powder + 60% cardboard	12,89	d
20% wood powder + 80% cardboard	9,89	c
100% cardboard	7,89	ab
BNJ 5%	**1,88**	

Number followed by the same alphabet in every coloum showed unreal different to BNJ 5% test.

Table 4.5 shows the average number of oyster mushroom fruit caps that are mostly in the treatment of 40% sawdust and 60% cardboard, which is 12.89 pieces, while the lowest number of oyster mushroom bodies in the treatment of 100% wood powder is 7.39 pieces, but not significantly different from the treatment of 80% wood powder and 20% cardboard and 100% cardboard. From the available data shows that the growth time of mycelium and the growing time of the fruit body does not affect the number of hoods. Oyster mushrooms are produced because in this process the more important role is the nutrients contained in the media. Cardboard and sawdust contain the cellulose needed for the growth of oyster mushrooms. In addition to containing high cellulose, cardboard also contains lignin which is quite high which is needed by fungi in the formation of the body and mushroom hood. This is in accordance with Periadnadi's statement (2013), that high cellulose content will increase the production of cellulose enzymes which help in the formation of the mushroom fruit body. The number of hoods produced has an effect on the diameter of the hood, because the more hoods produced, the smaller the diameter of the hood, this is due to competition in nutrient absorption.

Besides that, potassium produced by coconut water can also help the formation of primodia so that the number of fruit caps that are formed are also many. This is in agreement with Muffarihah (2009) that the content of potassium produced by coconut water

also affects the work of enzymes so that the enzyme's work becomes smooth and the fungus obtains sufficient energy, so that in the formation of primodia smoothly and automatically the number of fruit hoods is formed too much.

The results of the variance analysis showed that the substitution of wood powder planting media with cardboard and coconut water had a very significant effect on the body weight of white oyster mushroom fruit. The results of further testing of BNJ level of 5% on body weight of white oyster mushroom fruit are listed in Table 4.6.

Table 4.6. The average body weight of white oyster mushrooms

Treatment	**Fruit body weight (g)**	
100% wood powder	100,89	a
80% wood powder+ 20% cardboard	137,06	bc
60% wood powder+ 40% cardboard	116,61	ab
40% wood powder+ 60% cardboard	117,22	ab
20% wood powder+ 80% cardboard	155,56	c
100% cardboard	215,17	d
BNJ 5%	**31,90**	

Number followed by the same alphabet in every coloum showed unreal different to BNJ 5% test.

Tabel 4.6 shows that the lowest body weight of oyster mushroom fruit is on 100% wood powder which is 100.89 grams,

but not significantly different from the medium of 60% wood powder and 40% cardboard (116.61 grams) and 40% wood powder and 60% cardboard (117.22 grams). The highest body weight of oyster mushroom fruit is on 100% cardboard media which is 215.17 grams. This is because cardboard contains high levels of cellulose and lignin needed by fungi in the formation of the body and a mushroom hood. This is in accordance with Periadnadi's statement (2013), that high cellulose content will increase the production of cellulose enzymes which help in the formation of the mushroom fruit body.

In addition, the body weight of the fruit is also influenced by the number and diameter of the hood produced. The smaller the number of hoods produced, the greater the resulting stem diameter. More nutrients are absorbed which affect the body weight of the fruit produced.This is in line with the results of Murti's research (2015), wherein oyster mushrooms grown on cardboard media were 375 grams and 50 ml of cherry water produced the lowest average hood, which was 6 pieces but had the highest average hood weight of 79.16 grams.

CONCLUSION

Based on the results of the study can be concluded as follows:

1. There is an effect of substitution of wood powder planting media with cardboard on the growth and yield of white oyster mushrooms.
2. The effect of treatment without combination are 100% wood powder give the fastest time and length of mycelium distribution (6 weeks) and the fastest fruit body growth time (15.17 days) or (± 57.17 days) and in the treatment without combination, 100 % cardboard gives the highest yield on fruit weight (215.17 grams). Whereas in the treatment with a combination gives an effect on the highest number of caps (12.89 strands) in the treatment of 40% wood powder and 60% cardboard.

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The Transmission of Citrus Vein Phloem Degeneration (CVPD) Disease by *Diaphorina citri* Kuwayama (Homoptera : Psyllidae) in Siam Citrus Plants

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Abstract

The spreading rate of CVPD among citrus plants in Langkan and Katung villages (Subdistrict of Kintamani, Bangli Regency) reaches 32% per year, while the population of *D. citri* as a vector is relatively low, so a research must be conducted on the minimum number of the insects that effectively acts as a vector. This study aimed to determine the minimum number of *D. citri* insects which can transmit the CVPD disease, the incubation period of the disease in Siam citrus plants and the pathogenic properties of the insects. The research was done in Greenhouse Station, Department of Plant Pests and Diseases, Faculty of Agriculture, Udayana University. The result showed that one imago of *D. citri* could transmit the disease, therefore, when the number of vectors were increased, the faster the signs of the disease arose. *Liberobacter asiaticum* has a persistent nature inside the vectors.

Keywords: *D. citri*, incubation period, persistent, CVPD, citrus plants

Introduction

Citrus Vein Phloem Degeneration (CVPD) disease is classified as one of the most important diseases in citrus plants which has spread widely in Bali and has become the main obstacle of developmental efforts to increase citrus production. The cause of CVPD disease, which is often called by citrus greening or huanglongbin, is *Liberobacter*. This organism is in the subdivision of Protobacteria (Sandrine *et al*. 1996). *Liberobacter* lives inside the phloem of citrus plants and causes typical signs. It has not been able to be bred on artificial media (Wirawan, 2001).

The transmission of this disease is done by *Diaphorina citri* Kuw. (Homoptera: Psyllidar) (Tirtawidjaja & Suharsojo 1990, Wirawan 2000). The factors influencing the transmission of this disease are the number of *D. citri* population as the vectors and the presence of the inoculums sources (Chen, 1998).

Besides *D. citri* vector, CVPD disease can also be transmitted by the infected seedlings. The seeds of citrus plants which look healthy from the outside may be infected by the pathogen of CVPD because the incubation period of this pathogen in the host plants is around three to five months (Tirtawidjaja & Suharsodjo, 1990), hence the early detection of the pathogen's presence is needed to be done accurately and immediately.

The population of *D. citri* in Langkan and Katung villages is relatively low, approximately 0.12 – 9.9 organisms per plant, but the spreading rate of the CVPD disease is very high, which reaches 32% each year. Thus, a research must be conducted on the minimum number of the insects that effectively acts as a vector.

The objective of this study

The objective of this study are to determine the minimum amount of *D. citri* insects which can transmit the CVPD disease, the incubation period of the disease in Siam citrus plants and the pathogenic properties of the insects.

Materials and Tools

The materials and tools used in this research are: (1) the seeds of Siam citrus, (2) plastic glasses with a diameter of 7 cm and a height of 10 cm as containers, (3) cylindrical enclosures made of mylar plastic with a diameter of 7.5 cm and a height of 13 cm with a cloth cover, (4) fertilizers made from cow dung, (5) aspirators, (6) PCR analysis tools.

Implementation of Research

The amount of *D. citri* as a vector in CVPD disease

The infective imagoes of *D. citri* were introduced into the cylindrical enclosure of mylar plastic, where the four-week-old-

seeds of citrus plants were inside, with the interventions of 1, 3 and 5 organisms per plants. The imagoes were taken from the result of CVPD infected citrus plants breeding. Every intervention was done six times. The previously inoculated plants then were saved inside boxes which were insect resistant. Two weeks after the inoculation, *D. citri* imagoes were killed using insecticide. The maintenance and observation of the plants were done every day. After showing some signs of the disease, the leaves of the plants were taken and analyzed using PCR.

DNA Extraction

DNA from all citrus leaves samples were extracted using 'Genomic DNA Kit from Plant Maccherey-Nagel' conducted at the Genetic and Biomolecular Resource Laboratory, Udayana University, Denpasar. A total of 100 mg of the mother's bone leaves were cut into small pieces and added with liquid Nitrogen and crushed until smooth in a mortar. The sample was put into an Eppendorf tube, added with 400 µl GP1 and 5 µl RNase, lysis buffer, and then was mixed using vortex. Sample was incubated at 60°C for 10 minutes. During the incubation, the tube was inverted every 5 minutes. At the same time, a 200 µl per sample of elusion buffer was heated at 60oC. This elusion buffer was used for 4 stages of DNA elusion. Sample was added with 100 µl GP2 buffer then was mixed on vortex and incubated on ice for 3 minutes, then was transferred into filter column which has been installed on 2 ml

of collection tube, then was centrifuged at 1000 x g for 1 minute. The filter is removed. Supernatant was transferred into a new Eppendorf tube and added with 1.5 times GP3 buffer and mixed on a vortex until homogeneous. A visible pellet was then suspended using a pipette. A total of 700 µl of the mixture was transferred into a GD column that had been installed in the collection tube, and then was centrifuged at 14,000 -16,000 x g for 2 minutes. The supernatant was discarded. GD columns that have been placed on 2 ml collecting tubes was added with 400 µl W1 buffer into GD columns then centrifuged at 14,000 -16,000 x g for 30 seconds. The liquid was removed and the GD column was reassembled in 2 ml of the collection tube, then a 600 µl washed buffer was added to GD columns and centrifuged at 14,000 -16,000 x g for 30 seconds. The liquid is removed and the GD column is reassembled in 2 ml of collection tube and then centrifuged 14,000 -16,000 x g for 30 seconds for drying process. Move the dried GD columns to the Eppenorf tube, then added with 100 µl
elusion buffer right in the middle of GD columns and stand for 3-5 minutes, then centrifuged at 14,000 - 16,000 xg for 30 seconds , then the DNA was obtained for further test.

DNA amplification

DNA was amplified using specific 16S rDNA primers. Forward Primer was OI1: 5'GCG CGT ATG CAA TAC GAG CGG C 3 'and reverse primer was OI2c: 5' GCC TCG CGA CTT

CGC AAC CCA T 3 '. PCR program used was: Pre-treatment at temperature 92 oC for 30 seconds for one cycle. Denaturation at 92 °C for 60 seconds; annealing (tempering primer on template DNA) at 60 °C for 30 seconds andElongation (DNA synthesis) at 72 °C for 90 seconds for 40 cycles. Extention (adjustment of DNA double threads) at 72 °C for 90 seconds for one cycle. Visualization of PCR-amplified DNA was done as follows. PCR-treated DNA fragments of 1 µl were electrophoresed on 1% TBE agarose gel. Buffer for electrophoresis used was 1% TAE buffer containing 40 Mm sodium EDTA. Electrophoresis was carried out at 100 volts for 1-2 hours (Sambrook *et al*, 1989).

Persistent Test of Pathogen in The Insects

One-day-old-imagoes of *D. citri* produced by the breeding in the healthy plants were fed with the Siam citrus plants infected by CVPD for three days. Then one insect was moved using an aspirator to the four-week-old-healthy plant which was planted inside the covered plastic glass. The insects were moved every day in the healthy plants until they died. The intervention was repeated for six time and the glasses were all covered by clothes to avoid contamination from the other insects. As a control, the author used insects which were not fed with infected plants. The observation of the disease development was done every day.

Result and Discussion

The amount of *D. citri* organisms needed to transmit CVPD disease

The amplifications of DNA, which came from the inoculated plants by CVPD through 1, 3 and 5 infected organisms of *D. citri*, showed a result of DNA sequences with 1160 base pairs. The DNA sequences were not inoculated in plants given the non-infected organisms (Figure 1). This can be concluded that only one organism is needed to get infected by the disease.

The specificity and sensitivity of PCR method with primer used in this research had already been tested to amplify 16S rDNA from *L. asiaticum* strain. This primer had been tried to detect some diseases of other citrus plants, such as cancer (*Xanthomonas campestris*), puru disease (*Agrobacterium tumefaciens*) and tristeza virus, but ultimately did not amplify 16S rDNA from those bacteria and virus (Sandrine *et al*. 1996). This fact suggests that PCR method using the specific primer of *Liberobacter* is effective and accurate in diagnosing the infection of *Liberbacter* in citrus plants.

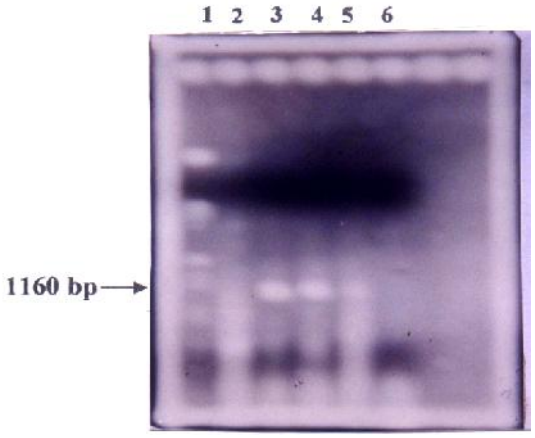

Figure 1. The amplification result of *L. asiaticum* DNA from the vector insects (*D. citri*) with PCR Technique using Primer 16S rDNA on Agarose Gel. Strip 1) DNA marker, 2) transmission using five organisms without pathogen, 3) transmission using five infected organisms, 4) transmission using three infected organisms, 5) transmission using one infected organism, 6) healthy citrus leaves

The infected plants with CVPD show signs of the occurrence of chlorosis or a yellowing leaf tissues process due to the lack of chlorophyll, the color of the leave stems turn into dark green, the leaves become thicker, rigid and smaller in size (Figure 2). The same conditions were reported by Sarwono (1995). Chlorosis happens due to the lesser production of chlorophyll than before which affects the photosynthesis activity. The infected plants by CVPD show necrosis activity and falling of the leaves (Marlina, 1998). The chlorosis process begins with the infection of leaf tissues by the pathogens through the insects as vectors while

they suck the plant liquid. Furthermore, the pathogens in the phloem spread to the plants body parts together with the organic translocation. The presence of these pathogens in relatively large amount can cause signs of chlorosis and even the necrosis process in the phloem of the stems (Diah, 2002).

Figure 2. The symtom of CVPD on citrus leaves

The Incubation Period of Disease in Infected Plants

The correlation analysis done in this study showed the presence of significant correlation (r = -0,93 **) between the number of vectors and the incubation periods of the disease. This means that the more number of vectors infecting the plants, the lesser time needed for the incubation period to happen.

The shortest incubation period occurred in the plants inoculated by five imagoes of *D. citri* in 32.67 days, and the longest was by one imago in 45.75 days (Table 1). This is because the more infected insects, the more pathogens are transmitted into

the plants, resulting in a faster incubation period. Mahfud states that the pathogen concentration in the plants is one of the contributing factors in spreading the disease. The spreading rate of this disease depends on the number of *D. citri* population as the vectors, the numbers of the inoculums sources, the time needed to eat the acquisitions and the period of the inoculations (Chen, 1998). The signs of transmission start to emerge after three to five months (Tirtawidjaja & Suharsojo, 1990). The result of Marlina's study (1998) suggested that the incubation period of CVPD pathogen in Rough Lemon was around 62.30 – 79.20 days.

Table 1. The Incubation Period of CVPD Pathogens Inoculated By Vectors of *D. citri* (Imago Stadium) in Siam Citrus Plants

Number of *D. citri* Imago	Incubation Period (day) $\bar{x}$ ± SE	Information
1	45,75 ± 1,89	r = - 0,93**
3	38,33 ± 2,42	
5	32,67 ± 2,25	

Subandiyah *et al* (1993) reported that the transmission of CVPD pathogen in *Catharantus roseus* (L.) G. Don showed signs of infection in three to four weeks after the process using *Cassytha filliformis* L. as the vector. This indicates that Siam citrus plant is more susceptible to CVPD disease than other plants.

The Pathogen Nature of *L. asiaticum* in *D. citri* Imagoes

The research shows that the imagoes can transmit the CVPD disease after sucking liquid of the infected plants in 72 hours and then infect healthy plants for 21 days (Table 1). So, it can be concluded that the pathogen is very persistent in nature.

The transmission process begins with the sucking of pathogens together with the plant liquid by the vectors, and then they go inside the digestive tracts and penetrate the intestinal wall, circulate inside the hemolymph and finally contaminated the saliva. The bacteria go through a latent period inside the vectors and after that they turn infective (Carter 1973, Oka 1993).

The transmission of the pathogens through the food chain is called by Hurd (2003) as “trophic transmission from host to host via the food chain”. The symbiotic mutualism occurs for a long time. There are some hypotheses proposed, namely: 1) Selection pressure leans towards the plant factor which can provide the best foods for vectors, 2) Selection pressure leans towards the vector which can be the best place to breed the pathogens, 3) Selection pressure leans towards the manipulation to the vectors in order to effectively transmit the disease. These hypotheses support this research because of the persistency of the CVPD pathogens inside the vectors.

Conclusion

D. citri is an effective CVPD vector because of its ability to transmit a disease in citrus plants using only one organism. This pathogen also transmits the disease during its whole life. The more vectors included, the faster the occurrence of the disease.

Advice

Considering that a single *D. citri* imago has been able to transmit CVPD pathogens, it is necessary to control the transmission, starting from nursery process to planting process, especially in endemic areas of CVPD disease. In areas which are still free of CVPD, controlling process is emphasized on the natural function of utilizing natural enemies.

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THE POTENTIAL OF TEMULAWAK CAPSULE TO INCREASE ANTIBODY TITER IN KINTAMANI'S PUPPIES AFTER RABIES VACCINATION

POTENSI KAPSUL TEMULAWAK UNTUK MENINGKATKAN TITER ANTIBODI PADA ANAK ANJING KINTAMANI PASCA VAKSINASI RABIES

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ABSTRACT

Rabies is a zoonotic disease. By giving vaccination the rabies disease could be prevent . Not all vaccines will produce high antibody titers. It need ingredients that can improve the body's immune response (*immunostimulator*), one of the compounds is curcuminoid that is contained in temulawak (*Curcuma xanthorrhiza* Roxb.). The aims of this research is to determine the potencyumption of temulawak capsule to increase antibody titer in kintamani's puppies post rabies vaccination. This study used 25 kintamani's puppies blood serum which 2 to 6 months puppies , divided into 5 groups consisting of: P_0 (control), P_1 (100 mg/kgBB/day), P_2 (200 mg/kgBB/day), P_3 (300 mg/kgBB/day), and P_4 (400 mg/kgBB/day). The capsule treatment is given once a day for 2 weeks. On the 15th day a vaccination with rabies type

rabisin. After 1 or 2 weeks from the last vaccination, blood sample test is taken. Examination of rabies antibody titer was performed by *Enzyme Linked Immunosorbent Assay* (ELISA). The results showed that temulawak can increase antibody titer, but it was not significant (P> 0,05). While the blood taking time has significant effect (P<0,05) to the titer's increasing in 2 weeks after the rabies vaccination.

Keywords : *Rabies; kintamani's puppy; temulawak (Curcuma xanthorrhiza Roxb.); antibody titers.*

ABSTRAK

Rabies merupakan penyakit zoonosis. Pencegahan penyakit rabies dilakukan dengan vaksinasi. Tidak semua vaksin akan menghasilkan titer antibodi yang tinggi. Diperlukan pemberian bahan yang dapat meningkatkan respons imun tubuh (*imunostimulator*), salah satu senyawanya adalah kurkuminoid yang banyak terkandung pada temulawak (*Curcuma xanthorrhiza* Roxb.). Penelitian ini bertujuan untuk mengetahui potensi pemberian kapsul temulawak untuk meningkatkan titer antibodi pada anak anjing Kintamani pasca vaksinasi rabies. Penelitian ini menggunakan serum darah 25 anak anjing Kintamani berumur 2 sampai dengan 6 bulan, yang dibagi ke dalam 5 kelompok yang terdiri dari : P_0 (kontrol), P_1 (100 mg/kgBB/hari), P_2 (200 mg/kgBB/hari), P_3 (300 mg/kgBB/hari), dan P_4 (400 mg/kgBB/hari). Pemberian kapsul temulawak satu kali sehari selama 2 minggu. Pada hari ke-15 dilakukan vaksinasi dengan vaksin rabies jenis rabisin. Satu dan dua minggu pasca vaksinasi dilakukan pengambilan darah. Pemeriksaan titer antibodi rabies dilakukan dengan uji *Enzim Linked Immunosorbent Assay* (ELISA). Hasil penelitian menunjukkan pemberian kapsul temulawak dapat meningkatkan titer antibodi, tetapi tidak signifikan (P>0,05). Sedangkan waktu pengambilan darah berpengaruh nyata (P<0,05) terhadap peningkatan titer antibodi pada 2 minggu pasca vaksinasi rabies.

Kata kunci : *Rabies; anjing kintamani; temulawak (Curcuma xanthorrhiza Roxb.); titer antibodi.*

INTRODUCTION

The main reservoir of rabies is dogs. This disease has a predictive rate of poor recovery. If rabies patients are not vaccinated, death can reach 100% (Tanzil, 2014). The first rabies case in Bali was reported in the Bukit Peninsula, Badung Regency in November 2008. Until now the outbreak has spread to all regencies or cities in Bali (Dibia et al, 2015). Bali Province has made efforts to eradicate rabies. Rabies eradication efforts in Bali are carried out using technical approaches and socio-cultural approaches such as: vaccination, dog maintenance monitoring, dog traffic monitoring, and selective elimination (Putra, 2011). Less successful efforts are caused by multifactors, such as: unavailability of emergency response funds, not optimal coordination of leadership and decision making, dog population in Bali which is quite dense. Field factors that greatly affect the success of dog vaccination, especially kintamani dogs that have aggressive nature, cause officers to be troubled in terms of restrain. Difficulty in restrain greatly affects the stress level of a dog, which later affects the immune response.

To eliminate or prevent rabies outbreaks, at least 70% of the dog population must get immunity (Cleaveland et al., 2003; Reece and Chawla, 2006). The study showed that there was still a low antibody response in vaccinated dogs, where antibody titers from the ELISA test showed 50% protective and 50% samples showed

negative titers in Gulingan Village, Mengwi District, Badung Regency, Bali (Prasatya et al., 2018). One effort that can be done to overcome this is by providing ingredients that can increase the body's immune response or commonly called immunostimulators. One of the natural ingredients that are as immunostimulators is temulawak. The main content in temulawak is curcuminoid. The curcuminoid fraction consists of desmetoxycurcumin, curcumin and bis-desmetoxycurcumin (BPOM, 2005). Antony, et al. (1999) showed that besides functioning as an anticancer compound, curcumin also showed activity as an immunomodulator, which can restore immune system imbalances. According to Sufiriyanto and Indradji (2007), temulawak extract is an immunostimulant and has a constructive effect that is able to repair damaged tissue and glands. Therefore, it is deemed necessary to conduct research on the potential of temulawak capsule to increase antibody titer in kintamani's puppies after rabies vaccination.

RESEARCH METHODS

The object used in this study was blood serum of 25 kintamani's puppies aged 2 to 6 months. Blood is taken from the anterior antebrachii cephalica vein. Temulawak capsules are given orally. Kintamani's puppies were divided into five groups randomly: negative control group (P0) given capsules without temulawak flour, P1 dose 100 mg / kgBB / day, P2 dose 200 mg / kgBB / day, P3 dose 300 mg / kgBB / day, and P4 dose 400 mg /

kgBB / day. Temulawak capsules are given once a day for 2 weeks. On the 15th day vaccination was carried out with Romindo production type rabies vaccine. 1 and 2 weeks after vaccination blood was taken and tested by the Enzyme Linked Immunosorbent Assay (ELISA) test to find out the antibody titer. The results of the data were tested using variance analysis (ANOVA). If there is a real difference, followed by Duncan's test, to determine the differences in the effect of temulawak capsules from each dose given.

RESULTS AND DISCUSSION

The results of the study are presented in table 4.1.

Table 4.1. Antibody Titer Results of Rabies ELISA Test on Kintamani's Puppies Before Treatment and Vaccination, One Week, and Two Weeks After Vaccination.

Number	Weight	Treatment Dose	Age	Titers		
				Before Treatment & Vaccination	**1 week after vaccination**	**2 weeks after vaccination**
P0						
1	5kg	0 mg/kg	6months	0,5 IU	0,1 IU	1,0 IU
2	3kg	0 mg/kg	2 months	1,0 IU	1,0 IU	0,5 IU
3	3kg	0 mg/kg	2 months	2,5 IU	1,3 IU	0,9 IU
4	4kg	0 mg/kg	5 months	0.4 IU	0,4 IU	0,4 IU
5	5kg	0 mg/kg	4 months	0,6 IU	0,4 IU	0,3 IU
Average				**1,0 IU**	**0,6 IU**	**0,6 IU**
P1						
1	3kg	100 mg/kg	3 months	0,3 IU	1,3 IU	0,2 IU
2	3kg	100 mg/kg	2 months	0,3 IU	0,4 IU	0,4 IU
3	3kg	100 mg/kg	2 months	0,4 IU	0,6 IU	0,5 IU
4	3kg	100 mg/kg	2 months	0,4 IU	0,5 IU	0,5 IU
5	3kg	100 mg/kg	2 months	0,2 IU	0,7 IU	0,8 IU
Average				**0,3 IU**	**0,7 IU**	**0,5 IU**
P2						
1	3kg	200	3	0,8 IU	2,3 IU	2,5 IU

		mg/kg	months			
2	3kg	200 mg/kg	3 months	0,2 IU	0,2 IU	5,1 IU
3	3kg	200 mg/kg	2 months	0,4 IU	0,3 IU	0,5 IU
4	3kg	200 mg/kg	3 months	0,3 IU	0,8 IU	1,0 IU
5	3kg	200 mg/kg	2 months	0,4 IU	0,5 IU	0,8 IU
Average				**0,4 IU**	**0,8 IU**	**2,0 IU**
P3						
1	4kg	300 mg/kg	3 months	8,5 IU	3,6 IU	4,4 IU
2	4kg	300 mg/kg	4 months	0,4 IU	3,9 IU	5,8 IU
3	4kg	300 mg/kg	3 months	0,6 IU	1,0 IU	1.4 IU
4	4kg	300 mg/kg	4 months	0,3 IU	2,5 IU	3,8 IU
5	4kg	300 mg/kg	3 months	0,4 IU	4,0 IU	4,5 IU
Average				**2,0 IU**	**3,0 IU**	**4,0 IU**
P4						
1	4kg	400 mg/kg	3 months	8,4 IU	4,0 IU	17,1 IU
2	4kg	400 mg/kg	4 months	0,2 IU	0,2 IU	4,0 IU
3	4kg	400 mg/kg	3 months	0,3 IU	0,2 IU	0.3 IU
4	4kg	400 mg/kg	4 months	0,4 IU	0,5 IU	0,5 IU
5	4kg	400 mg/kg	4 months	0,3 IU	1,0 IU	1,5 IU
Average				**1,9 IU**	**1,2 IU**	**4,7 IU**

Description : Titers $\geq$ 0,5 IU : SEROPOSITIVE **;** Titers < 0,5 IU : SERONEGATIVE

The results showed that administration of temulawak capsules (Curcuma xanthorrhiza Roxb.) And rabies vaccination against kintamani's puppies did not have a significant effect (P> 0.05) on the increase in antibody titers of kintamani's puppies. The time in taking blood of kintamani's puppies significantly (P <0.05) on the increase in antibody titers. Graphs of mean antibody titers after rabies vaccination in kintamani's puppies are presented in

Graph 4.1.

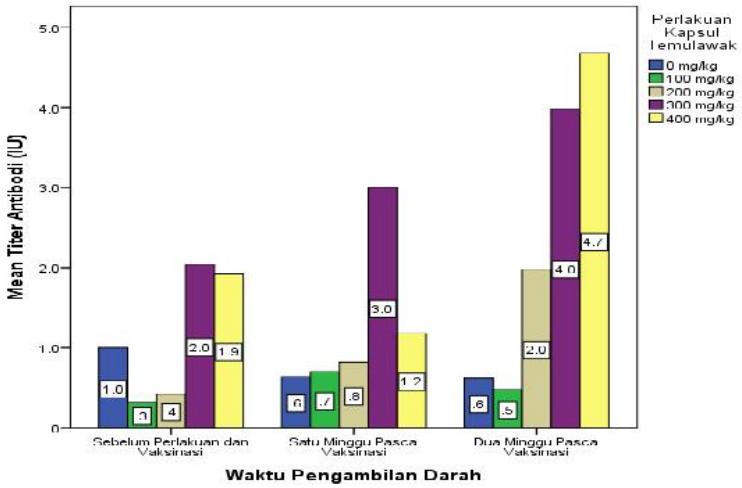

Chart 4.1 Graph of Meaning of Antibody Titer on Kintamani Dogs Before Treatment and Vaccination, One and Two Weeks after Rabies Vaccination and Temulawak capsule on Dose Treatment P0 = 0 mg / kgBB / day, P1 = 100 mg / kgBB / day, P2 = 200 mg / kgBB / day, P3 = 300 mg / kgBB / day, and P4 = 400 mg / kgBB / day.

Based on the analysis of variance (ANOVA), the time of blood taking of kintamani's puppies had a significant effect (P

<0.05) on the increase of antibody titers, so it was continued with Duncan's test. Table of duncan test results is presented in Table 4.2.

Table 4.2. Average Duncan Test Meaning of Antibodies on Kintamani Dogs Ages 2-6 Months, Before Treatment and Vaccination, One Week, and Two Weeks Post.

Number	Time	Average	Significance (0,05)
1	Before Treatment and Vaccination	1.140	a
2	One Week Post Vaccination	1.268	a
3	Two Weeks Post Vaccination	2.348	b

Description: The same letter in the same column indicates that it is not significantly different, while the same letter does not show any significant difference.

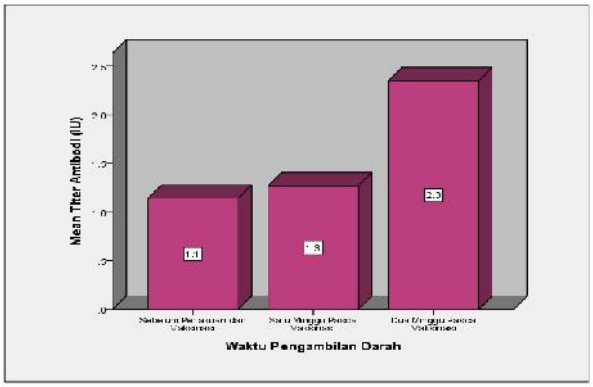

Chart 4.2 Average Graph of Antibodies Titer on Kintamani Dogs Before Treatment and Vaccination, One Week, and Two Weeks After Vaccination.

In this study showed that the administration of temulawak capsules can increase the antibody titers of kintamani's puppies, but not statistically significant (Graph 4.1). This is because curcumin can modulate the immune system by increasing the ability of T cell proliferation (Varalakshmi et al., 2008). T cells generally play a role in inflammation, activation of macrophage phagocytosis, activation and proliferation of B cells in antibody production (Baratawidjaja, 2013). The administration of temulawak capsules has no significant effect after it is seen from the results of variance analysis (ANOVA). This is due to the administration of temulawak capsules which are too short, only for 14 days before rabies vaccination is carried out.

Increased antibody titers are less significant due to the age of dogs used is 2-6 months where at this age the immune system is undergoing development, this is in line with the research Utami, et al. (65%) had protective antibody titers 18.6 times higher when compared with puppies vaccinated for the first time at 1-6 months (9.1%) Besides other causes that make the increase of antibody titer is less significant that dogs aged 1-6 months get natural immunity or still have maternal antibodies. Dog immunity after rabies vaccination is affected by several factors, including age, sex, nationality, vaccine type, and postvaccination period (Ohore et al., 2007). Another factor that affects the dog's immune level against rabies is the state of the dog's high maintenance environment with a high population (Widdowson et al., 2002).

In this study that significantly affect the increase of antibody titer is the time of taking blood (Table 4.2). The first measurements of puppies were performed before the treatment of temulawak capsules and before vaccination. This titer measurement aims to find out the effect of before giving temulawak capsules with temulawak capsules after being given, and to find out whether there are maternal antibodies possessed by the puppies. Antibody titers data show mixed results (Table 4.1). The ELISA test results on the measurement of the first antibody showed eight of the 25 samples in each treatment showed high antibody titers and were expressed as seropositive, meaning the puppy had high maternal antibodies obtained fro the puppy's mother (Utami et al., 2012). The presence of high maternal antibody titers in puppies is due to the fact that the mother has high antibodies.

Measurement of the second antibody titer in puppies was given out after temulawak capsules for 14 days and one week after rabies vaccination. The measurement of the antibody titer aims to determine the effect of temulawak capsules on the immune response of kintamani’s puppies. Data on antibody titers from the ELISA test showed a trend toward an increase in antibody titers one week after vaccination, but there were six dog samples that showed a decrease in antibody titers (Table 4.1). The decrease in antibody titers results from the neutralization reaction between maternal antibodies and rabies vaccine antigens, so puppies that initially have high maternal antibodies will neutralize the vaccine

antigen, which causes vaccine antigens to not stimulate antibodies. This is in line with the results of Sarosa's (2004) study of vaccination too early, with maternal antibodies still high, there will be a neutralization reaction between maternal antibodies to the vaccine virus that enters the body.

Measurement of third antibody titers in puppies was carried out after temulawak capsules were given for 14 days and two weeks after rabies vaccination. Data on antibody titers from the ELISA test showed a tendency to increase antibody titers at two weeks after vaccination (Table 4.1). From the Duncan test results two weeks after vaccination showed antibody titer in kintamani's puppies that were significantly different, which experienced a significant increase of blood taking before treatment and before vaccination and 1 week after vaccination. This is because in the two weeks after rabies vaccination there has been a process of proliferation and differentiation of T cells and B cells.

The process of forming antibodies is divided into three phases, namely, the logarithmic phase, the flat phase, and the phase of decline. Two weeks post vaccination is in the logarithmic phase, where there is an increase in antibody levels logarithmically. In this phase, the time needed to double the concentration is about 5-8 hours. This is due to the increasing number of plasmasites as a result of repeated division of B cells (Subowo, 1993). This is in line with data from studies showing that the rheumatic vaccine responded immune to day 14 after vaccination of 87% (Minke et

al., 2009). There are puppies of kintamani who experience a decrease in the antibody titer of four (Table 4.1), which is influenced by the individual factors of each different dog in terms of responding to the incoming vaccine (Utami et al., 2012).

CONCLUSION

Temulawak capsule administration and rabies vaccination against kintamani's puppies, can increase antibody titers but not significantly, statistically no significant effect ($P > 0.05$), while the time of blood taking of kintamani's puppies significantly ($P < 0.05$) to increase antibody titer at two weeks post-rabies vaccination.

SUGGESTION

Further research is needed regarding the optimal dosage of temulawak capsules to increase antibody titers after rabies vaccination, and about the time to give temulawak capsules before rabies vaccination of 6-12 months old kintamani dogs.

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INFLUENCE OF TEMPERATURE ON THE QUALITY OF CACAO BEANS (*Theobroma cacao* L.) ON VARIOUS FERMENTATION METHODS CONDUCTED BY LOCAL FARMERS

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Abstract

Fermentation of cacao beans (*Theobroma cacao* L.) is very important and determines the quality of the cacao beans. Various fermentation methods have been done by local farmers, however there have been no report on the quality of cacao beans from those fermentation methods. This study aims to know the influence of temperature on the quality of cocoa beans on various fermentation methods conducted by local farmers. The research conducted in Tabanan Regency, Province of Bali by implementing field survey with observation and interview method supported with literature study. According to field observation and analysis of fermented cacao beans taken from the local farmers, it can be concluded that most of local farmers did not fermented their cacao beans. Thus, the fermentation done by utilizing a plastic sack or a bamboo basket or a wooden box lined and covered with banana leaves. Temperature is very influential on fermentation results. Fermentation with basket 1 and fermentation with wooden box 2 gave the highest temperature (42.33^{o}C and 47.33^{o}C) with the best fermentation results (cut test value> 3.0; pH 5.2 - 5.8) and the highest ratio of total sugar to reducing sugar (0.14 and 0.12)

Keywords: Cacao bean, Fermentation, Wooden Box, Bamboo basket, Temperature

Background

Indonesian cocoa has the advantage that it is not easy to melt. So it is suitable for use as a blending (Baon *et al*., 2005). Indonesian cocoa has a flavor that is equivalent to cocoa from Ghana when fermented properly. Seed fermentation is the most important process in handling post-harvest cocoa. Seed fermentation is very important and beneficial. Seed fermentation aims to release the cocoa beans from the pulp, turn off the seeds, improve and form a delicious and pleasant taste of cocoa and reduce the bitter taste of the seeds (Hatmi *et al*, 2015, Widyotomo *et al*, 2004; Wood, 1975). Unfermented cocoa or imperfect fermentation causes the distinctive flavor of chocolate to not form, and the seeds taste sour, bitter, twisted, stingy, and the taste as soil (Atmawinata, *et al*, 1998). With fermentation there will be chemical and biochemical reactions in the pieces of seeds that encourage the formation of precursors of taste and color of cocoa (Haryadi and Supriyanto, 1991 in Ganda Putra, 2009; Wood, 1975).

Pulp contains 80% water, 15% glucose and fructose, about 5% non-volatile acid, lagerly citric and small amounts of sucrose, starch, volatile acids and salt. With the presence of high sugar and acidity (pH 3-5) from the pulp it will be a good condition for the development of microorganisms. The first stage of yeast converts sugar to alcohol. Cells from the pulp begin to decompose and the liquid pulp flows out as sweating. Yeast activity causes a lot of CO2 to form and the atmosphere becomes anaerobic. This condition causes the development of lactic acid bacteria that help break down sugar. With the flow sweating conditions become more aerobic and acidity decreases with loss of citric acid. The presence of O2 allows acetic acid bacteria to take over yeast to convert alcohol into acetic acid (Illeghems *et al*, 2015 in Pelaez *et al*., 2016). This is achieved when stirring or turn the cacao bean. Micro organism activity causes temperature to rise. Rising temperatures and the entry of acetic acid into the seeds will kill the seeds and activate enzymes in the seeds so that chemical changes will occur which will improve the taste, color and aroma of cocoa

Good quality seeds will be obtained if fermentation goes well. Fermentation will take place well if the fermentation temperature is reached 45-48 ^{o}C. To get a good temperature, it takes a large quantity of seeds and takes a long time to ferment. This situation causes farmers feel lazy to ferment because they want to get money faster. However, sometimes there is also a farmer who carries out a simple fermentation to remove pulp from seeds but there is no research that studies the quality of the seeds from fermentation methods carried out by the farmer.

This study aims to know the influence of temperature on the quality of cocoa beans on various fermentation methods conducted by local farmers

Materials and Methods

The study was conducted in Tabanan Regency. The material used is the fermented beans of farmers. The method used is a field survey with observation and interviews. Data sources are secondary data and primary data. Secondary data was obtained from the Tabanan Regency Forestry and Plantation Service and users of cocoa products. Primary data is obtained from direct observation and interviews in the field and analysis of fermented seeds obtained from farmers in the field

The research consisted of four stages of activities, namely (1) secondary data collection, (2) field survey in Tabanan Regency based on secondary data, (3) measuring fermentation temperature carried out by farmers and (4) laboratory analysis of fermented grain yield obtained in the field

The variables observed were: fermentation temperature, cut test (the method of Senanayake et al., 1996 in Kustyawati and Setyani, 2008), and the chemical quality of dry cocoa beans, including:, pH, total acid and reducing sugar ratio by total sugar. All analyzes were carried out at the Agricultural Product Analysis Laboratory, Faculty of Agricultural Technology, Udayana University.

Results and Discussion

In Tabanan Regency, cocoa plant is most commonly found in Selemadeg Barat District. Farmers rarely ferment cocoa beans because, 1) the beans obtained are few, 2) farmers want to get money faster, and 3) the price of fermented cocoa beans is not much different from unfermented seeds. However, some farmers carry out so-called fermentation by storing wet seeds in plastic sack for 3-4 days then drying immediately (Method 1). Data obtained from a cocoa bean processing company located in Cau Village, Marga District, Tabanan Regency, there are several farmers and farmer groups that ferment because the company provides certain requirements for seeds that can be received and given an adequate price. These farmers and farmer groups ferment using bamboo baskets and wooden boxes. The methods are: Bamboo basket size 70 cm high 60 cm high, 80 kg wet seed weight, lined and covered with banana leaves and thick plastic on the top. The seeds was turned on the second day, the third day and on the fourth day fermentation was complete, the seeds are then washed and then dried (Method 2); Bamboo basket 2 is 70 cm in diameter, 20 cm in height, with a weight of 50 kg of wet seeds. The seeds are lined and covered with banana leaves, the top is closed with a plastic sack. The seeds was turned on the second, third and fourth days then washed and then dried (Method 3); Bamboo basket 3 diameter 70 cm, height 20 cm, with a weight of 30 kg of wet seeds. The seeds are lined and covered with banana leaves, the top is closed with a plastic sack. Reversal is done on the second, third and fourth day then washed and then dried (Method 4); The wooden box is sealed with a size of 40cm x 40cm x 60cm with a weight of 40 kg of wet seeds. Seeds are lined and covered with banana leaves. Reversal is done on the third day and the fourth day is immediately washed and dried (Method 5); Wooden box with a size of 50cm x 60cm x 50cm, with 100 kg wet seed weight, lined and covered with banana leaves and a plastic on the top. Reversal is done on the third day and the fourth day, and on the fifth days are washed and dried immediately (Method 6).

Of the six ways of fermentation carried out by farmers the temperature obtained varies. The higher the quantity of seeds fermented, the higher the temperature. The highest temperature is 47.33^{o}C obtained in fermentation with wooden box 2, with a wet seed weight of 100 kg, then basket 1 (42.33^{o}C) with 80 kg of wet seed weight (Table 1). Wood (1975) states that the wet seed weight needed for complete fermentation is not less than 90 kg. According to Kongor *et al*., 2016 (in Pelaez *et al*., 2016), during fermentation bacteria change occurs with temperature changes in addition to pH and O2 availability. Fermentation temperature affects the degree of fermentation.

Table 1. Results of fermented cocoa beans obtained from farmers

Ways Of Fermentation	**Temperature (^{O}c)**	**Cut-Test Value**	**Ph**	**Total Acid (%)**	**Reduction Sugar/Total Sugar**
Recomendation	**45-49**	> 3	**5,2-5,8**	**-**	-
Plastic sack	36,00 a	0,92 a	6,39 a	1,02 b	0,08 c
Bamboo basket 1	42,33 d	3,20 e	5,03 d	1,63 a	0,12 b
Bamboo basket 2	40,33 c	2,76 d	6,42 a	1,05 b	0,06 e
Bamboo basket 3	38,67 bc	2,44 c	5,94 b	1,03 b	0,07 d
Wooden Box 1	40,00 bc	1,64 b	6,42 a	1,05 b	0,08 c
Wooden Box 2	47,33 e	3,12 e	5,58 c	1,07 b	0,14 a

Remarks: the numbers followed by the same letter in the same column mean that they are not significant in the 5% BNT test

The degree of fermentation is measured by the cut-test value. The cut-test value is used to determine whether the seeds have been fermented perfectly or not. According to Senanayake *et al*., 1995 (in Kustyawati and Setyani, 2008), a good cut-test value is above 3 where the seeds are purplish brown with a more dominant brown color. When associated with fermentation temperature, a cut-test value >3 is obtained in fermentation with a maximum temperature exceeding 40^{o}C (bamboo basket 1: 42.33 ^{o}C, and wooden box 2: 47.33 ^{o}C). The temperature that is suitable for the perfect fermentation is related to the weight of the wet seed during fermentation. The weight of wet seeds in a bamboo basket 1 is approximately 80 kg, in a wooden box 2 is 100 kg. Fermentation with plastic sack, bamboo basket 2, bamboo basket 3 and wooden box 1 shows a cut-test value below 3 which means that the purple color dominant to the brown color. This shows that fermentation is less than perfect. This situation is thought to be related to the weight of the seeds that do not qualify to reach the optimal temperature for perfect fermentation. The weight of wet seeds in these fermentation methods is below 50 kg and the maximum temperature is below 40 ^{o}C.

Judging from the pH, the method of fermenting a bamboo basket 1 and a wooden box 1 produces a pH that is in accordance with the requirements of 5.2 - 5.8. According to Dumadi (2000), the pH of cocoa beans must be above 5.0 in order to produce a distinctive cocoa flavor. Cocoa beans with a relatively low pH will eliminate the formation of potential scents, but the pH of seeds that are too high (more than 6.0) will cause aroma damage caused by the growth of microorganisms that can degrade or damage amino acids. With increased aeration due to cocoa pulp drainage and fermentation temperature above 37 ° C, this condition is suitable for the growth of acetic acid bacteria which oxidize ethanol formed by yeast and lactic acid formed by lactic acid bacteria into acetic acid (Illeghams *et. Al*., 2015 Pelaez *et al*., 2016). Amores *et al*., 2009 (in Pelaez *et al*., 2016) stated that acetic acid enters cotyledons and decreases pH from 6.4 to 4.5 during fermentation with temperatures above 45^{o}C. This acidification destroys cell space and ultimately causes cell death, permeable pulp to acetate acid so acetate acid enters the cotyledons, kills

embryos and decreases pH. Furthermore, it is said that the pH value is negatively correlated with the total acid value, where the lower the pH, the total acid tends to increase. This can be seen from fermentation with basket 1 where the seed pH is 5.03 is the lowest pH compared to other fermentation methods but the highest total acid value is 1.63% which is significantly different from other treatments.

Reducing sugar plays a role in the formation of brown color on the seeds and also plays a role in the formation of the distinctive flavor and aroma of chocolate. During the fermentation process, sucrose which is a form of sugar before fermentation, by fermentation will be converted into glucose and fructose (reducing sugar) by the enzyme invertase in the seed coat. Thus, incompletely fermented or unfermented cocoa beans will contain more sucrose and less reducing sugar. Rohan and Stewart, 1967b (in Winahyu, *et al*, 2002) states the ratio of reducing sugar and total sugar can be used as a fermentation index where the higher the ratio the better because it is the maximum level of sugar breakdown during fermentation. Ratio values can be seen in Table 1. From the results of seed analysis, fermentation with wooden box 2 resulted in the highest ratio of reducing sugar to total sugar, which was 0.14 which was significantly different from other treatments, then basket 1 was 0.12, while fermentation with skill , basket 2, basket 3 and wooden box 1 only give values of 0.08; 0.06; 0.07 and 0.08.

Conclusions

From the results of observations and interviews in the field and from the results of the analysis of fermented cocoa beans taken from farmers, it can be concluded as follows:

1. The methods of fermentation carried out by farmers are by plastic sack, bamboo baskets, and wooden boxes which are lined and covered with banana leaves and covered with plastic or plastic sacks on top.
2. Temperature is very influential on fermentation results. Fermentation with basket 1 and fermentation with wooden box 2 gave the highest temperature (42.33oC and 47.33oC) with the

best fermentation results (cut test value> 3.0; pH 5.2 - 5.8 and the ratio of total sugar to reducing sugar the highest is 0.14 and 0.12)

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BB/day = P0
BB/day = P1
BB/day = P2
BB/day = P3
BB/day = P4