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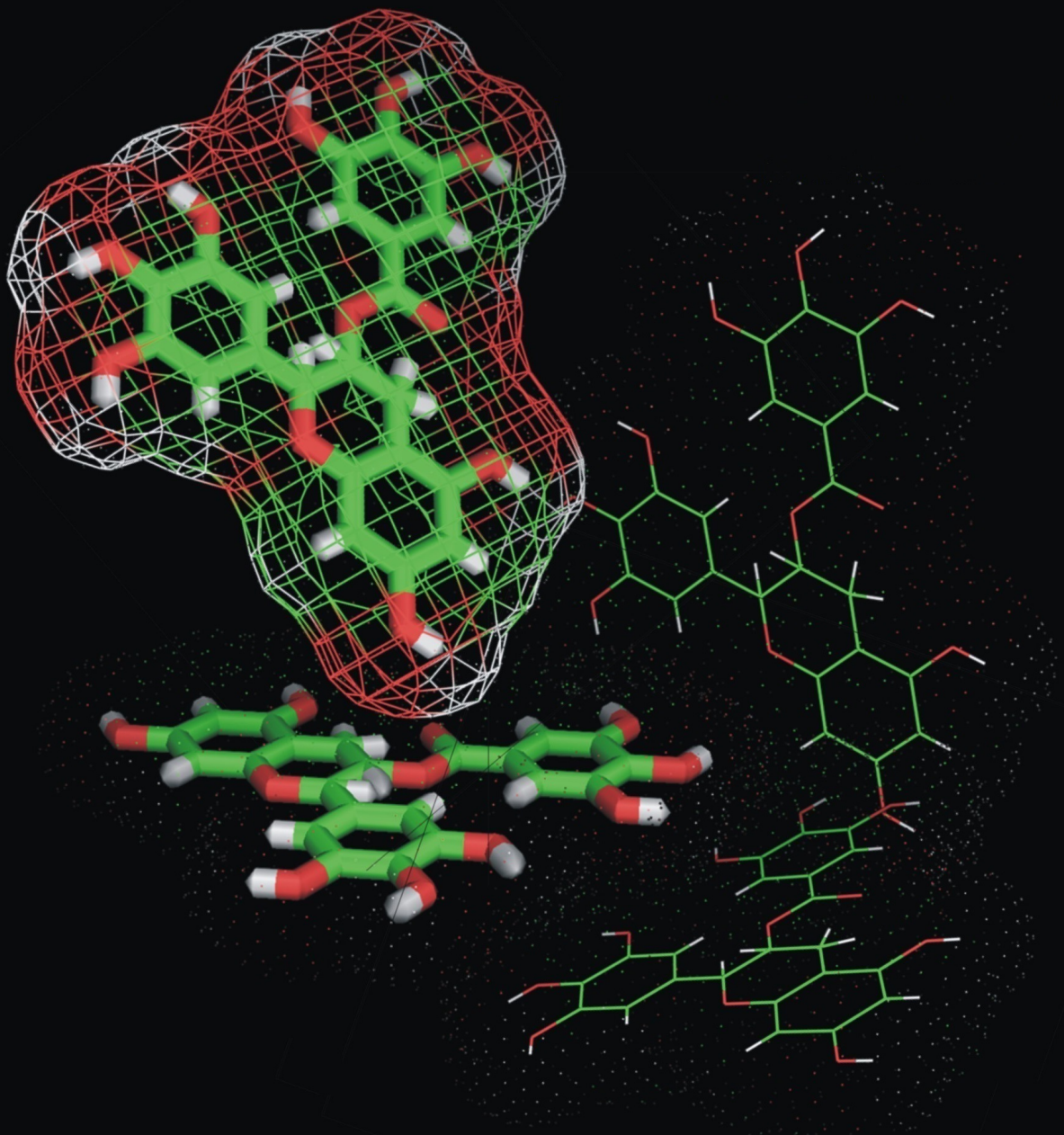

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The Effect of Difference Temperature on Cortisol, Glucose and Glycogen level of Uceng Fish (*Nemacheilus fasciatus*)

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Abstract

Nemacheilus is one of the genera of freshwater fish. The existence of *Uceng* fish (*Nemacheilus fasciatus*) in public waters is increasingly rare. Changes in water temperature in the maintenance medium will affect the physiological processes of fish. The purpose of this study was to analyze physiological responses (blood glucose levels, cortisol, and glycogen hormones) of *Uceng* Fish (*N. fasciatus*) which were incubated at different temperatures and find out the initial time of *Uceng* fish adaptation to its new environment. Total of 200 fish were collected from wild catches in the Lekso River, Blitar. Fish tissue collection was carried out to test the profile of glucose, cortisol, and glycogen. The research activities at this stage were carried out by raising the *Uceng* fish from nature to a cultivation container in an aquarium of size 50 x 30 x 30 cm that given the different temperatures (20°C, 24°C, 28°C and 32°C), and each aquarium is filled with 10 Uceng fish and will be kept for 14 days. ELISA method was used to quantify the parameter. The result of this study showed highest peak of cortisol levels and blood glucose levels was achieved in the treatment of 24°C on the 10th day and the lowest in the treatment temperature of 28°C on the 6th day. The highest glycogen peak was reached at 32°C on day 10 and the lowest peak was reached at 28°C on day 2. We conclude that temperature affects the physiological response (cortisol, blood glucose, and glycogen) of *Uceng* fish (*N. fasciatus*). High levels of cortisol and blood glucose indicate stressful fish.

Keywords: Fish, *Nemacheilus fasciatus,* Physiological, Temperature, Uceng.

INTRODUCTION

Nemacheilus is one of the genera of freshwater fish, all of which are endemic in Asia. Genus *Nemacheilus* consists of 33 species, estimated 18 species of which are found in Indonesia, i.e. Eight species on the island of Sumatra, two species on the island of Java and eight species in Borneo [1]. However, the number of species from the genus *Nemacheilus* spread throughout Indonesia is still uncertain, due to the lack of collection and extensive and comprehensive taxonomic examination of these fish groups [2].

In Indonesia, *Uceng* fish (*N. fasciatus*) is a specific type of local fish that has a high economic value, these fish are usually sold in processed form in the form of fried flour at a price of IDR 200.000 - IDR 300.000 per kilogram; this determination is the main potential of cultivation [3]. *Uceng* fish is also sold as an ornamental fish commodity. The overfishing of these fish in natural waters environment without regard to the aspects of sustainability causing this fish to be threatened with extinction [4].

Until now, there is not much information that supports the domestication of *Uceng* fish, especially for aspects of cultivation. In supporting the domestication of uceng fish, one aspect that must be studied well is the ability of fish to adapt in the ex-situ environment. Especially for water temperature. Changes in water temperature in the cultivation medium will affect the physiological processes of fish [5]. As a result of significant temperature changes, causing fish difficulty in the acclimatization process, which will cause fish stress that can increase the secretion of catecholamines and cortisol. Both hormones at high levels negatively affect the fish's immune system, because increased cortisol in plasma will inhibit the formation of interleukins I and II [6]. It will also affect fish activity and even death due to failure in response to changes in its new environment [7]. Changes in environmental temperature will also have a direct effect on the metabolic process. Therefore, temperature changes will affect the high demand for blood glucose supply for thermogenesis [8].

The purpose of this study was to analyze physiological responses (blood glucose levels, cortisol, and glycogen hormones) Uceng Fish (*N. fasciatus*) which were incubated at different temperatures and find out the initial time of *Uceng* fish adaptation to its new environment.

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MATERIALS AND METHODS

This research was conducted from March to April 2018. For fish rearing activities carried out at the Fish Cultivation Laboratory, Faculty of Fisheries and Marine Sciences, Brawijaya University and blood glucose test, cortisol and glycogen hormones were carried out at FAAL Laboratory, Faculty of Medicine, Brawijaya University.

The test animals used were *Uceng* fish (*N. fasciatus*) obtained from wild catches in the Lekso River, Blitar Regency. The experimental design in this study uses a Completely Randomized Design (CRD) which is temperature with values of 20°C, 24°C, 28°C and 32°C. Fish that are raised for about 14 days.

Data Collection

The Fish Test

Total of 200 fishes was collected from wild catches in the Lekso River, Blitar Regency. The research sample from three fishes as control and the rest will be maintained in an aquarium container with a treatment temperature. Each aquarium contains 15 *Uceng* fishes.

Fish Tissue Collection

Fish tissue collection is carried out to test the profile of blood glucose, cortisol, and glycogen. Fish tissue collection method based on Guest et al. [9].

Treatment

The research activities at this stage were carried out by collecting the *Uceng* fish from nature to a maintenance container in an aquarium of size 50 x 30 x 30 cm that given the different temperatures modified from Syawal and Ikhwan research [10] (20°C, 24°C, 28°C, and 32°C) each aquarium is filled with 10 fishes and will be kept for 14 days. The aquarium is aerated to supply oxygen and is equipped with modified artificial currents with the help of a water pump. Every two days a sampling of a fish is sampled for testing its physiological profile (blood glucose, cortisol, and glycogen).

Calculation of Cortisol Levels

Calculation of cortisol hormone levels was measured using method based on Velasco-Santamaría et al. [11].

Calculation of Blood Glucose Levels

Calculation of blood glucose levels was based on Syawal et al [10] method.

Calculation of Glycogen Levels

Calculation of glycogen levels is using the method based on Suarsana et al. [12]. The calculation of glycogen in the fish meat is calculated by Elabscience formula [13].

Data Analysis

Data retrieval techniques are carried out by direct observation, by observing directly the observed subject. The data obtained from the research were analyzed statistically using ANOVA (Analysis of Variance) according to the completely randomized design (CRD). Furthermore, LSD (Least Significance Different) test was carried out. The LSD test was carried out if the test results showed the F value was significantly different or very real different, to determine the treatment that gave the best response at 5% and 1% confidence intervals [8]. Then proceed with regression tests and orthogonal polynomial calculations. Furthermore, the overall data obtained from the observations and calculations are analyzed and the results are recorded.

RESULTS AND DISCUSSION

Cortisol

Base on Figure 1, on temperature treatment 20°C, changes in cortisol levels continued to increase until reaching the highest peak on the 12^{th} day of 239.83 $ng.mL^{-1}$ then decreased to 228.5 $ng.mL^{-1}$ on the 14^{th} day. At temperature treatment 24°C, Cortisol increased to the highest point on day 10, which was 192.5 $ng.mL^{-1}$ then continued to decline until the 14^{th} day of 169.83 $ng.mL^{-1}$. Temperature treatment 28°C has almost the same results as temperature treatment 24°C namely, cortisol levels continued to increase from the first day of maintenance to reach the highest peak on the 10^{th} day, which was 193.66 $ng.mL^{-1}$, then there was a decrease until the 14^{th} day which was 187.33 $ng.mL^{-1}$. However, at temperature treatment 32°C, cortisol levels continue to increase until the 14^{th} day is 267.83 $ng.mL^{-1}$.

Changes in cortisol levels in plasma, often used as the main indicator of stress, while the second indicator is an increase in glucose levels [14]. The results of this study, at maintenance temperatures 20°C, 24°C, and 28°C similar to the results of research which state that fish are kept for 14 days, cortisol levels continue to increase at each maintenance temperature [14]. However, at maintenance temperatures 32°C, Cortisol levels continue to increase, presumably, the displacement of the *Uceng* fish habitat from natural waters to a maintenance container in the laboratory causes prolonged stress.

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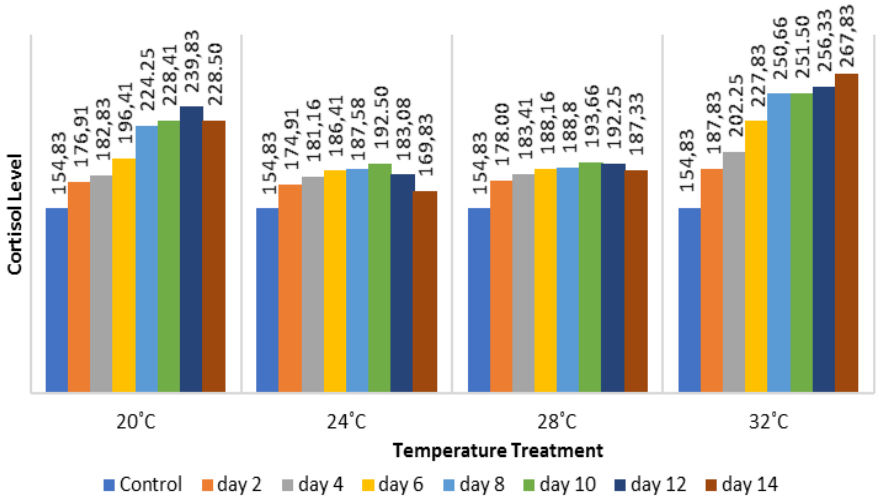

Figure 1. Cortisol Levels for 14 Days of Cultivation

Stressed fish will increase the secretion of catecholamines and cortisol [14]. Both hormones at high levels negatively affect the fish's immune system, because increased cortisol in plasma will inhibit the formation of interleukins I and II. As a result, fish will lose their immunity and are easily infected with pathogens, thus, can cause a high mortality rate. Furthermore, increased cortisol and glucose levels in plasma indicate that fish experience prolonged stress, which results in specific and non-specific immune reactions of fish decreasing [14].

Cortisol levels in the blood due to stress, will mobilize glucose from reserves stored by the body into the blood, so that glucose in the blood increases. High cortisol levels cause metabolic effects such as modulation of carbohydrate metabolism through gluconeogenesis, increased turnover protein, regulation of amino acid metabolism, ammonia production and increased lipolysis [15], thus increasing blood glucose. Fish will repeatedly have stressful situations and can show physiological responses such as accumulation of cortisol [16] which causes physiological conditions to decline, immunity decreases, even survival and growth of fish become threatened [17].

Glucose

Base on Figure 2, at a treatment temperature of 20°C, changes in blood glucose levels continued to increase until they reached the highest peak on the 12th day for 192.87 mg.L^{-1} then decreased to 183.93 mg.L^{-1}. At 24°C, the changes in glucose levels increased to the highest point on the 10th day which was around 193.93 mg.L^{-1} and then continued to decline until the 14th day, which was 165.27 mg.L^{-1}. At a temperature treatment of 28°C, it was almost the same as the 24°C temperature treatment, i.e. blood glucose levels continued to increase from the first day of maintenance to reach the highest peak on the 10th day, namely 202.60 mg.L^{-1}, then a decline until the 14th day, namely 182.13 mg.L^{-1}. However, at 32°C, the blood glucose levels continued to increase until the 14th day which was around 233.27 mg.L^{-1}. It can be said that at a temperature of 32°C, it is not suitable for the life of the *Uceng* fish due to the temperature range, fish stress continues to increase. In accordance with the results of a study, states that Tapah fish which are kept at 31°C produce increased blood glucose levels or hyperglycemia [18]. This condition reflects that the temperature of 31°C is not able to reduce stress or failure to achieve glucose homeostasis due to the occurrence of stress at very high levels [19].

Blood glucose levels that continue to increase indicate the flow of glucose into the blood that is greater than the entry of blood glucose into cells. Conversely, glucose levels will decrease if the flow of glucose into the blood is lower than entering blood glucose into cells. Thus, the peak blood glucose level occurs when the flow of glucose into the blood and the entry of blood glucose into the cell reach the point of balance [20].

Glucose that has entered the cell will be immediately metabolized to meet energy needs thus avoiding the use of a number of amino acids as a source of metabolic energy [21]. The availability of glucose in cells is used to meet the physiological needs of the body and energy needs, after fulfilling high glucose intake will stimulate the occurrence of glycogenesis and lipogenesis [22].

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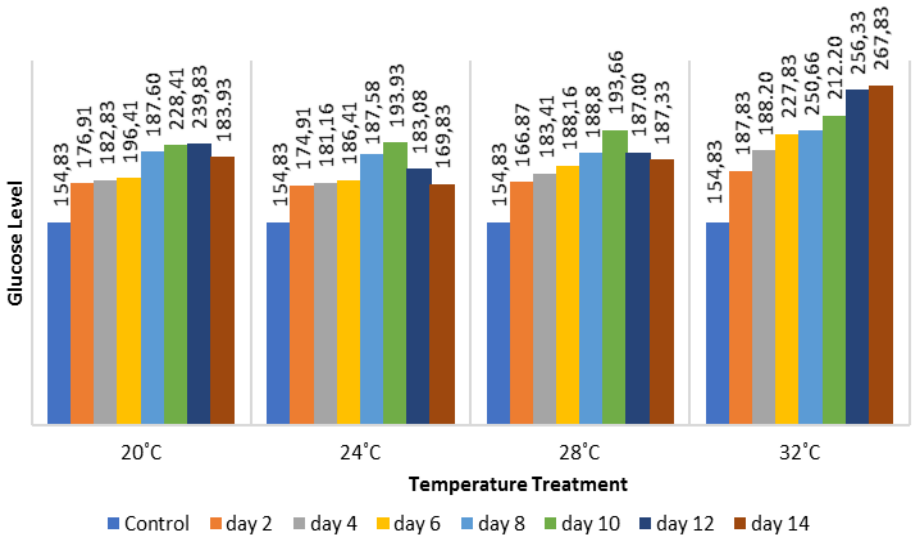

Figure 2. Glucose Levels for 14 Days of Cultivation

Glycogen

Base on Figure 3, at a temperature of 20°C, changes in glycogen levels from the first day continued to increase until the 14th day was 29.16 mg.g^{-1}. This is because fish do not experience prolonged stress so glycogen is not needed as an energy requirement. At 24°C, the glycogen changes decreased on the 2nd day (26.74 mg.g^{-1}) until the 4th day (26.36 mg.g^{-1}), then on the 6th day (29.17 mg.g^{-1}) until the 12th day (30.88) continued to increase. There was a decrease on the 14th day which was 31.13 mg.g^{-1}. At a temperature treatment of 28°C, it was almost the same as the treatment temperature of 24°C, namely glycogen levels on the 2nd day (26.69 mg.g^{-1}) until the 4th day (25.51 mg.g^{-1}) decreased. Then on day 6 (28.13 mg.g^{-1}) until day 14 (30.50 mg.g^{-1}) continued to increase. This is suspected, even though *Uceng* fish has an increase in blood glucose and cortisol, which is thought to be stressful, the fishes are still consuming a lot of feed given so that glycogen levels will increase.

An increase in glycogen levels indicates an excess of blood glucose after metabolic energy needs are met, which is immediately converted to glycogen and then stored in the muscles and liver [23]. Carbohydrates consumed by fish will be digested become glucose. At a temperature treatment of 32°C glycogen levels on day 2 (26.19 mg.g^{-1}) until day 4 (25.50 mg.g^{-1}) decreased because the fish has prolonged stress and took glycogen levels that had been stored previously. Then there was an increase until the 8th day (27.64 mg.g^{-1}) then decreased again on the 12th day (26.85 mg.g^{-1}) and increased again on the 14th day which was 27.83 mg.g^{-1}.

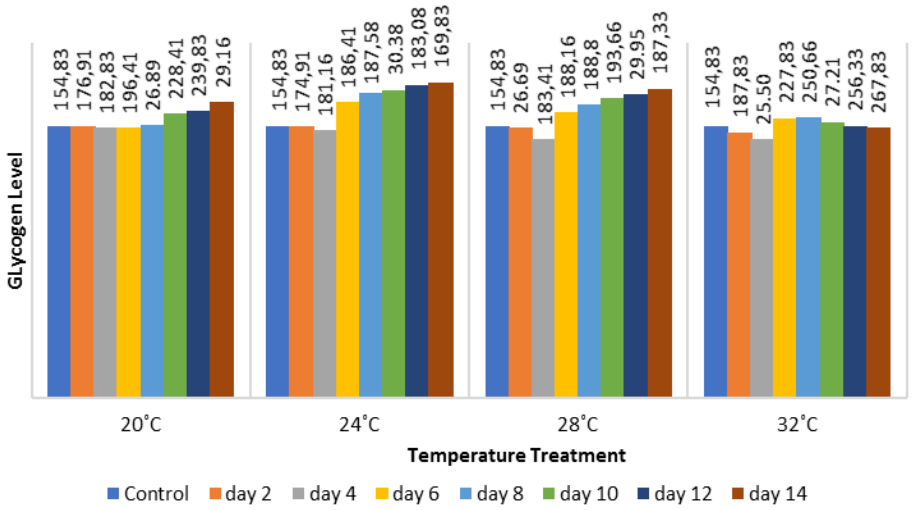

Figure 3. Glycogen Levels for 14 Days of Cultivation

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In freshwater, decreased glycogen levels can lead to the need for glucose as long as the fish experience stress, resulting in glycogenolysis activity, which is the breakdown of glycogen into glucose. In *Oncorhynchus mykiss* (Rainbow Trout), the highest glycogenolysis activity occurs in the liver, which is 60% compared to muscle organs and other organs [24]. Glycogen comes from excess glucose in the blood. Carbohydrates consumed by fish will be digested in the digestion to become glucose. Glucose will be absorbed by the intestinal wall and then transported in the blood. In the liver, glucose will enter the hepatocyte cells easily and then converted into glycogen [25].

Temperature greatly affects blood glucose levels but the pattern is inconsistent [26]. The Blood glucose level of fish rapidly increases within a short period after stress primarily due to the activation of hepatic β-adrenoceptors and glycogenolysis [27,28]. Fish subjected to stress challenges increase energy metabolism to cope with stress response, and glucose is used as the main energy fuel [27,29]. Glucose that enters the blood beside being used as a source of energy that is entering the citric acid cycle to produce energy, the excess will be stored in the form of glycogen and will be used as a source of energy through the process of glycogenicity after being converted back into blood glucose. Liver glycogen is strongly associated with hexose deposits and deliveries to maintain blood glucose levels, especially when between meals. After 12-18 hours of fasting, almost all of the liver's glycogen deposits were depleted. So that the glycogen content in the liver is related to the efficiency of digestion and absorption of glucose from the feed used. The liver glycogen content varies from each treatment combination.

The glycogen content that had accumulated will shrinkage. In the liver, glycogen degradation and synthesis play major roles in regulating blood glucose homeostasis and supplying energy to other tissues [30,31]. When environmental temperature decreased to 8°C, however, the energy utilization strategy of sea bream changed to the production of nonpolar and polar lipids, whereas the glycogen content in the livers was not significantly changed. In environments with much lower temperatures, the production of lipids was altered to maintain membrane fluidity, and glycogen storage decreased in the liver of the sea bream [32,33].

CONCLUSION

Temperature affects the physiological response (cortisol, blood glucose, and glycogen) of *Uceng* fish (Nemacheilus fasciatus). High levels of cortisol and blood glucose indicate stressful fish.

REFERENCES

[1] Kottelat, M. 2012. Conspectus cobitidum*: an inventory of the loaches of the world (Teleostei: Cypriniformes: Cobitoidei). *Raffless Bull. Zool*. 26. 1-199.

[2] Hadiaty, R.A. 2014. Taxonomic study of the Genus *Nemacheilus* (Telestei : *Nemacheilidae*) in Indonesia. PhD Thesis. Graduate Scool of Engineering and Science University of the Ryuskyus. Taiwan.

[3] Prakoso, V.A., J. Subagja, A.H. Kristanto. 2017. Aspek biologi reproduksi dan pola pertumbuhan ikan Uceng (*Nemacheilus fasciatus*) dalam pemeliharaan di akuarium. *Jurnal Media Akuakultur*. 12(2). 67-74.

[4] Fajarwati, E.M. 2006. Studi mengenai aspek eko-biologi ikan Lalawak (*Barbodes balleroides*) pada berbagai ketinggian tempat di kabupaten Sumedang, Jawa Barat. Bachelor Thesis. Bogor Agricultural University. Bogor.

[5] Nofrizal, K. Yanase, T. Arimoto. 2009. Effect of temperatur on the swimming endurance and post-exercise recovery of jack macke-rel *Trachurus japonicus* as determined by monitoring. *Fish Sci.* 75. 1369-1375.

[6] Syawal, H., Y. Ikhwan. 2011. Respon fisiologis ikan Jambal Siam (*Pangasius hipoptalamus*) pada suhu pemeliharaan yang berbeda. *Berkala Perikanan Trubuk.* 39(1). 51-57.

[7] Masjudi, H., M.U. Tang, H. Syawal. 2016. Kajian tingkat stres ikan Tapah (*Wallago leeri*) yang dipelihara dengan pemberian pakan dan suhu yang berbeda. *Berkala Perikanan Terubuk.* 44(3). 69-83.

[8] Brown, J. A. 1993. Endocrine responses to environmental pollutions. In: Rankin, J.F., F.B. Jemsen (Eds). Fish Ecophysiology. Chapman & Hall. London. 276-292.

[9] Guest, T.W., R.B. Blaylock, A.N. Evans. 2016. Development of a modified cortisol extraction procedure for intermediately sized fish not amenable to whole-body or plasma extraction methods. *Fish Physiol. Biochem.* 42(1). 1-6.

[10] Syawal, H., Y. Ikhwan. 2011. Respon fisiologis ikan Jambal Siam (*Pangasius*

hipoptalamus) pada suhu pemeliharaan yang berbeda. *Berkala Perikanan Trubuk*. 39(1), 51-57.

[11] Velasco-Santamaría, Y., P. Cruz-Casallas. 2007. Methodology for determination of plasma cortisol in fish using competitive enzyme-linked immunosorbent assay (ELISA). *Rev. MVZ Cordoba.* 12(1). 869-877.

[12] Suarsana, I.N., B.P. Priosoeryanto, T. Wresdiyati, M. Bintang. 2010. Sintesis glikogen hati dan otot pada tikus diabetes yang diberi ekstrak tempe. *Jurnal Veteriner.* 11(3). 190-195.

[13] Elabscience. 2018. Glycogen assay kit. 8th Ed. revised in February, 2018. Elabscience.

[14] Aslamyah, S. 2006. Penggunaan mikroflora saluran pencernaan sebagai probiotik untuk meningkatkan pertumbuhan dan kelang-sungan hidup ikan Bandeng. PhD Thesis. Postgraduate School, Bogor Agricultural University. Bogor.

[15] Mommsen, T.P., M.M. Vijayan, T.W. Moon. 1999. Cortisol in teleosts: dynamics, mecha-nisms of action, and metabolic regulation. *Rev. Fish Biol Fish.* 9(3). 211-268.

[16] Koakoski, G., L.C. Kreutz, M. Fagundes, T.A. Oliveira, D. Ferreira, J.G.S. da Rosa, L.J.G. Barcellos. 2013. Repeated stressors do not provoke habituation or accumulation of the stress response in the catfish Rhamdia quelen. *Neotrop. Ichthyol.* 11(2). 453-457.

[17] Wedemeyer, G.A. 1996. Physiology of fish in intensive culture systems. Chapman and Hall.

[18] Suarez, M.D., A. Sanz, J. Bazoco, M.G. Gallego. 2002. Metabolic effects of changes in the dietary protein: carbohydrate ratio in eel (*Angilla anguilla*) and trout (*Oncorhynchus mykiss*). *Aquac. Int.* 10. 143–156.

[19] Stryer, L. 2000. Biokimia. 4th Ed. VoL. 2. EGC. Jakarta.

[20] Handayani, S. 2011. Uji toleransi glukosa dan uji toleransi insulin glukosa pada ikan Gurame yang diberi pakan mengandung kadar protein dan karbohidrat yang berbeda. Bogor Agricultural University. Bogor.

[21] Hadim, E., M.I. Djawad, M.Y. Karim. 2002. Kondisi glikogen dalam hati juvenil ikan Bandeng (*Chanos Chanos* Forskall) yang dibantut. Laboratory of Ecotoxicology and Physiology of Marine Biota, Hasanuddin University. Makassar.

[22] Adam, H.M. 2004. Comparative studies on the effect of water quality on haemato-logical of *Orechromis niloticus* under culture condition. PhD. Thesis. Sudan University of Science and Technology.

[23] Svobodova, Z., B. Vykusova, H. Modra, J. Jarkovsky, M. Smutna. 2006. Haematolo-gical and biochemical profile of harvest-size carp during harvest and post-harvest stroge. *Aquac. Res.* 37. 959-965.

[24] Mehrani, H., K.B. Storey. 1993. Control of glycogenolysis and effects of exercise on phosphorylase kinase and cAMP-dependent protein kinase in rainbow trout organs. *Biochem. Cell Biol.* 71(11-12). 501-506.

[25] Junqueira, L.C., J. Carneiro. 1995. Histologi Dasar, 3rd Ed. EGC. Jakarta.

[26] Ottolenghi, C., A.C. Puviani, D. Ricci, L. Brighenti, E. Morsiani. 1995. Comparative biochemistry and physiology part A: physiology. 111(2). 229-235.

[27] Wendelaar B.S.E. 1997. The stress response in fish. *Physiol. Rev.* 77. 591-625.

[28] Fabbri, E., A. Capuzzo, T.W. Moon. 1998. The role of circulating catecholamines in the regulation of fish metabolism: an over-view. *Comp. Biochem. Physiol. C.* 120. 177-192.

[29] Lupatsch, I., G.A. Santos, J.W. Schrama, J.A.J. Verreth. 2010. Effect of stocking density and feeding level on energy expenditure and stress responsiveness in European sea bass *Dicentrarchus labrax*. *Aquaculture.* 298. 245-250.

[30] Vornanen, M., J. Haverinen. 2011. Season-ality of glycogen phosphorylase activity in crucian carp (*Carassius carassius* L.). *J. Comp. Physiol. B.* 181(7). 917-926.

[31] Polakof, S., S. Panserat, J.L. Soengas, T.W. Moon. 2012. Glucose metabolism in fish: a review. *J. Comp. Physiol. B.* 182(8). 1015-1045.

[32] Ibarz, A., J. Blasco, M.A. Gallardo, J. Fernández-Borràs. 2010. Energy reserves and metabolic status affect the acclimation of gilthead sea bream (*Sparus aurata*) to cold. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 155(3).319-326.

[33] Melis, R., R. Sanna, A. Braca, E. Bonaglini, R. Cappuccinelli, H. Slawski, T. Roggio, S. Uzzau, R. Anedda. 2017. Molecular details on gilthead sea bream (*Sparus aurata*) sensitivity to low water temperatures from ¹H NMR metabolomics. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 204. 129-136.

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Transoceanic Disperse of the White-lipped Island Pit Viper (*Cryptelytrops insularis*; Kramer,1997) from Sundaland to Lesser Sunda, Indonesia

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Abstract

White-lipped island pit viper (*Cryptelytrops insularis*) is one of the most distributed Viperidae in Indonesia, especially in eastern part of Sundaland and Lesser Sunda. To investigate the evolutionary history and the dispersal pattern of *C. insularis,* we collected 23 samples from 11 localities. Four simultaneous genes composing two mitochondrial genes (16S rRNA & ND4) and two nuclear genes (7IβFIB & 3ITBP) have been successfully amplified and sequenced. Bayesian inference was performed to reconstruct the phylogeny tree. Furthermore, time divergence and the population demography analyses were estimated. The phylogeny tree of *C. insularis* exhibits monophyletic group, with four geographically structured lineages. The time divergence estimation indicated that *C. insularis* evolved at approximately 7 million years ago (mya). Population demography was inferred by Bayesian Skyline Plot analysis, it shows that the population increased constantly from the past to recent time. The evolutionary history of *C. insularis* can be explained by a pattern of the time divergences estimation that indicating movement from West (Java) to East (Lesser Sunda). We expected that the dispersal factor of *C. insularis* into many different islands (in Lesser Sunda) is caused by the animal helped and also oceanic rafting which could be the stepping stones to another island.

Keywords: *Cryptelytrops insularis*, dispersal patterns, phylogeny, population demography, time divergence

INTRODUCTION

Indonesia was one of some biggest hotspot biodiversity in the world, especially the Sundaland and Wallacea area [1]. There are 5 large islands, Sumatra, Java, Kalimantan, Sulawesi, Papua New Guinea, and around 17.000 small islands. [2]. These islands have unique geographical history leading to unique biological evolution.

During Pliocene approximately 5 million years ago (mya) and Pleistocene (12000 years ago) Epoch, there were several sea level fluctuations due to the glaciations [3]. At that time, the Sundaland area including Sumatra, Java, and Borneo was in a changing phase, and also the unity of Sulawesi and the appearance of Lesser Sunda were formed due to the collisions of Philipines and Australian plates [4]. These periods were suggested to affect recent animal compositions [3]. The lowering of sea level caused the emergence of some landbridges for dispersal. In contrast, the raising of sea level will inundate some islands and coastal plain, and restrict the dispersals of some fauna [5]. During the period of isolation, organisms would evolve independently such as snake [6].

Insular species was very interesting to study the evolution and biogeography because they are separated by islands [7]. *Cryptelytrops insularis (Trimeresurus insularis)* or white-lipped island pit viper was one of the insular species that distributed in Java, Bali, Adonara, Alor, Flores, Komodo, Lombok, Padar, Rinca, Romang, Roti, Sumba, Sumbawa, Timor, and Wetar [8,9]. Based on the Reptile Database this species has type locality from Timor Island [8]. This species was well-distributed in Java, Bali (Sundaland) and Lesser Sunda, but in fact between Sundaland and Leser Sunda are never been connected. Thus, we raised a question, how can *C. insularis* dispersed into those islands?

Robust phylogeny on the genus level of *Trimeresurus* (previous scientific genus name) were done by Maholtra & Thorpe [10], Creer et al [11], Maholtra & Thorpe [12], Creer et al [13], and Maholtra et al [14], but intra-species level on each species still unclear. Based on molecular assessment using Cyt B David et al [15] showed that *C. insularis* has a close relationship with *Cryptelytrops fasciatus* from Tanahjampea Island, South Sulawesi. Interestingly, there is another viper whose distribution overlapped with *C. insularis*, namely *Cryptelytrops albolabris*. But, the relationship is closer to *C. fasciatus* than *C. albolabris* which have similar morphology [14]. We hypothesize that geographical history might influence their relationships as well. Therefore,

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using more genes and more samples we have aimed to evaluate the phylogeny and biogeography of white-lipped island pit viper, and also to estimate their dispersal patterns.

MATERIALS AND METHODS

Sample collection

We collected a total of 23 samples including one outgroup (*Cryptelytrops albolabris*) and two samples from Genbank (NCBI) [16]. Sample collections were conducted during May 2016 - January 2017, in Java, Bali, and Lombok Islands. Visual Encounter Survey (VES) method was used to collect the sample by looking around the tracking area [17]. Additional sequences were obtained from Genbank NCBI (Supplementary 1). Tissues collection was taken from muscle, tail tips, and liver then preserved into 1.5 mL tubes containing 98% ethanol or lysis buffer (Tris, EDTA, and SDS). Specimen's whole body were preserved into 70% ethanol and stored in the room temperature.

Molecular protocols

DNA extraction was conducted by QIAGEN kit (QIAamp DNA Minikit, Hilden, Germany) and following their protocol, also by manual procedure with phenol and chloroform. Tissue digestion conducted with 330 µL STE buffer (cell lysis buffer) (10 mM Tris-HCl pH 5.0, 0.1 NaCl, 1 mM EDTA), 40 µL 20% SDS, and 20 µL of 20 $mg.mL^{-1}$ Proteinase K [18]. DNA pellet was dissolved with 50 µL ddH_2O, then kept in -20°C freezer for later use.

We used two mitochondrial genes (16S rRNA and ND4) and two nuclear genes (7IβFIB and 3ITBP). These genes were succeeded to amplify Asian pit viper and generated well-resolved phylogeny [13]. Primers used were shown in Supplementary 2. PCR mixture composition was followed by the 2x Taq DNA Polymerase Master mix RED reaction composition (BIONOVAS, Toronto, Ontario). Thermal cycles used were: pre-denaturation at 94°C for 1-minute, then followed by 40 cycles of 30 seconds denaturation at 94°C, 1-3 minutes annealing at 50-57°C and 1-minute extension at 72°C. then 10-15 minutes final extension at 72°C. We have a difficulty in the nuclear genes amplification, the PCR products were amplified with an unstable successful rate. In order to increase the sensitivity, a nested PCR approach was applied [19]. PCR products were cleaned up with PCR Advanced Clean-Up System (VIOGENE). These four genes were completely sequenced from both directions with the following primers in Biomedical Research Center at Academia Sinica and Mission Biotech (Taiwan), using ABI PRISM Model 3730 Version 3.2.

Sequences analyses

Sequences were aligned by Clustal W and modified implemented in MEGA 7 [20], then double-checked manually with FinchTV 1.4.0 [21]. Phylogenetic trees were reconstructed using Bayesian inference (BI), with the best fit model of GTR+I+G (general time-reversible + Proportion invariable sites + gamma distribution) was selected with Akaike information criterion (AIC) by jModeltest 2.1.10 [22,23]. The parameter for Proportion invariable was 0.6583 and for gamma/shape was 0.7690. BI was estimated using MrBayes 3.2.3 with two runs of four Monte Carlo Markov chains for 100.000.000 generations and a tree was sampled every 100 generations [24]. The consensus topology was summarized with burn-in first 25% trees.

Time divergence was estimated using BEAST v1.8.4 [25]. The parameters were set up in BEAUti v1.8.4 as GTR substitution model, empirical base frequencies, 6 number of gamma categories, random local clock model, Coalescent; constant size tree prior. At the Prior column, fossil records calibration used are: (i). *Cryptelytrops insularis* divergence around 8.5 million years ago (mya), (ii). *Cryptelytrops albolabris* divergence around 7.15 mya [26]. For the alpha parameter used is gamma distribution; Initial value/shape=0.6583), and for the Pinvariable the parameter used is lognormal distribution with Initial value/mean=0.6583, stdev=0.32915) then using 200.000.000 chain length. The BEAST was performed with two independent runs. The BEAST log result was checked by TRACER 1.6 [27] estimations to check the validity of convergence and effective sample size (ESS). Two BEAST tree files then combined by the LogCombiner v1.8.4, then the combined tress would summarize by TreeAnnonator v.1.8.4. then the time-calibrated tree was visualized and modified by FigTree v.1.4.3.

Bayesian skyline plot (BSP) was performed by BEAST v.1.8.4 [25] to estimates changes in effective population size through the time. The analysis was conducted at all individuals which belong to *Cryptelytrops insularis* (22 individuals). The parameter for the tree prior was used the Coalescent; Bayesian Skyline, and for the time calibration used was 7.15 mya for *C. insularis* [26]. The BEAST file was double generated in

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3.000.000 chain length. The BSP result was analyzed and visualized by TRACER 1.6 [27].

RESULTS AND DISCUSSION

Phylogenetic tree and genetic distances

To determine the phylogeny of 22 individuals in *Cryptelytrops insularis,* Bayesian inference tree was constructed based on 3575 base pairs sequences from 4 simultaneous genes (16S rRNA, ND4, 7IβFIB, and 3ITBP).

Our phylogenetic tree based on 4 simultaneous genes showed a monophyletic group with four clades (Fig. 1). Clade A containing *C. insularis* samples from Lesser Sunda area such as Lombok and Timor, while Clade E consisting samples from Sundaland such as Java and Bali. In detail, Clade B consisted of *C. insularis* from East Java, Clade C are composed of samples from Bali Island, and Clade E consisted of samples from East Java. Most of the posterior probability on the nodes were weakly supported by Bayesian Inference, however, there were some nodes received high supports such as Clade C and the common ancestor of Clade B, C, and D.

The genetic distances among clades are varied between individuals (Table 1), but there are no significant genetic differences. The highest genetic differences were 0.009 and 0.008 which showed on samples from East Java (sample no. 1) with the sample from Bondowoso, East Java (sample no. 22), and samples from Bondowoso, East Java (sample no. 22) with the sample from Tanah Lot, Bali (sample no. 56), respectively.

Divergence times estimation

Divergence time estimation indicated that *C. insularis* was younger than *C. albolabris* (Fig. 2). Most ancient *C. insularis* evolved around 7 mya (node a), then followed by first diversification on the node b, around 6.7 mya in Sundaland area (Java and Bali). Samples from Lesser Sunda (node c) diverge around 4 mya. Based on our estimations we expected that there is an eastward movement of *C. insularis.*

Population demography (Bayesian skyline plot)

To estimate the effective population size through the time, we performed the Bayesian skyline plot (BSP) on Figure 3. BSP analysis on *C. insularis* population showed increased constantly from the past to recent time. At the first diversification of *C. insularis* around 7-5 mya, the effective population size was increased slightly. Moreover, around 5-3 mya the population size was increased significantly. Then around 3 mya to recent time, the population size estimation showed the most stable with slightly increase.

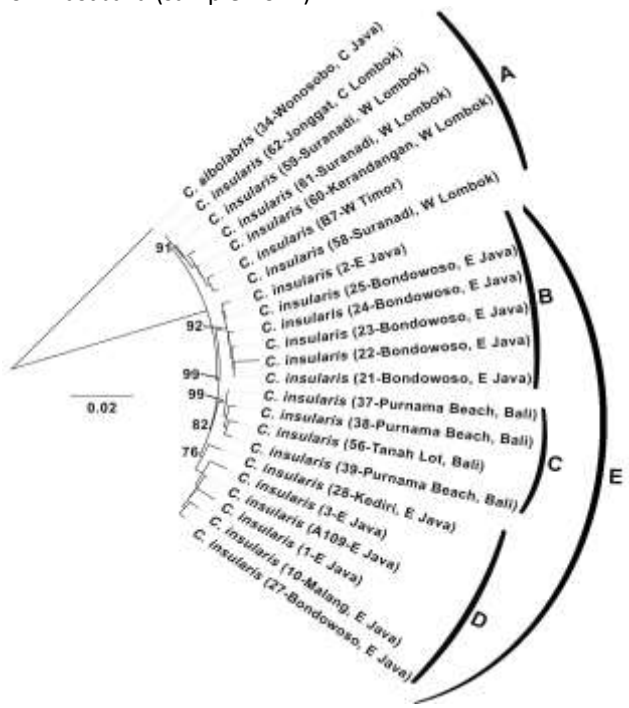

Figure 1. Bayesian phylogeny semi circular-tree based on 4 simultaneous genes (16S rRNA, ND4, 7IβFIB & 3ITBP). *C. albolabris* was used as an outgroup. The number on the nodes represent the Bayesian posterior probability (pp). Sample number and locality were showed in parentheses. (C: Central, E: East, W: West).

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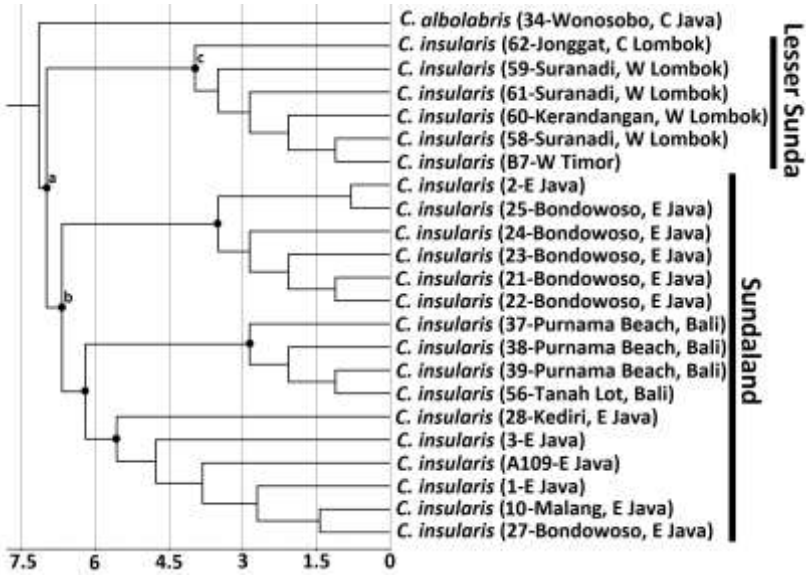

Figure 2. Time-calibrated tree based on 4 simultaneous genes (16S rRNA, ND4, 7IβFIB & 3ITBP). Sample number and locality were showed in parentheses. The scaled line below the trees represents the time in million years ago. The alphabets on the nodes represent the divergence time which explained in the results section (C: Central, E: East, W: West).

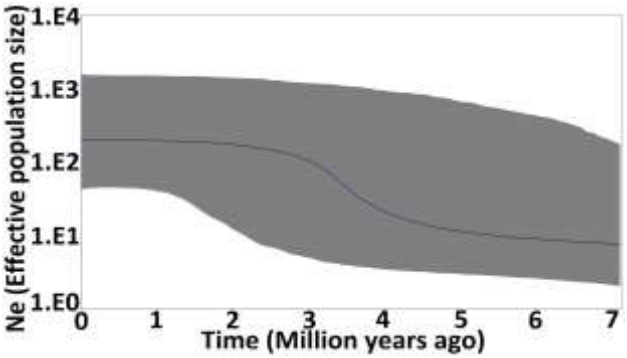

Figure 3. Bayesian Skyline Plot illustrating effective population size (Ne) through the time on *Cryptelytrops insularis.* Grey solid color represent 95% posterior probability range. Blackline represents the median population size.

Historical biogeography and population demography of *Cryptelytrops insularis*

Java and Bali emerged to the surface by 10 mya and then followed by Lombok and Sumbawa by 5 mya [28]. The emergence of younger islands to the east, starting from Bali to Lombok, Sumbawa, Flores, and Wetar were corresponding to the relationship in our phylogeny tree [29]. Seems, our time divergences analysis also demonstrate the same condition, which samples from Java-Bali was oldest then followed by Lesser Sunda's sample.

Timor was emerged around 2 mya, as the result of the collision between Australian continental margin and the Banda volcanic arc. [3,30-32]. These reports strengthen our findings, that sample from Timor is the youngest one than another which is included in Lesser Sunda.

During Miocene Epoch, warm temperature, humid climate, and rainforest habitat were predominated thus provided organisms suitable habitats [34]. However, started from Pliocene (around 3 mya) to recent the temperature was cold, even extremely cold [35,36]. In such conditions, some organisms might experience population fluctuation and even extinction [37]. But, *C. insularis* population are constantly increased. We suggested that this species has a strong survival rate to adapt in several conditions and habitat, especially cold temperature [35].

Dispersion probability

Java and Bali's islands have experience of being connected by the shallow sea during Pleistocene [3]. Java and Bali shared a lot of well-distributed fauna such as avians and mammals [38]. Other evidence showed in freshwater fishes, a total of 227 species spread in Java and Bali [39].

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Table 1. Genetic distances with the selected parameters based on jModeltest. Evolutionary model= GTR+I+G, Proportion of invariable sites (I) = 0,6583 and Gamma distribution shape parameter = 0,7690. The number in bold showed the most significant genetic differences. Sample number and locality were showed in parentheses. (C: Central, E: East, W: West).

		1	2	3	4	5	6	7	8	9	10	11	12
1	*Cryptelytrops insularis* (1-E Java)	-											
2	*C. insularis* (2-E Java)	0.004	-										
3	*C. insularis* (3-E Java)	0.003	0.003	-									
4	*C. insularis* (10-Malang, E Java)	0.003	0.004	0.002	-								
5	*C. insularis* (21-Bondowoso, E Java)	0.004	0.001	0.003	0.003	-							
6	*C. insularis* (22-Bondowoso, E Java)	**0.009**	0.004	0.005	0.006	0.003	-						
7	*C. insularis* (23-Bondowoso, E Java)	0.005	0.001	0.003	0.004	0.000	0.004	-					
8	*C. insularis* (24-Bondowoso, E Java)	0.005	0.001	0.003	0.003	0.000	0.004	0.000	-				
9	*C. insularis* (25-Bondowoso, E Java)	0.005	0.000	0.003	0.003	0.000	0.004	0.000	0.000	-			
10	*C. insularis* (27-Bondowoso, E Java)	0.003	0.002	0.002	0.002	0.001	0.004	0.001	0.001	0.001	-		
11	*C. insularis* (21-Kediri, E Java)	0.004	0.003	0.003	0.003	0.003	0.006	0.003	0.002	0.002	0.003	-	
12	*C. insularis* (21-Purnama Beach, Bali)	0.004	0.003	0.003	0.004	0.002	0.006	0.003	0.002	0.002	0.003	0.002	-
13	*C. insularis* (38-Purnama Beach, Bali)	0.005	0.004	0.003	0.004	0.004	0.006	0.004	0.004	0.004	0.003	0.003	0.001
14	*C. insularis* (39-Purnama Beach, Bali)	0.003	0.004	0.002	0.003	0.003	0.006	0.004	0.004	0.004	0.002	0.003	0,.001
15	*C. insularis* (56-Tanah Lot, Bali)	0.004	0.004	0.003	0.004	0.004	**0.008**	0.005	0.005	0.004	0.004	0.005	0.002
16	*C. insularis* (58-Suranadi, W Lombok)	0.005	0.003	0.003	0.005	0.002	0.006	0.003	0.003	0.002	0.004	0.003	0.003
17	*C. insularis* (59-Suranadi, W Lombok)	0.004	0.002	0.002	0.004	0.001	0.006	0.002	0.002	0.001	0.003	0.003	0.003
18	*C. insularis* (60-Kerandangan, W Lombok)	0.004	0.003	0.003	0.004	0.003	0.006	0.003	0.003	0.002	0.004	0.004	0.003
19	*C. insularis* (61-Suranadi, W Lombok)	0.005	0.003	0.003	0.005	0.003	0.007	0.003	0.003	0.003	0.004	0.004	0.003
20	*C. insularis* (62-Jonggat, C Lombok)	0.004	0.002	0.002	0.003	0.001	0.006	0.002	0.002	0.001	0.003	0.003	0.002
21	*C. insularis* (A109-E Java)	0.005	0.002	0.000	0.001	0.002	0.002	0.003	0.002	0.002	0.002	0.001	0.002
22	*C. insularis* (B7-W Timor)	0.006	0.003	0.003	0.004	0.003	0.002	0.003	0.003	0.002	0.004	0.005	0.003
23	*Cryptelytrops albolabris* (34-Wonosobo, C Java)	0.052	0.052	0.053	0.067	0.057	0.072	0.063	0.064	0.062	0.067	0.066	0.067

Table 1. ***Continue***

		13	14	15	16	17	18	19	20	21	22	23
13	*C. insularis* (38-Purnama Beach, Bali)	-										
14	*C. insularis* (39-Purnama Beach, Bali)	0.002	-									
15	*C. insularis* (56-Tanah Lot, Bali)	0.002	0.002	-								
16	*C. insularis* (58-Suranadi, W Lombok)	0.003	0.003	0.004	-							
17	*C. insularis* (59-Suranadi, W Lombok)	0.003	0.003	0.003	0.001	-						
18	*C. insularis* (60-Kerandangan, W Lombok)	0.003	0.003	0.003	0.001	0.001	-					
19	*C. insularis* (61-Suranadi, W Lombok)	0.003	0.004	0.004	0.002	0.002	0.001	-				
20	*C. insularis* (62-Jonggat, C Lombok)	0.003	0.003	0.003	0.001	0.000	0.001	0.002	-			
21	*C. insularis* (A109-E Java)	0.003	0.002	0.003	0.003	0.002	0.001	0.003	0.001	-		
22	*C. insularis* (B7-W Timor)	0.003	0.003	0.002	0.001	0.001	0.001	0.001	0.001	0.002	-	
23	*Cryptelytrops albolabris* (34-Wonosobo, C Java)	0.067	0.053	0.053	0.051	0.052	0.050	0.051	0.058	0.060	0.042	-

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The existence of landbridge between Java and Bali might play an important role in *C. insularis* dispersal [3].

Between Bali and Lombok, there is a deep strait which is known as fauna discontinuation, called by Wallace's line [38]. But this Wallace line has no effect on *C. insularis*, this species is well distributed eastward to Timor, despite being separated by inter-island barriers [40]. Therefore, we suggested that there is an intervention of another animal, which influenced the movement of *C. insularis* from Bali to Lombok.

Komaki et al suggested that there are two possibilities for terrestrial animal transoceanic dispersal, ocean flow or carry by other animals with high dispersal ability (birds or human) [41]. However, at that time (around 4 mya) humans did not exist yet [42]. Therefore, we convinced that the dispersal pattern from Bali to Lombok was helped by birds. *C. insularis* categorized in the Viperidae Family which usually less-mobile compared to other families [9]. This behavior could also enhance the chance for birds to catch this species then moved to another place.

Zoologists explained that insects, mammals, and reptiles have been demonstrated the transoceanic dispersal by rafting [43-45]. Some floating stuff such as macroalgae, pumice, wood, and vegetation could be a substrate for the snake to hold up [41,46]. The ocean drift usually following the ocean throughflow and winds [46].

The eastward distribution of *C. insularis* from Lombok until Timor is more likely to be helped by ocean flow from the Pacific Ocean [28]. Water flows from the north (Makassar Strait) head eastward from Lombok to Timor. Then the throughflow circled Timor (to the south of Timor) and turned west toward the Indian Ocean [28,47,48]. Another viper species which exhibit a similar distribution is *Cryptelytrops albolabris* [8]. However, there is no report or genetic data of *C. albolabris* from Lesser Sunda.

CONCLUSION

Our phylogeny showed monophyletic group, Sundaland and Lesser Sunda were geographically separated with independent common ancestor. *Cryptelytrops insularis* started to evolve around 7 million years ago, then followed by first diversification around 6.7 mya in Sundaland area (Java and Bali). Samples from Lesser Sunda diverge around 4 mya. The *C. insularis* transoceanic dispersal are observed with eastward movement. We tend to believe that, the overseas dispersal is helped by other animal and rafting by the ocean drift. We still don't have complete samples representing all the islands in the Lesser Sunda. Therefore, more samples from other localities were needed for further research to explore the biogeography of *C. insularis.*

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REFERENCES

[1] Sodhi, N.S., L.P. Koh, B.W. Brook, P.K.L. Ng. 2004. Southeast Asia biodiversity: an impending disaster. *Review in TRENDS in Ecology and Evolution.* 19.

[2] Iskandar, D.T., W.R. Erdelen. 2006. Conservation of amphibians and reptiles in Indonesia: issues and problems. *Amph. Reptile Conserv.* 4. 60-87.

[3] Lohman, DJ., M. de Bruyn, T. Page, K. von Rintelen, R. Hall, K.L. Ng, Peter, H.T. Shih, G.R. Carvalho, T. von Rintelen. 2011. Biogeography of the Indo-Australian Archipelago. *Annu. Rev. Ecol. Evol. Syst.* 42. 205-226.

[4] Hall, R. 1998. The plate tectonics of Cenozoic SE Asia and the distribution of land and sea. Biogeography and Geological Evolution of SE Asia. 99-131.

[5] Voris, H.K. 2000. Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. *J. Biogeogr*. 27. 1152-1167.

[6] Molengraaff, G., M. Weber. 1921. On the relation between the Pleistocene glacial period and the origin of the Sunda Sea (Java and South China-Sea), and its influence on the distribution of coral reefs and on the land and freshwater fauna. *Proc. R. Acad. Amsterdam.* 23. 395–439.

[7] Boback, S.M. 2003. Body size evolution in snakes: evidence from island populations. *Copeia*. 1. 81–94.

[8] Uetz, P., T. Etzold. 1996. The EMBL/EBI Reptile Database. *Herpetol. Rev.* 27. 174-175.

[9] Marlon, R. 2014. Panduan visual dan identifikasi lapangan: 107+ ular Indonesia. Indonesia Nature and Wildlife Publising.

[10] Maholtra, A., R.S. Thorpe. 2000. A phylogeny of the Trimeresurus group if pit vipers: new evidence from a mitochondrial

ISSN. 2087-2852
E-ISSN. 2338-1655

gene tree. *Mol. Phylogenetics Evol.* 16. 199-211.

[11] Creer, S., A. Maholtra, R.S. Thorpe. 2003. Assessing the phylogenetic utility of four mitochondrial genes and a nuclear intron in the Asian Pit Viper genus, *Trimeresurus*: separate, simultaneous, and conditional data combination analyses. *Mol. Biol. Evol*. 20. 1240-1251.

[12] Maholtra, A., R.S. Thorpe. 2004. A phylogeny of four mitochondrial gene regions suggests a revised taxonomy for Asian pitvipers (*Trimeresurus* and *Ovophis*). *Mol. Phylogenetics Evol.* 32. 83-100.

[13] Creer, S., C.E. Pook, A. Maholtra, R.S. Thorpe. 2006. Optimal intron analyses in the *Trimeresurus* radiation of Asian Pitvipers. *Syst. Biol.* 55. 57-72.

[14] Maholtra, A., S. Creer, C.E. Pook, R.S. Thorpe. 2010. Inclusion of nuclear intron sequence data helps to identify the Asian sister group of New World pitvipers. *Mol. Phylogenetics Evol.* 54. 172-178.

[15] David, P., G. Vogel, N. Vidal. 2003. On *Trimeresurus fasciatus* (Boulenger, 1896) (Serpentes: Crotalidae), with a discussion on its relationships based on morphological and molecular data. *Raffles Bull. Zool.* 51. 149-157.

[16] Benson, D.A., I. Karsch-Mizrachi, D.J. Lipman, J. Ostell, D.L. Wheeler. 2005. GenBank. *Nucleic Acids Res*. 33. 34-38.

[17] Menegon, M.2007. Methods for surveying and processing reptiles and amphibians of Alpine springs. In: Cantonati, M., E. Bertuzzi, D. Spitale (Eds). The Spring Habitat: Biota and Sampling Methods. Museo Tridentino di Scienze Naturali, Trento. 275-285.

[18] Kocher, T.D., W.K. Thomas, A. Meyer, S.V. Edwards, S. Paabo, F.X. Villablanca, A.C. Wilson. 1989. Dynamics of mitochondrial DNA Evolution in animals: Amplification and sequencing with conserved primers. *Proc. Nati. Acad. Sci. USA.* 86. 6196-6200.

[19] Boon, N., W.D. Wind, W. Verstraete, E.M. Top. 2002. Evaluation of nested PCR-DGGE (denaturing gradient gel electrophoresis) with group-spesific 16S rRNA primers for the analysis of bacterial communities from different wastewater treatment plants. *FEMS Microbiol. Ecol.* 39. 101-112.

[20] Kumar, S., G. Stecher, K. Tamura. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol*. 33. 1870-1874.

[21] Patterson, J., B. Chamberlain, D. Thayer 2006. Finch TV Version 1.4.0. Available at: http://www.geospiza.com/Products/finchtv.shtml

[22] Guindon, S., O. Gascuel. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst. Biol.* 52. 696-704.

[23] Darriba, D., G.L. Taboada, R. Doallo, D. Posada. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods.* 9. 772.

[24] Huelsenbeck, J.P., F.R. Ronquist. 2001. MrBayes: Bayesian Inference of Phylogenetic Trees. *Bioinformatics.* 17. 754-755.

[25] Drummond, A.J., M.A. Suchard, D. Xie, A. Rambaut. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol*. 29. 1969-1973.

[26] Zhu, F., Q. Liu, J. Che, L. Zhang, X. Chen, F. Yan, R. Murphy, C. Guo, P. Guo. 2016. Molecular phylogeography of white-lipped tree viper (*Trimeresurus*; Viperidae). *Zoologica Scripta.* 45. 252-262.

[27] Rambaut, A., M.A. Suchard, D. Xie, A.J. Drummond. 2014. Tracer v1.6. Institute of Evolutionary Biology, University of Edinburgh.

[28] Hall, R. 2009. Southeast Asia's changing palaeogeography. *Blumea.* 54. 148-161.

[29] Hall, R., I. Sevastjanova. 2012. Australian crust in Indonesia. *Aust. J. Earth Sci.* 59. 827-844.

[30] Carter, D.J., M.G. Audley-Charles, A.J. Barber. 1976. Stratigraphical analysis of island arc-continental margin collision in eastern Indonesia. *J. Geol. Soc*. 132. 179-189.

[31] Hamilton, W. 1979. Tectonics of the Indonesian region, Geological Survey Professional Paper 1078. U.S. Government Printing Office, Washington.

[32] Audley-Charles, M.G. 2011. Tectonic post-collision processes in Timor. In: Hall, R., M.A. Cottam, M.E.J. Wilson (Eds). The SE Asian gateway: history and tectonics of the Australia–Asia collision. Geological Society of London, Special Publications. 355. 241–266.

[33] Wilson, M.E.J. (Eds). 2011. The SE Asian gateway: history and tectonics of the Australia-Asia collision, Geological Society of London, Special Publication. 355. 241-166

ISSN. 2087-2852
E-ISSN. 2338-1655

[34] Woodruff, D.S. 2010. Biogeography and conservation in Southeast Asia: how 2.7 million years of repeated environmental fluctuations affect today's patterns and the future of the remaining refugial-phase biodiversity. *Biodiv. Conserv.* 19. 919-941.
[35] Kershaw, A.P., S. van der Kaars, J.R. Flenley. 2007. The quaternary history of far eastern rainforests. In: Bush, M.B., J.R. Flenley (Eds). Tropical rainforest responses to climate change. Springer, Berlin. 77-115.
[36] Haywood, A.M., H.J. Dowsett, P.J. Valdes, D.J. Lunt, J.E. Francis, B.W. Sellwood. 2009. Introduction. Pliocene climate, processes and problems. *Philos. Trans. R. Soc. A*. 367. 3-17.
[37] Heaney, L.R. 1991. A synopsis of climatic and vegetational change in Southeast Asia. *Clim. Change.* 19. 53-61.
[38] Wallace, A.R. 1860. On the zoological geography of the Malay Archipelago. *J. Linn. Soc. London.* 4. 172-184.
[39] Dahruddin, H., A. Hutama, F. Busson, S. Sauri, R. Hanner, P. Keith, R. Hadiaty, N. Hubert. 2016. Revisiting the ichthyodiversity of Java and Bali through DNA barcodes: taxonomic coverage,identification accuracy, cryptic diversity and identification of exotic species. *Mol. Ecol. Resour.* 17(2). DOI: 10.1111/1755-0998.12528.
[40] Mayr, E. 1944. Wallace's Line in the light of recent zoogeographic studies. *Am Mus. Nat. Hist*. 19. 1-14.
[41] Komaki, S., S.M. Lin, M. Nozawa, S. Oumi, M. Sumida, T. Igawa. 2016. Fine-scale demographic processes resulting from multiple overseas colonization events of the Japanese stream tree frog, *Buergeria japonica*. *J. Biogeogr*. 1-12.
[42] van den Bergh, GD., J. de Vos, P.Y. Sondaar. 2001. The late quaternary palaeogeography of mammal evolution in the Indonesian Archipelago. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 171. 385-408.
[43] Heatwole, H., R. Levins. 1972. Biogeography of the Puerto Rican Bank: floatsam transport of terrestrial animals. *Ecology.* 53. 112-117.
[44] Prescott, J.H. 1959. Rafting of jack rabbit on kelp. *J. Mammal.* 40. 443.
[45] Censky, E.J., K. Hodge, J. Dudley. 1998. Over-water dispersal of lizards due to hurricanes. *Nature.* 395. 556.
[46] Thiel, M., L. Gutow. 2005. The ecology of rafting in the marine environment. I. The floating substrata *Oceanogr. Mar. Biol. Annu. Rev.* 42. 181-263.
[47] Godfrey, J.S. 1996. The effect of the Indonesian throughflow on ocean circulation and heat exchange with the atmosphere: a review. *J. Geophys. Res.* 101. 12217-12238.
[48] Metzger, E.J., H.E. Hurlburt. 1996. Coupled dynamics of the South China Sea, the Sulu Sea and the Pacific Ocean. *J. Geophys. Res.* 101. 12331-12352.
[49] Arévalo, E., W.S. Jack Jr., S.K. Davis. 1994. Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in Central Mexico. *Syst. Biol.* 43. 387-418.

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Supplementary 1. List of the sample used in this study

Species	Code*	Locality	Accession number			
			16S	ND4	7IβFIB	3ITBP
C. insularis	1	E Java	MG561477	MG561528	MG561620	MG561575
C. insularis	2	E Java	MG561478	MG561529	MG561621	MG561576
C. insularis	3	E Java	MG561479	MG561530	MG561622	MG561577
C. insularis	10	Malang, E Java	MG561486	MG561537	-	MG561584
C. insularis	21	Bondowoso, E Java	MG561497	MG561548	MG561632	MG561594
C. insularis	22	Bondowoso, E Java	MG561498	MG561549	-	-
C. insularis	23	Bondowoso, E Java	MG561499	MG561550	-	MG561595
C. insularis	24	Bondowoso, E Java	MG561500	MG561551	-	MG561596
C. insularis	25	Bondowoso, E Java	MG561501	MG561552	-	MG561597
C. insularis	27	Bondowoso, E Java	MG561503	MG561554	-	MG561599
C. insularis	28	Kediri, E Java	MG561504	MG561555	-	MG561600
C. insularis	37	Purnama beach, Bali	MG561513	MG561564	-	MG561608
C. insularis	38	Purnama beach, Bali	MG561514	MG561565	-	MG561609
C. insularis	39	Purnama beach, Bali	MG561515	MG561566	MG561638	MG561610
C. insularis	56	Tanah Lot, Bali	MG561518	MG561568	MG561639	MG561613
C. insularis	58	Suranadi, W Lombok	MG561520	MG561570	MG561641	MG561615
C. insularis	59	Suranadi, W Lombok	MG561521	MG561571	MG561642	MG561616
C. insularis	60	Suranadi, W Lombok	MG561522	MG561572	MG561643	MG561617
C. insularis	61	Suranadi, W Lombok	MG561523	MG561573	MG561644	MG561618
C. insularis	62	Jonggat, C Lombok	MG561524	MG561574	MG561645	MG561619
C. insularis	A109/A96	E Java/Indo	AY352738	AY352833	GQ428347	-
C. insularis	B7	W Timor	AY059550	AY059586	DQ116978	DQ117500
C. albolabris	34	Wonosobo, C Java	MG561510	MG561561	MG561636	MG561606

*The code start with a number were obtained in this study. Whereas the code start with alphabet was obtained from Genbank. (C: Central, E: East, W: West)

Supplementary 2. Primers and sources used in this study

Genes	Primers	Primer sequences	References
16S	16Sf4	5'- CTTGTACCTTTTGCATCATGGT-3'	This study
	16Sr2	5'-CCGGTCTGAACTCAGATCACGT-3'	This study
ND4	ND4	5'-CACCTATGACTACCAAAAGCTCATGTAGAAGC-3'	Arevalo et al [49]
	LEU	5'-CATTACTTTTACTTGGATTTGCACCA-3'	Arevalo et al [49]
	ND4-R	5'-TTACCAGATTGAAGAGTTAGCAGGTCTT-3'	This study
7IβFIB	TRIMFIB-BI7U	5'-AGAGACAATGATGGATGGTAAG-3'	Creer et al [13]
	TRIMFIB-B17L	5'-CCTTTTGGGATCTGGGTGTA-3'	Creer et al [13]
	7IβFIB-2F	5'-TATAGCACCTGCAAAACG-3'	This study (Nested PCR)
	7IβFIB-2R	5'-CTTGCCTTATCTTACTATGTG-3'	This study (Nested PCR)
3ITBP	TRIMTBPI3F	5'- CCTTTACCAGGAACCACACC-3'	Creer et al [13]
	TRIMTBPI3R	5'-GAAGGGCAATGGTTTTTAG-3'	Creer et al [13]
	3ITBP-2F	5'-CAGGTTCTACTCTGCTCACA-3'	This study (Nested PCR)
	3ITBP-2R	5'-AATGGACTCCAGAAGATG-3'	This study (Nested PCR)

Role of Active Compounds of *Bohadschia argus* Inhibit Cancer Cell Survival

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Abstract

Sea cucumber is marine biota with a high economic value and also has potential for anti-cancer. The purpose of this study was to explore the mechanism of active compound of *Bohadschia argus* on regulating cancer cell survival. The *B. argus* samples were collected from the sea of Kamal Village, West Seram Maluku, then extracted by water. The constituents of water extract of *B. argus* were examined by LC-MS. The network among active compound and its protein target were determined by Cytoscape app. The result shows that *B. argus* has several active compounds, such as chondroitin sulfate, holothurin A, holothurin B, and scabraside that might play a role in cancer cell apoptosis, proliferation, and metastasis.

Keywords: Active compound**,** *Bohadschia argus*, LC-MS.

INTRODUCTION

Indonesia is archipelago country which surrounded by the ocean and has the potential natural resources, especially marine biota. One of the marine biotas that have high economic value is sea cucumber (Holothuriidae). Sea cucumber is an invertebrate animal which included into the phylum Echinodermata and has characters such as soft body, elongated body and rough skin [1].

The previous study has shown that some active compound of sea cucumber contains anti-cancer and anti-inflammatory properties include Monosulfated, Triterpenoid Glycoside, 12-Methyltetradecanoic, Frondoside A, Frondoside B, and Frondoside C [2]. Frondoside A is capable of the treatment for breast cancer [3]. *In vitro* study showed that frondoside A was able to fight cancer by reducing cell viability, inducing apoptosis (activated caspase-3) [4]. This study aimed to investigate the bioactive compounds in the crude extracts of sea cucumber *Bohadschia argus.*

MATERIAL AND METHOD

Bohadschia argus sample in this study was obtained from Kamal Village, West Seram, Mollucas Indonesia. The sample was washed with water to remove dirt and sand. Then the sample was stored on a cold state.

Bohadschia argus Extraction

Extraction of *B. argus* carried out by maceration method. *B. argus* was weighed 25 g and crushed. The sample was macerated into the hot water 80°C ± 500 mL for ± 2 hours. Maceration results were filtered using filter paper. After filtered, the extraction is then separated from water contain using *freezdry.*

Analysis Using LC-MS

The analysis of compound extract *B. argus* was performed by the LC-MS (Shimadzu LCMS - 8040 LC/MS), 1 µL sample was injected into the column 2 mm D x 150 mm 3 µm, Capillary voltage 3.0 kV, with column temperature 35°C and Flow rate 0,5 $mL.min^{-1}$. Desolvation gas flow 6 $L.hr^{-1}$, run time 120 minutes.

Network Construction

Network analysis was used to understanding the effect of the active compound in sea cucumber *B. argus.* The network analyzing active compound with protein was constructed using App Cytoscape 3.6.0.8 [20]. Six proteins related cancer cell was obtained by STRING network diseases then active compounds and protein interaction was established with STICH proteins/ compound network. In the network graphic, proteins and active compounds were presented as nodes, while compounds-proteins and proteins-proteins interaction were presented as edges.

RESULT AND DISCUSSION

Based on the results of LC-MS, the active compound of *B. argus* extract are shown in Table 1. Chondroitin sulfate is an active compound of *B. argus* extract that may act as anti-metastasis [5]. Another compound like Holothurin B and Holothurin A that included in the Triterpene glycoside [6]. Triterpene glycoside is the most abundant compound in sea cucumber [7]. It was

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secondary metabolite on sea cucumber which plays a role in cancer cells with inducing apoptosis by activating caspase, anti-proliferation and arrest cell cycle on S or G2/M [8].

Table 1. Sea cucumber *B. argus* active compound induce apoptosis and cell cycle arrest in breast cancer cell

Compound	Activity	Ref
Frondoside A	Induce Apoptosis	[9]
Holohurin A	Induce apoptosis, Anti-metastasis	[10]
Holohurin B	Induce Apoptosis	[13]
Echinoside A	Induce Apoptosis G0/G1 arrest	[11][12]
Cucumarioside	S phase Arrest	[13]
Chondoitin sulfate	Inhibit proliferation, Anti-Metastasis	[5]
Scabraside	Activated Caspase 3	[4]

Induction apoptosis is one of the most prominent markers of cytotoxic antitumor agents. Some of the natural compounds from sea cucumber induce apoptotic pathways to inhibit cancer progression. Frondoside A induces apoptotic cell through increased expression of P53, and induction CASP9, CASP3, CASP7 cell death in breast cancer cells [4]. Cucumarioside demonstrated anticancer effects through its ability to cause the arrest of the cell cycle during the S phase and was shown to induce apoptosis [13].

Analysis of *B. argus* Active Compound Target Network

Sea cucumber contains several active compounds that potentially for anti-cancer [8]. The interaction among active compound and its protein target have been constructed (Fig. 1). The active compounds interacted with proteins that involve in the apoptosis, metastasis, and proliferation.

The network constructed is shown that several compounds from sea cucumber targets are proteins which play a role in breast cancer like MMP9, CASP9, TP53, CASP3, CHSY1. Some of the active compounds have direct interaction with a protein related to breast cancer and other compounds have indirect interaction. Frondoside A has direct interaction with Matrix metallopeptidase 9 (MMP9), and inhibit the growth of cancer cell [4,9,15]. MMP9 have interaction with several proteins such as TP53, BCL2, NFKB1, and BAX. The compund is capabale of inducing apoptosis through various mechanisms, including the intracellular caspase, decreasing BCL2 and increasing CASP3 [16].

Chondroitin sulfate is one of the active compounds found in *B. argus* interact directly with several proteins like CHSY1, ARSB, and CSPG4. The compound is able to inhibit Chondroitin synthase-1 (CHSY1) expression that links to cell apoptosis and proliferation [17]. CHSY1 interact with CSPG4 responsible for regulating apoptosis and suppressing cell proliferation and metastasis [18]. Several compounds like Echinoside A whereas indirect interact with a protein that has a function for controlling cell cycle and apoptosis [12]. The active compounds in *B. argus* interacted with many proteins that involved in the cell cycle, metastasis, and apoptosis [19]. The ability of the active compounds in sea cucumber compounds to inhibit cancer cell can be modified to fit the genetic profile of cancer cells for the purpose of treatment.

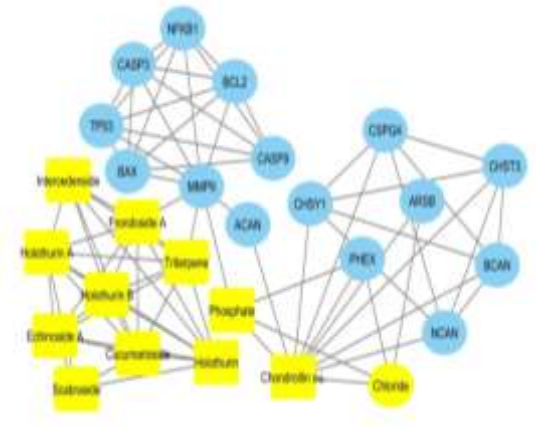

Figure 1. Active compound-protein related cell cycle arrest and apoptosis pathway in the breast cancer cell. The yellow rectangular represents active compound from *B. argus.* The blue circle represents proteins in breast cancer.

CONCLUSION

The *B. argus* contains active compound such as Frondoside A, chondroitin sulfate, echinoside A, holothurin A, holothurin B, and scabraside. The compound has interaction with some of the proteins like MMP9, CASP9, TP53, CASP3, BCL2, BAX, NFKB1, and CHSY1 that play a role on cancer cell apoptosis, cell cycle and metastasis. The mechanism for killing cancer cells using the active compounds contained in sea cucumber *B. argus* has been investigated. Future researchers may conduct a study that develops treatment using the active compounds in *B. argus.*

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REFERENCES

[1] Lovatelli, A., C. Conand, S. Purcell, S. Uthicke, J.F. Hamel, A. Mercier. 2004. Advances in sea cucumber aquaculture and management. FAO Fisheries Technical. Rome, Italy.

[2] Janakiram, N., A. Mohammed, C. Rao. 2015. Sea cucumbers metabolites as potent anti-cancer agents. *Mar. Drugs*. 13(5). 2909-292.

[3] Park, S.Y., Y.H. Kim, Y. Kim, S.J. Lee. 2012. Frondoside A has an anti-invasive effect by inhibiting TPA-induced MMP-9 activation via NF-κB and AP-1 signaling in human breast cancer cells. *Int. J. Oncol.* 41(3).933-940.

[4] Al Marzouqi, N., R. Iratni, A. Nemmar, K. Arafat, M.A. Al Sultan, J. Yasin, S. Attoub. 2011. Frondoside A inhibits human breast cancer cell survival, migration, invasion and the growth of breast tumor xenografts. *Eur. J. Pharmacol.* 668(1-2). 25-34.

[5] He, M., J. Wang, S. Hu, Y. Wang, C. Xue, H. Li. 2014. The effects of fucosylated chondroitin sulfate isolated from Isostichopus badionotus on antimetastatic activity via down-regulation of Hif-1α and Hpa. *Food Sci. Biotech.* 23(5). 1643-1651.

[6] Silchenko, A.S., V.A. Stonik, S.A. Avilov, V.I. Kalinin, A.I. Kalinovsky, A.M. Zaharenko, G. Cimino. 2005. Holothurins B(2), B(3), and B(4), new triterpene glycosides from mediterranean sea cucumbers of the genus holothuria. *J. Nat. Prod.* 68(4). 564-567.

[7] Li, Y.X., S. Himaya, S.K. Kim. 2013. Triterpenoids of marine origin as anti-cancer agents. *Molecules*. 18(7). 7886-7909.

[8] Aminin, D., E. Menchinskaya, E. Pisliagin, A. Silchenko, S. Avilov, V. Kalinin. 2015. Anticancer activity of sea cucumber triterpene glycosides. *Mar. Drugs*. 13(3). 1202-1223.

[9] Adrian, T.E., P. Collin. 2018. The anti-cancer effects of frondoside A. *Mar. Drugs*. 16(2).

[10] Zhao, Q., Y. Xue, Z. Liu, H. Li, J. Wang, Z. Li, Y. Wang, P. Dong, C. Xue. 2010. Differential effects of sulfated triterpene glycosides, holothurin A1, and 24 dehydroechinoside A, on antimetastasic activity via regulation of the MMP-9 signal pathway. *J. Food Sci.* 75(9). H280-288.

[11] Li, M., Z.H. Miao, Z. Chen, Q. Chen, M. Gui, L.P. Lin, J. Ding. 2010. Echinoside A, a new marine-derived anticancer saponin, targets topoisomerase2alpha by unique interference with its DNA binding and catalytic cycle. *Ann. Oncol.* 21(3). 597-607.

[12] Zhao, Q., Y. Xue, J. Wang, H. Li, T. Long, Z. Li, Y. Wang, P. Dong, C. Xue. 2012. In vitro and in vivo anti-tumour activities of echinoside A and ds-echinoside A from *Pearsonothuria graeffei*. *J. Sci. Food Agric.* 92(4). 965-974.

[13] Menchinskaya, E.S., E.A. Pislyagin, S.N. Kovalchyk, V.N. Davydova, A.S. Silchenko, S.A. Avilov, V.I. Kalinin, D.L. Aminin. 2013. Antitumor activity of cucumarioside A2-2. *Chemother.* 59(3). 181-191.

[14] Assawasuparerk, K., T. Rawangchue, R. Phonarknguen. 2016. Scabraside D derived from sea cucumber induces apoptosis and inhibits metastasis via iNOS and STAT-3 expression in human cholangiocarcinoma xenografts. *Asian Pac. J. Cancer Prev.* 17(4). 2151-2157.

[15] Kotipatruni, R.R., A.K. Nalla, S. Asuthkar, C.S. Gondi, D.H. Dinh, J.S. Rao. 2012. Apoptosis induced by knockdown of uPAR and MMP-9 is mediated by inactivation of EGFR/STAT3 signaling in medulloblastoma. *PloS One.* 7. e44798.

[16] Bruey, J.M., N. Bruey-Sedano, F. Luciano, D. Zhai, R. Balpai, C. Xu, C.L. Kress, B. Bailly-Maitre, X. Li, A. Osterman, S. Matsuzawa, A.V. Terskikh, B. Faustin, J.C. Reed. 2007. Bcl-2 and Bcl-XL regulate proinflammatory caspase-1 activation by interaction with NALP1. *Cell.* 129. 45-56.

[17] Zeng, L., J. Qian, X. Luo, A. Zhou, Z. Zhang, Q. Fang. 2018. CHSY1 promoted proliferation and suppressed apoptosis in colorectal cancer through regulation of the NFκB and/or caspase-3/7 signaling pathway. *Oncol. Lett.* 16. 6140-6146.

[18] Wang, X., T. Osada, Y. Wang, L. Yu, K. Sakakura, A. Katayama, S. Ferrone. 2010. CSPG4 protein as a new target for the antibody-based immunotherapy of triple-negative breast cancer. *J. Natl. Cancer Inst.* 102(19). 1496-1512.

[19] Szklarczyk, D., J.H. Morris, H. Cook, M. Kuhn, S. Wyder, M. Simonovic, A. Santos, N.T. Doncheva, A. Roth, P. Bork, L.J. Jensen, C. von Mering. 2017. The STRING database in 2017: Quality-controlled protein-protein association networks, made broadly accessible. *Nucleic Acids Res.* 45. D362-368.

[20] Shannon, P., A. Markiel, O. Ozier, N. Baliga, S., Ramage, D. 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* 13(11). 2498-2504.

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Anatomical Characters of Shoot Apical Meristem (SAM) on Bulbil Porang (*Amorphophallus muelleri* Blume) At the End of Dormancy Period

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Abstract

Porang is one species member of the genus *Amorphophallus*. In Indonesia, Porang is cultivated extensively because it has a high economic value. There is three seeded material available for planting. One of seeded material is bulbil. Bulbil is a vegetative reproductive organ of Porang which is located in the branch of the leaf. Bulbil has 3 - 4 months dormancy period. The dormancy period will end marked with shoot growth. Morphologically, many tubercles were found on the bulbil peel. The tubercle scattered throughout the bulbil peel and become bud growth locus. Shoots on bulbil will grow from the tubercle and only in the adaxial part, contrary do not grow from the abaxial part. This study aim to compare the anatomy of shoot apical meristem (SAM) in the adaxial and abaxial parts of bulbil at the end of the dormancy period, i.e 100 days after harvest (DAH), 110 DAH, 120 DAH, and 130 DAH. Anatomical structures of bulbil were observed by semi-permanent preparations. The results showed that the anatomical structures of the adaxial and abaxial parts of bulbil Porang had differences in SAM development, leaf primordia, primary thickening meristem (PTM) and procambium.

Keywords: Anatomy, Bulbil Porang, Dormancy, Shoot Apical Meristem.

INTRODUCTION

Porang (*Amorphophallus muelleri* Blume) is a member of Araceae [1] which in Javanese society is known as*iles-iles* [2]. At least three growth periods are needed to be harvested. Since the first planting period it can produce aerial tubers called bulbil. It grows on leaf branches which can reach 20 bulbils per plant [3]. Porang can be found in tropical and subtropical regions. These scattered from Africa to Asia such as Japan and China. Previously it was found in India (Andaman), then scattered eastward in Myanmar, Thailand, and Indonesia [4].

In Indonesia, porang were distributed from western regions such as Sumatra to West Nusa Tenggara (NTB) and East Nusa Tenggara (NTT). Furthermore *A. muelleri* can be propagated both generative by seeds and vegetative by tubers and bulbils [5]. Extensively propagation of *A. muelleri* were applied by bulbil [6] because it has ability to grow independently into new plants [7]. Information regarding seeds and tubers has been widely reported [8], whereas studies related to bulbil need to be expanded to supply porang references. Morphologically, bulbil has characteristics such as tubers but smaller in size. On the surface of bulbils peel, there are rough brown tubercles and these are bud growth locus [9]. Bulbil has a lot of tubercles scattered both adaxial and abaxial sides, but the shoots only arise from adaxial part. Morphologically, it were observable from the tubercle. However, anatomical structures of bulbils need to be investigated to obtain information of porang.

The terms adaxial and abaxial refers to Ezeabara [10]. At the end of the dormancy period, the tubercle in the adaxial part will transform into buds. Based on these empirical facts, this study aim to investigate the anatomy of bulbil both adaxial and abaxial parts after 3 months / 92 days after harvest (DAH) until the end of the dormancy period.

MATERIALS AND METHODS

Bulbils

The bulbil used has approximately 92 DAH (3 months). The age was determined (calculated) since it was harvested from Porang. The bulbil was obtained from the district of Madiun, East Java. The bulbil weight ranged from 1.4-3.75 g with diameter of 14.5-19.5 mm. Then the bulbils were germinated on the germination tray and placed in the laboratory at room temperature. When the bulbils

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were reacheds 100 DAH, it begins to be soaked in the FAA solution then followed periodically at 120 and 130 DAH in the same way.

Observation of Bulbil Anatomy

The whole bulbil were cut into two equal parts (adaxial and abaxial). Both parts were sliced vertically with a razor blade than each slice was sliced with a microtome (Figure 1). Each slice was taken at a bud growth locus / tubercle in the bulbil peel. Sliced in the tubercle region intend to obtain the anatomical character of shoot apical meristem (SAM) in bulbil. The anatomy of bulbil was observed using semi-permanent slide which was preparations based on the modified Ruzin method [11]. The stages for making slides start from fixed the bulbil with FAA solution (Formaldehyde Acetic Acid Alcohol), bulbil which has been cut as described above clamped with carrot media and made using a microtome (clamp on hand microtome), sliced bulbil was soaked in 5% NaOH during 5 minutes, followed by washing by distilled water for 5 minutes, then staining with Safranin 1% for 3 minutes, then washing again with distilled water for 5 minutes. Furthermore, the slice-tissue were placed on a sliding glass, mounting with glycerin and covered by cover glass. To prevent evaporation of glycerin, sealed cover glass with transparent nail polish. Now the slides ready to observe with the light microscope and documented with a digital camera.

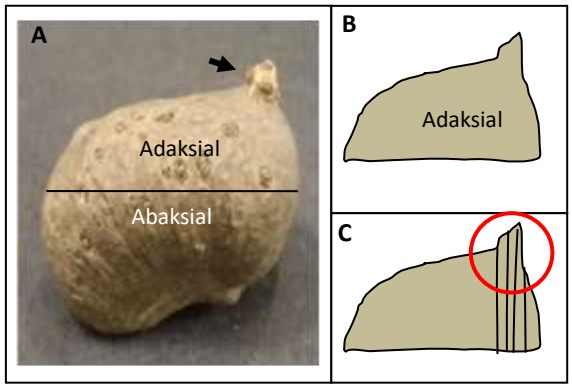

Figure 1. Cutting the bulbil Porang. (A: whole bulbil and its part, B: a part that will be sliced, C: slice in the tubercle area).

Data Analysis

The data obtained in this study are qualitative and quantitative. Qualitative data are the anatomical characters of the adaxial and abaxial parts that have been documented. Quantitative data was the result of the measurement of height and width of SAM. Both of these data were analyzed descriptively referring to various references.

RESULT AND DISCUSSION

Anatomical Character of Bulbil in Adaxial and Abaxial Parts

There are differences in SAM development in the adaxial and abaxial parts. The anatomical structure of bulbil at 100 DAH has been observed the presence of SAM in the adaxial part (Fig. 2A). SAM organized as a dome and surrounded by two small leaf primordia. At the tip of SAM, tunica cells can be observed (Fig. 2A). It was composed of a 2-3 layer of cells. Tunica cells can divide quickly to intensify the surface and also differentiate to advance the development of SAM. At the bottom of SAM, procambium and primary thickening meristem (PTM) were observed. PTM is composed of semicircular cells. PTM cells and procambium surrounded byergastics substance organized as a black spot. The abaxial part of bulbil at 100 DAH has not observed the development of SAM. Candidate SAM emerge in the epidermis and has a conical shape (Fig. 2E). Candidate of SAM was not growing well and incomparable with adaxial parts (Fig. 2A). It was indistinguishable between the dome of SAM and leaf primordia. Cell space was observed among groups of cells of various sizes and several points of ergastic substances.

Bulbil's anatomy in adaxial and abaxial parts at 110 DAH has indicated the differences in SAM development, leaf primordia, and procambium. The anatomical structure of the adaxial part was observable the development of SAM and leaf primordial surrounding the SAM (Fig. 2B). Adaxial anatomy of bulbil aged 100 DAH (Fig. 2A) has a SAM height of 621.05±142.05 µm and a width of 670.47±194.16 µm. While the anatomy of SAM aged 110 DAH increased in height and width, i.e. 629.91±30.14 µm and 633.76±168.50 µm. Based on these measurements, there has been an increase in the height and width of SAM. However, in the abaxial part, SAM developments were unobserved (Fig. 2F). But both have similarities to the PTM structure. Around PTM there are meristematic cells with a small size (Fig. 2F). These meristematic cells may develop into SAM. But the development of SAM did not occur as in the adaxial part (Fig. 2B), only SAM candidates are observed around the

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epidermis. The SAM candidate has conical shaped and was bigger than the candidate of SAM at bulbil 100 DAH (Fig.2E).

Bulbil's anatomy at 120 DAH has shown the development of SAM structure. In the adaxial part, the average height was 638.54±126.64 µm and 774.43±230.67 µm in width. In addition, leaf primordia were observed dome-circling (Fig. 2C). SAM structure and leaf primordia can be used as an indicator to determine the development of shoots. Furthermore, the presence of procambium under SAM was observed and the composition of the PTM was composed of semicircular cells. In contrast to the abaxial part, the development of SAM was unobserved (Fig. 2G). It was observed a candidate for a SAM like conical shaped in the epidermis. In addition, the presence of intercellular space and ergastic substances around the cell were observed.

Bulbil's anatomy at 130 DAH has shown the significant development of SAM. It has an average height of 655.06± 446.66 µm and width of431.91± 272.61 µm. The average width value has decreased but the high value of SAM increase continuously (Table 1). The SAM structure looks so prominent, leaf primordia look wider and bigger (Fig. 2D). Procambium was observed under SAM and has sinuous shaped. Primary thickening meristem (PTM) was observed as previously and morphologically, shoot sprouts have emerged. The shoots emerge on the peel of the bulbil in white color. The shoots emerge from one of the tubercles on adaxial part of bulbil. Anatomically, SAM development has an impact morphologically on the growth of shoots.

An abaxial part at 130 DAH has also shown the existence of SAM. However, SAM was unobserved in the dome shape as in the adaxial part (Fig. 2H). It structures are undeveloped and leaf primordia were observed like a circle around the SAM. The structure of the leaf primordia was different from the adaxial part. Leaf primordia form a semicircle and emerge from two sides of SAM.

Comprehensively anatomical structures on adaxial at various ages (100, 110, 120, and 130 DAH) was observablethe SAM structure, the presence of procambium, PTM, leaf primordia, tunica-corpus structure, and the emergence of several ergastic substances. Whereas in the abaxial part, the existence of SAM was unobserved. Only a few SAM candidates were observed around the tubercle-shaped epidermal cells. It was observed at 130 DAH but did not indicate a dome. The anatomical structure of the abaxial part was also observed in the presence of intercellular spaces that are spread throughout the cell and the presence of ergastic substances. According to Steeves [12], SAM is formed since the embryogenesis stage and then develops into various important organs such as shoots, leaves, and stems. It can be observed with a microscope, composed of meristematic cells with different parts and functions. The PTM [13] is formed by several regular cell layers such as a circle of radius circles and is found in the peripheral zone. In some tubers and rhizomes, PTM is a collection of cells which can develop into the parenchyma, collateral vessels, and amphivasal vessels that produce adventitious roots.

Procambium is derived from vascular bundles association [13] then Krauss [14] states that the procambium is a tissue of primary meristems that will develop into the main vessels of the xylem and primary phloem vessels. Based on the observations of the anatomical structure of bulbil Porang at all ages, shows that meristematic cells are differentiated well and show a more mature phase in the adaxial part (Fig. A-D) compared to the abaxial part (Fig. E-H). The association of meristematic cells in the adaxial part was rows arranged to form a layered pattern with much smaller cell size. This shows that cells around SAM develop faster in the adaxial part. The existence of cells with a smaller size makes meristem cells have smaller vacuoles. The size can be an indicator when its staining. Meristematic cells can absorb color well because vacuoles were relatively small. While dyes will be difficult to penetrate cells other than meristematic because they have a larger vacuole. It has an effect on the color of the colored cells which paler tendency. Another factor that also important in the development of SAM according to Ganjari [9] was caused by internal factors in an accumulation of auxin hormones. Accumulation of auxin hormones in adaxial part was less than the abaxial portion.

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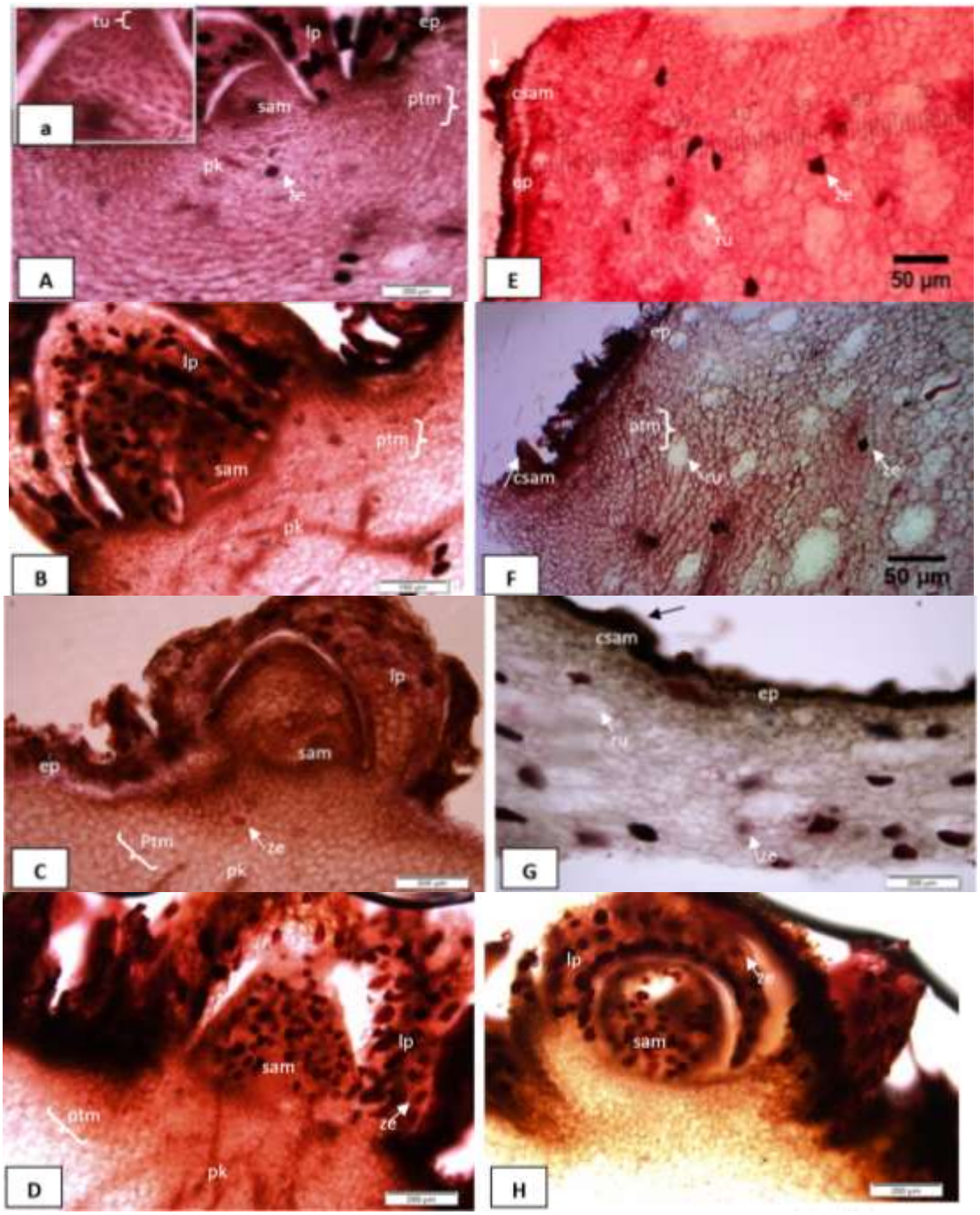

Figure 2. Anatomical structures of bulbil at various ages. A and E: 100 DAH, B and F: 110 DAH, C and G: 120 DAH, D and H: 130 DAH. (A-D anatomical structure of the adaxial part), (E-F anatomical structure of the abaxial part). sam; shoot apical meristem, ptm; primary thickening meristem, pk; procambium, tu; tunica, lf; leaf primordia, ze; ergastic substance, ru; cell space, ep; epidermis, csam; candidate of sam.

Based on Sorce's [15], the potato tubers during dormancy have very much accumulated auxin hormones in the apical meristem and leaf primordia which are characterized by dark color when observed with a microscope. However, the tuber has sprouted then auxin appears to spread basipetally from the meristematic tissue to the proximal direction at the base of the shoot. It is

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also stated that there were a difference in auxin hormone level in the apical part of an organ with other organs that auxin movements occur basipetally from the apical to the basal, resulting in more accumulation of auxin in the apical portion compared to auxin in the basal [16]. The process of emergence of a shoot in the adaxial part of the bulbil was supported by apical dominance theories [9] that if the apical shoots have grown, the other shoot candidates will be eliminated. Anatomically, based on observations on bulbil of all ages can be observed epidermal cell structure. The epidermis of monocot plants has a multilayer epidermal structure as the outermost tissue [17] and has a solid cell with a neat structure as a characteristic often found in the Araceae family [18].

Length and Width of SAM

Based on the observation table the high value of SAM continues to increase from 100-130 DAH (Table 1). The increase in value indicates that there was a long increase in SAM cells.

Table 1. Table of length and wide bulbils.

Bulbil age, (the day after harvest)	Width (μm) ± SD	Height (μm) ± SD
100	670.47 ± 194.16	621.05 ± 142.05
110	633. 76 ± 168.50	629.91 ± 30.14
120	774.43 ± 230.67	638.54 ± 126.64
130	431.91 ± 272.61	655.06± 446.66

However, there was a variation in the value of the SAM width, which was the highest value obtained in SAM aged 120 and the lowest in SAM aged 130 DAH. Theoretically, there will be an increase in cell weight in the growth process, but there may be constraints in the technique of sample slicing and the layout of bulbil ridges that are not the same at the time of slicing.

CONCLUSION

There are differences in the anatomical character of bulbil Porang in various ages. There are differences development of shoot apical meristems leaf primordia, primary thickening meristem, tunica, and procambium cells. The existence of various anatomical characters causes the growth of shoots in the adaxial part develops better than the abaxial part.

REFERENCES

[1] Mastuti, R., N. Harijati, E.L. Arumingtyas, W. Widoretno. 2018. Effect of bulbils position on leaf branches to plant growth responses and corms quality of *Amorphophallus muelleri* Blume. *J.Exp.Life Sci.* 8(1). 1-6.

[2] Harijati, N., R. Mastuti. 2014. Estimation of diverse porang (*Amorphophallus muelleri* Blume) age in the forest is based on the branching pattern of leaf petiolule. *Res. J. Life Sci.* 1(1). 20-26.

[3] Sumarwoto., Maryana. 2011. Pertumbuhan bulbil Iles-Iles (*Amorphophallus muelleri* Blume) berbagai ukuran pada beberapa jenis media tanaman. *Jurnal ilmu kehutanan.* 5(2). 91-98.

[4] Afifah, E., M.O. Nugrahani, Setiono. 2014. Peluang budidaya iles-iles (*Amorphophallus* spp.) sebagai tanaman sela di perkebunan karet. *Warta Perkaretan.* 33(1). 35-46.

[5] Gusmalawati, D., S. Indriyani, R. Azrianingsih. 2013. Anatomi dan histokimia organ generatif a*morphophallus muelleri*. *Floribunda*. 4(7). 175-180.

[6] Sulistiyo, H.R., L. Soetopo, Damanhuri. 2015. Eksplorasi dan identifikasi karakter morfologi porang (*Amorphophallus muelleri* B.) di jawa timur. *Jurnal Produksi Tanaman.* 3(5). 353-361.

[7] Yang, P., L. Xu, H. Xu, Y. Tang, G. He, Y. Cao, Y. Feng, S. Yuan, J. Ming. 2017. Histological and transcriptomic analysis during bulbil formation in *Lilium lancifolium*. *Plant Sci.* 8. 1508.

[8] Indriyani, S., W. Widoretno. 2016. The effects of photoperiod to break dormancy of porang's (*Amorphophallus muelleri* Blume) tuber and growth. *J. Life Sci.* 3(3). 166-167.

[9] Ganjari, L. E. 2014. Pembibitan tanaman porang (*Amorphophallus muelleri* Blume) dengan model agroekosistem botol plastik. *Widya Warta Journal.* 43-58.

[10] Ezeabara, A.C., C.U. Okeke, A.I. Izundu, C.D. Udechukwu, G.O. Ogbuozobe. 2015. Comparative leaf and root anatomical studies of five varieties of *Colocasia esculenta* (L.) Schott. *Int. J. Sci. Nat.* 6(4). 576-580.

[11] Ruzin, S.E. 1999. Microtechnique and Microscopy Plant. Oxford University Press. New York.

[12] Steeves, T.A., I.M. Sussex. 1989. Patterns in Plant Development. Cambridge University Press. New York.

ISSN. 2087-2852
E-ISSN. 2338-1655

[13] Rodrigues, C.A., M.E.M. Estelita. 2009. Morphoanatomy of the stem in Cyperaceae. *Acta Bot.* 23(3). 889-901.
[14] Krauss, B.H. 1948. Anatomy of the vegetative organs of the pineapple, *Ananas comosus* (L.) Merr. I. Introduction, organography, the stem, and the lateral branch or axillary buds. *Bot. Gazette.* 110. 159-217.
[15] Sorce, C., L. Lombardi, L. Giorgetti, B. Parisi. 2009. Indoleacetic acid concentration and metabolism changes during development in tubers of two potatoes (*Solanum tuberosum*) cultivars. *J. Plant Physiol.* 166. 1023-1033.
[16] Taiz, L., Zeiger. 2012. Plant physiology. Sinauer Associates, Inc. Publishers. Sunderland, Massachusetts.
[17] Kauff, F., P.J. Rudall, J.G. Gonran. 2000. Systematic root anatomy of asparagales and other monocotyledons, plant systematic an evaluation. *Plant Syst. Evol.*223. 139-154.
[18] Higaki, T., H.P. Rasmussen, W. J. Carpenter. 1984. A study of some morphological and anatomical aspects of *Anthurium andreanum* Lind. Hawaii Institute of Tropical Agriculture and Human Resources. The University of Hawaii,Manoa.

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Antifeedant Effect of *Moringa oleifera* (L.) Leaf and Seed Extract on Growth and Feeding Activity of *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae)

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Abstract

This study was conducted to evaluate the effect of *Moringa oleifera* leaf and seed extract on length, weight, and feeding activity of *Spodoptera litura*. Leaf and seed were extracted by maceration method in 70% ethanol. Bioassays were conducted by using five concentrations (0%, 5%, 10%, 15%, 20%) on 5 larvae by leaf-dip method with 5 times replication. The result showed that leaf and seed of Moringa extract contained alkaloid, flavonoid, and saponin which gave antifeedant effect to growth and feeding activity of larvae. The increased extract concentrations are lead to weight loss and the length shrinkage of larvae. The extracts promoted prolongation of the larval instar. Reduction of food intake of the larva is in line with the concentration increase of extracts. The antifeedant effect also affects time to stop feeding and give mortality of larvae.

Keywords: antifeedant, leaf, moringa, seed, *S. litura*

INTRODUCTION

Agriculture is one of the largest sectors for the economy in developing countries, including Indonesia. One of the efforts made in improving the quality and quantity of productivity is pest control. Insect pests are known to cause significant damage to crops and affect agricultural productivity because they eat the crops as their food [1].

Insect pests from order Lepidoptera are found throughout the world [2]. *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) which is known attack many types of crop, such as cotton, soybean, tobacco, onion, chili, corn, and rice [6]. The survey showed *S. litura* was found in 16 regencies in East Java with leaf damage rate above 12.5% [3]. The *S. litura* has a wide range of host, feeding nearly on 112 species worldwide [4]. It is causing considerable economic loss to many vegetable and fruit crops. Pest control use chemical insecticides has been a fundamental tool for pest control, but leads to serious consequences such as intoxication of people and animals, contamination of water, air, and soil, residues on food, high persistence in the environment, resistance in pest, and impact on beneficial insect [5] or non-target insect, and secondary pests explosion [6]. This impact encourages to use biological control methods without damaging the environment or harming other organisms. The holistic approach towards insect pest management which includes the use of botanicals such as plant products to pest control and combat resistance problem [12]. This is in line with the government program in the effort to protect crops productivity which applies the Integrated Pest Control technique in accordance with Presidential Instruction No 3 of 1998 [7].

Several types of plants have been known to have potential as botanical pesticides because of the content of phytochemical compounds that have an antifeedant impact and mortality on insect pests. Earlier studies have indicated that antifeedant compound derived from leaf, seeds, flowers, fruit, and root of the plants can be used as botanical pesticides to against the growth and metamorphose of the noxious insects [12]. This compound came from processed organ plants into various forms, such as raw materials in the form of flour, extras, or resins which are the result of extracting liquid secondary metabolites from plant parts.

Phytochemicals are a group of non-nutrient compounds that are naturally synthesized by plants. These compounds are in flowers, tubers, leaves, fruit, roots, tree bark, and in the rhizome. Phytochemicals act as a defensive mechanism for the plants against disease and many external attacks. Phytochemicals are the results of primary or secondary metabolites of plants [8]. These substances include saponins, tannins, alkaloids, flavonoids, and terpenoids. Moringa is a plant that contains active compounds alkaloids,

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flavonoids, saponins, steroids, and tannins [8]. In the study [9,10] Moringa seeds proved to be larvacidal against *Anopheles stephensi* and Moringa flowers had a larvacidal effect on the *Aedes aegypti*. Moringa seed extract contains high concentrations of flavonoids, and leaves contain flavonoids, alkaloids, saponins, and tannins [8]. Based on the results of previous studies, *Moringa oleifera* can be used as a candidate for botanical pesticides, especially in *S. litura.* The purpose of this study was to determine the antifeedant effect of *M. oleifera* leaf and seed extract on growth and feeding activity of *S. litura* larvae.

MATERIAL AND METHOD

The culture of *S. litura*

Spodoptera litura larvae were obtained from the Research Institute for Sweeteners and Fiber Plants (BALITTAS), Malang. Larvae are collected at the second instar stage and are ready for use at the third instar. The larvae are stored in jam bottles (5 larvae each bottle) that have been given mustard leaves as feed. The bottle is closed using a cloth. Feed is replaced every day and dirt cleaned using a brush.

Extraction of Moringa Leaf and Seed

Moringa leaves and seeds are powdered into simplicia. Total 500 grams of simplicia was extracted with 3750 mL of ethanol using the maceration method. The maceration vessel is closed and left for 3-4 days, protected from direct sunlight. The immersion was stirred in several times, so the diffusion process of dissolved compounds in ethanol more effective [11]. The immersion was filtered using a cloth. The resulting filtrate was then evaporated using a rotary evaporator [13] to evaporate the solvent. The resulting extracts were then stored in the refrigerator (≤4°C) until ready to used. The extract use for bioassay studies in five concentrations (0 %, 5%, 10%, 15%, and 20%).

Total Phytochemical Assay for Moringa Leaf and Seed

The tested phytochemicals were flavonoids [29], alkaloids, and saponins [30]. The total phytochemical content test was carried out by the spectrophotometric method. Each extract was tested in duplicate.

Bioassay of extract

The third instar *S. litura* larvae are exposed for 1-2 hours before testing. Each of the concentrations of the extracts was prepared and put in a small basin container. Mustard leaves that are still fresh and not contaminated with chemical pesticides are dipped in each concentration for ± 10 seconds. Testing is done by the deep-in method and then placed on a tray to be air dried. The leaves are inserted into all jam bottles which contain *S. litura* larvae [6]. Each concentration used 5 larval and repeated 5 times. The total number of *S. litura* larvae used was 250 individuals.

Observation of Antifeedant Effect

The development of larvae was observed starting from the 3rd instar larvae to the instar 5. Observation variables included the length, weight, feed residue, development time, observed at each turn of the larval instar stage, and the stopped eat time which was observed at 4, 8, and 12 hours after the application (HAA).

RESULTS AND DISCUSSION

The result of the total phytochemical compound of leaves and seeds showed that all samples positive contained flavonoids, alkaloids, and saponins (Table 1). In each extract, it was known that the content of flavonoids and alkaloid was relatively higher compared to saponins. These results are in line with the study [14], which showed that flavonoid content (3.46 ± 0.03 g.100g^{-1}) and alkaloid (3.30 ± 0.00 g.100g^{-1}) in *M. oleifera* was higher than saponin content (0.95 ± 0.03 g.100g^{-1}).

Table 1. The total compound of phytochemical of *M. oleifera*

Extract	Total (mg.100g^{-1})		
	Flavonoid	Alkaloid	Saponin
leaf	3157.095	153.711	106.159
	3205.656	154.542	108.814
seed	2075.002	205.134	65.553
	2041.744	209.240	63.703

The three secondary metabolites gave antifeedant effect to the development of *S. litura* larvae including length, weight, larval development time, food residue, and time to stop eating. Secondary metabolites in plants have an impact on growth, development, and reduced digestive capacity in insects [15].

Flavonoids, alkaloids, and saponins on Moringa leaves and seeds give significant different to the length of *S. litura* larval on various instars. The increased concentration of Moringa leaf and seed extract declined the mean length of *S. litura* larvae (Fig. 1). The 3rd instar larva on both treatment significantly different between control (0%) and with all

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concentrations, except 5%. The 4th instar and 5th instar were significantly different at all concentration. The shrinkage of larvae lenght is higher in larva treated by Moringa seed extract than the leaf. This is due to the higher alkaloid in Moringa seeds than Moringa leaves. Alkaloids become inhibitors of several metabolisms. Alkaloid inhibits the work of sucrose in the intestine in digestive metabolism and affects the growth of larvae [16].

The treatment of Moringa leaf and seeds extracts also affects the weight of larvae of *S. litura*. The increase of extract concentration in line with losing weight of larvae (Fig. 1). Alkaloids cause lose weight and a decrease in food intake of insects [17]. In the study [18] reported that the alkaloid from *P. harmala* was studied in *S. greraria* and it caused weight loss. Based on the previous research, it was explained that the presence of rapid neurotoxicity symptoms hampered larval feeding activity [17]. The weight of each larval instar growth has decreased which higher in the treated diet of Moringa seed extract. The weight of the 3rd instar larvae with both extracts was not significantly different in 3rd instar and 4th instar between control and 5% concentration. Alkaloids for insects act as stomach and contact toxic. Alkaloids in the form of salt can be degraded cell membranes in the digestive tract to enter and damage cells.

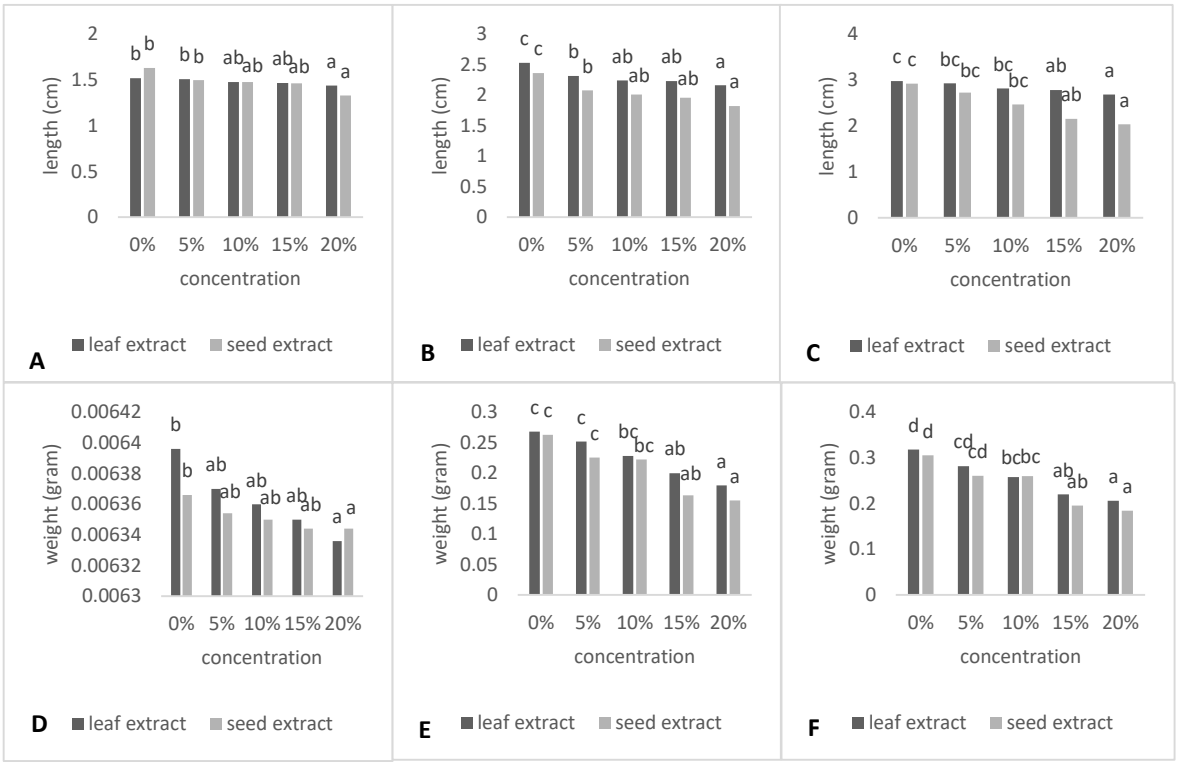

Figure 1. Mean length of *S. litura* on diet treated by moringa extract in 3rd instar (A), 4th instar (B), 5th instar (C); and mean weight of *S. litura* on diet treated by moringa extract in 3rd instar (D), 4th instar (E), 5th instar (F).

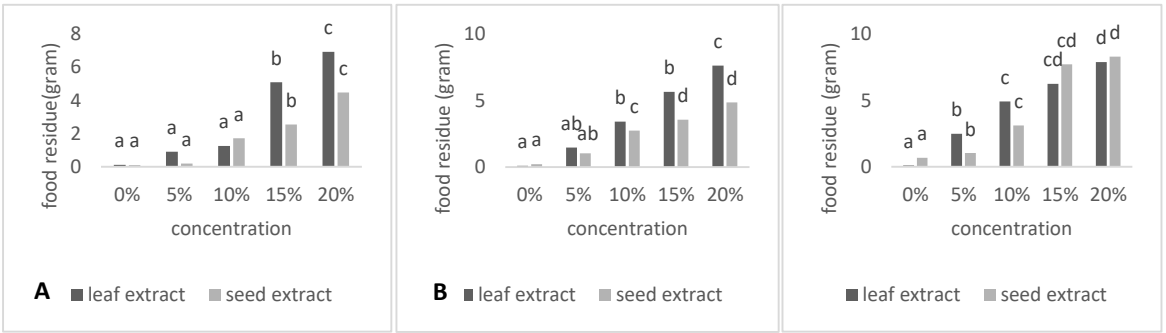

Figure 2. Mean food residue of *S. litura* on diet treated by moringa extract in 3rd instar (A), 4th instar (B), 5th instar (C)

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These results indicate that alkaloids interfere with neuroendocrine control by inactivating acetylcholinesterase in larvae [19]. Alkaloids from some plants have a similar effect to blocking the acetylcholinesterase enzyme. This enzyme cannot work in the body to continue sending the signal to larval digestive [20]. Higher alkaloid content in Moringa seed extract caused weight loss in larval higher than larvae given Moringa leaf extract.

In addition to alkaloids, flavonoid content also affected the length and weight of larvae. Flavonoids affect molting, reproduction, feeding, and insect behavior, including cutworms [21]. Flavonoids in plants can reduce digestive ability or can even be toxic [22]. This is related to food residue of *S. litura* larvae (Fig. 2). Food intake in control larvae was higher than the treatment of both extracts. In Figure 3, it can be seen that the higher concentration of the extract reduced the food intake of larvae. Feeding with the addition of leaf extract and seed extract caused a decrease in larval feed residue for each increase in concentration.

Alkaloid, flavonoids, and saponins give antifeedant effect in insects. Antifeedant compounds affect eating behaviour through the direct action of insect taste organs. The insect's reaction was refusing to eat or eating in small amounts. Increased feed residue at each increase in extract concentration because the food is not suitable and stops eating temporarily or permanently. This feeding inhibitory activity is related to the sensitivity of gustatory neurons and thus inhibits insect growth [27].

The activity of inhibiting eating by extracts of Moringa leaves and seeds is also shown by the results of the time to stop eating. In Figure 4, the time to stop eating of *S. litura* after feeding with the addition of Moringa leaf and seed extract increased.

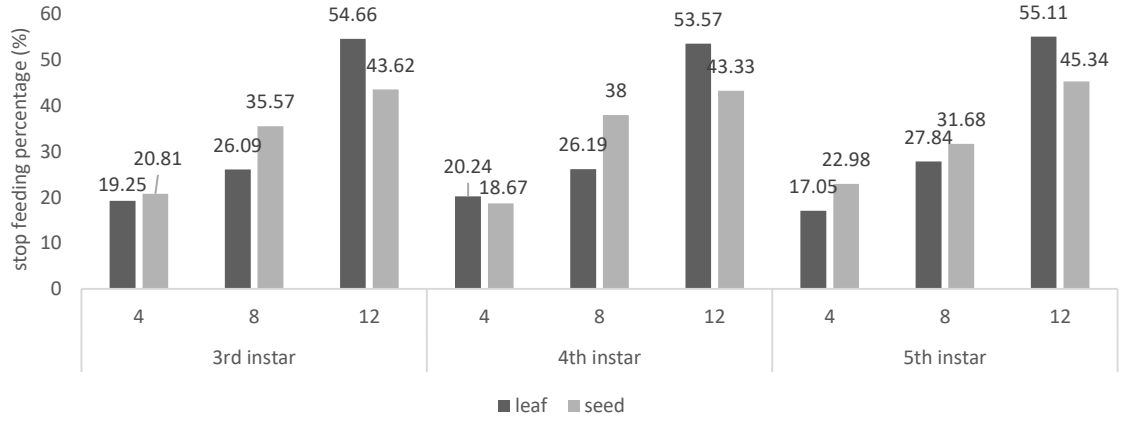

Figure 3. Percentage of stop feeding of *S. litura* on diet treated by moringa extract in 3rd instar (A), 4th instar (B), 5th instar (C) at 4 hours after application (HAA), 8 HAA, and 12 HAA

On the 3rd, 4th, and 5th instar, treatment of Moringa leaf and seeds extract caused the percentage of time to stop feeding at the 4th, 8th and 12th hours after application (HAA) increased. At 4, 8, and 12 HAA, the percentage of time to stop feeding with Moringa leaf extract treatment was higher than the seeds extract on the third and fifth instar. On the fourth instar, the percentage of time to stop eating was higher in Moringa seed extract treatment at 4 and 12 HAA. Leaf and seeds extract containing bioactive substances from secondary metabolites that inhibited larval activity, marked by slow motion, no response to the motion, and stop feeding [6].

The secondary metabolites also affect the duration of development of larvae. In Figure 4, it can be seen that the larval development duration did not differ significantly in the development of the 4th instar larvae in both extracts and 5th instar with the of Moringa leaf extract treatment. Plant secondary metabolites can disturb development, lead to malformations or malfunctions, extend the duration of developmental stages or act as repellents [23]. Diet treatment on larval gives development time prolonged compared to control, although statistically not significantly different. The extent of development time is due to a disruption in the insect hormone development, namely brain hormone (BH), juvenile hormone (JH), and the ecdysone hormone. This disruption caused by saponin in the extracts.

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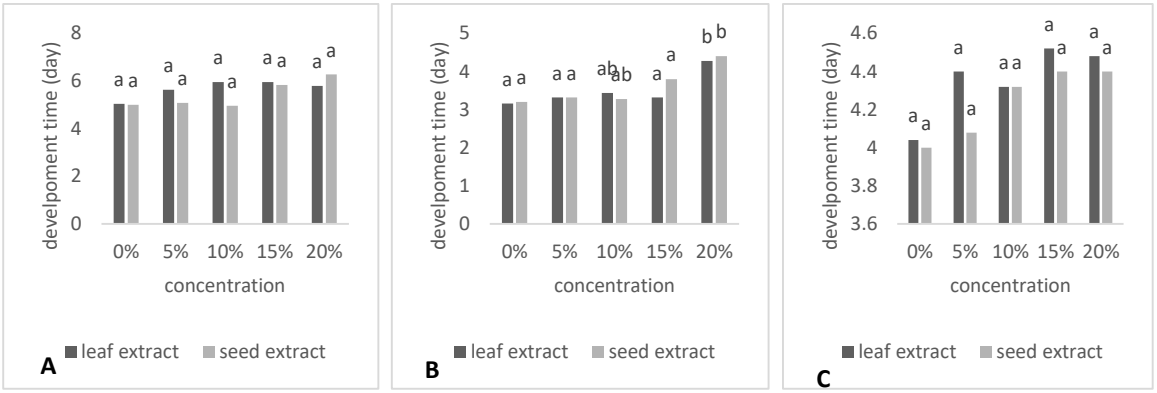

Figure 4. Mean development time of *S. litura* on diet treated by moringa extract in 3rd instar (A), 4th instar (B), 5th instar (C)

Saponins are terpenoid compounds. The activity of saponins in the insect's body is to bind free sterols in the digestive tract of food. Sterols are substances that function as precursors of the ecdysone hormone [25]. Larvae growth is affected by JH. As long as there is JH, the molting that occurs under the control of ecdysone hormone will only produce an immature stage of larval. If the concentration of JH is relatively higher compared to the ecdysone hormone, it will stimulate the larvae and prevent the formation of pupae [28]. It was reported that the saponin content of *S. litura* growth resulted in prolongation of the development stage, including at the larval stage [26]. In addition to giving prolongation of the development stage, the secondary metabolites in Moringa leaf and seed also causing mortality to larval. Mortality is characterized by reduced in eating activity and passive movements.

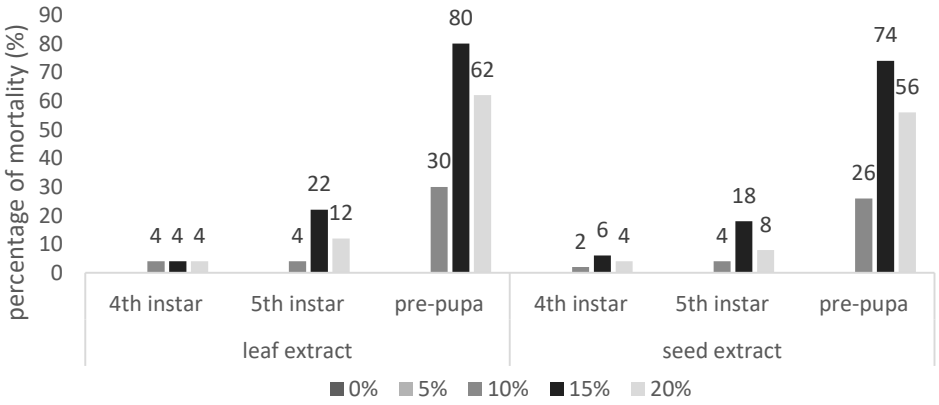

Figure 5. Percentage mortality of *S. litura* diet by moringa leaf and seed extract

Mortality began in 4th instar diet by 0%, 15%, and 20% concentration of extract (Fig. 5). The highest mortality occurred in 15% extract in pre-pupa phase. Mortality caused by quality and quantity of diet on stage larvae. The secondary metabolites in both extracts are toxic and the toxicity in larvae also increase due to feeding activities, the larvae were unable to neutralize toxins in the body. These toxic reduce metabolic activity resulting in mortality. The insect that is exposed to insecticides in lethal concentrations affected the physiological and behavioral changes, resulting in stunted growth and failure in pupae formation [31].

CONCLUSION

The treatment of leaf extract and Moringa seeds affect the length shringkage, lose weight of *S. litura* larvae. Both extracts also caused longer larval development in instar growth but reduce food intake. The number of larvae that consume feed that has been given extract also decreases with every increase in time. This antifeedant effect gives mortality to the larvae.

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REFERENCES

[1] Hadi, M., Aminah. 2012. Keragaman serangga dan perannya di ekosistem sawah (Insect diversity and its role in weatland ecosystem). *Jurnal Sains dan Matematika*. 20(3). 54-57.

[2] Elleuch, J., R.Z. Zghal, M. Jemaa, H. Azzouz, S. Tounsi, S. Jaoua. 2014. New *Bacillus thuringiensis* toxin combination for biological control of Lepidopteran Larvae. *International J. Biol. Macromol.* 65. 148-154.

[3] Tengkano, W., Suharsono. 2005. Ulat Grayak *Spodoptera litura* Fabricius (LEPIDOPTERA: NOCTUIDAE) pada tanaman kedelai dan pengendaliannya. *Buletin Palawija.* 10. 43-52.

[4] Badathu, M., V. Lingampally, A. Kaur. 2014. Effect of betulinic acid on *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). *Indian J. Sci. Res.* 8(1). 177-181.

[5] Kandagal, A., M.C. Khetagoudar. 2013. Study on larvacidal activity of weed extracts against *Spodoptera litura. J. Environ. Biol.* 34. 253-257.

[6] Yanuwiadi, B., A.S. Leksono, H. Guruh, M. Fathoni, Bedjo. 2013. Potensi ekstrak daun sirsak, biji sirsak, dan biji mahoni untuk pengendalian ulat Grayak (*Spodoptera litura* L.). *Natural B.* 2(1). 88-93.

[7] Sa'diyah, N.A., K.I. Purwani, L. Wijayawati. 2013. Pengaruh ekstrak daun Bintaro (*Carbera odollam*) terhadap perkembangan ulat Grayak *(Spodoptera litura* F.). *Jurnal Sains dan Seni Pomits*. 2(2). E111-E115.

[8] Aliyu, A., U.D. Chukwuna, E.H. Omoregie, K.O. Folashade. 2016. Qualitative phytochemical analysis of the leaf of *Moringa oleifera* lam. from three climatic zones of Nigeria. *J. Chem. Pharm. Res.* 8(8). 93-101.

[9] Prabhu, K., K. Murugan, A. Nareshkumar, N. Ramasubramanian, S. Bragadeeswaran. 2011. Larvacidan and repellent potential of *Moringa oleifera* against malaria vector, *Anopheles stephensi* Liston (Insecta: Diptera: Culicidae). *Asian Pac. J. Trop. Biomed.* 124-129.

[10] Pontual, E.V., T.H. Napoleao, C.R.D. Assis. R.D.S Bezzerra, H.S. Xavier, D. Navarro, L.C. Coelho, P.M.G. Paiva. 2012. Effect of *Moringa oleifera* flower extract on larval trypsin and acetylcholinesterase activity in *Aedes aegypti. Arch. Insect Biochem. Physiol*. 79(3). 135-152.

[11] Anas, Y., A. Imron, S.I. Ningtyas. 2016. Ekstrak daun kelor (*Moringa oleifera* Lam.) sebagai peluruh kalsium batu ginjal secara *In Vitro*. *Jurnal Ilmu Farmasi dan Farmasi Klinik*. 13(2). 7-15.

[12] Chauhan, D., V.K. Mishra. 2016. Effect of medicinal plant extract on growth and development of Tobacco Caterpillar, *Spodoptera litura* (Fabricius). *Int. J. Agr. Environ. Biotech.* 9(3).435-442.

[13] Wardani, E., H. Sunaryo, M.Z. Sopiani, M. Fatahillah. 2015. The antihypertriglyceride activity and antihyperglycemic of ethanol extract of Moringa leaves (*Moringa oleifera* Lam.) on hypertriglyceride diabetes rats. *Media Farmasi.* 22(2). 199-212.

[14] Ezeigbo, I.C., O.R. Ezeigbo. 2016. Phytochemical and nutritional evaluation of southeastern Nigerian grown *Moringa oleifera* leaf extract. *J. Complement. Altern. Med. Res.* 1(4). 1-8.

[15] Belete, T. 2018. Defense mechanisms of plants to insect pests: from morphological to biochemical approach. *Trends Technic. Sci. Res.* 2(2). 001-009

[16] Matsuura, H.N., A.G. Neto. 2015. Plant alkaloids: main features toxicity, and mechanism of action. *Plant Toxins Springer Science.* 1-15.

[17] Acheuk, F., B.D. Mitiche. 2013. Insecticidal activity of alkaloids extract of *Pergularia tomentosa* (Asclepiadaceae) against fifth instar larvae of *Locusta migratoria cinerasces* (Fabrisius 1781) (Orthoptera: Acrididae). *Int. J. Sci. Adv. Technol.* 6(3). 8-13

[18] Abbassi, K., Z.A. Kadiri, S. Ghaout. 2003. Biological effects of alkaloids extracted from three plants of Moroccan areas on the Desert Locust. *Physiol. Entomol.* 28. 232-236.

[19] Aniszewski, T. 2007. Alkaloids- secrets of life. Alkoloid chemistry, biological significance, applications and ecological role. Elsevier. Amsterdam. 185-186.

[20] Ahdiyah, I., K.I. Purwani. 2015. Pengaruh ekstrak daun mangkokan (*Nothopanax scutellarium*) sebagai larvasida nyamuk *Culex* sp. *Jurnal Sains dan Seni ITS.* 4(2). 32-36.

ISSN. 2087-2852
E-ISSN. 2338-1655

[21] Boue, S.M., S.K. Raina. 2003. Effects of plants flavonoids on fecundity, survival, and feeding of the formosan subterranean termite. *J. Chem. Ecol.* 29(11). 2575-2584.
[22] Mierziak, J., K. Kostyn, A. Kulma. 2014. Flavonoids as important molecules of plants interactions with the environment. *Molecules*. 19. 16240-16265.
[23] Chowanski, S., Z. Adamski, P. Marciniak, G. Rosinski, E. Buyukguzel, K. Buyukguzel, F. Patrizia, L. Scrano, E. Ventrella, F. Lelario, S.A. Bufo. 2016. Review: a review of bioinsecticidal activity of Solanaceae alkaloids. *Toxins.* 8(60). 1-28.
[24] Hasnah, Husni, A. Fardhisa. 2012. Effect of rhizome extract of sweet flag (*Acorus calamus* L.) on mortality of grayak caterpillar *Spodoptera litura. J. Floratek*. 7. 115-124.
[25] Aminah, N.S.S., S. Sigit, Partosoedjono, Chairul. 2001. *S. lerak, D. metel*, dan *E. prostata* sebagai larvasida *Aedes aegypti*. Cermin Dunia Kedokteran. 131. 7-9.
[26] Chaieb, I. 2010. Saponins as insecticides: a review. *Tunis. J. Plant Prot.* 5. 39-50.
[27] Khairul, A.U., C. Vemithasa. 2018. The Potential of *Vitex trifolia* (Verbenaceae) as a botanical insecticide to control *Crocidolomia pavonana* (Lepidoptera: Crambidae). Proceeding of Nasional Seminar of Indonesian Biodiversity Community. 4(2). 169-170.
[28] Lukman, A. 2009. Hormon role in insect methamorphosis. *Biospecies*. 2(1). 42-45.
[29] Chinelo, A., C.U. Okeke, O. Bibian, V. Cinyere, A. Emeka. 2014. Determination of saponin content of various Parts of six citrus species. *Int. Res. J. Pure Appl. Chem.* 4(1). 137-143.
[30] Tambe, V.D., R.S. Bhambar. 2014 Estimation of total phenol, tannin, alkaloid, and flavonoid in *Hibiscus tiliaceus* Linn. wood extract. *Res. Rev. J. Pharmacog. Phytochem.* 2(4). 41-47.
[31] Muta'ali, R., K.I. Purwani. 2015. Pengaruh ekstrak daun beluntas (*Pluchea indica*) terhadap mortalitas dan perkembangan larva *Spodoptera litura* F. *Jurnal Sains dan Seni ITS*. 4(2). E55-E58.

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Effect of Place and Time Storage on the Quality of Tubers *Amorphophallus muelleri* Blume

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Abstract

Porang tubers are plant commodities that physiologically susceptible to damage. Longtime storage can decrease the quality of tubers. Furthermore, the presence of fungi or bacteria makes tubers cannot be stored in a long time. Decreasing quality of Porang tuber became a problem because of the higher demand for Porang tubers. This research was conducted to determine the suitable place and time storage of Porang tubers. Therefore, farmers can control or reduce the decrease in tuber quality. Methods of this research were an observation of color change based on the *Munsell Color System*, odor observation, hardness observation using penetrometer, the rotten area of tuber was measured using Leaf Area Meter, shrinkage of tuber weight, the percentage of rotten and sprouted tubers, density, and moisture content by the oven method. The results of this study were analysed statistically by ANOVA test. The hardness of the tubers has a significant effect on the storage place (soil, rack, and floor) for period 14 weeks. Storage time has an effect on changes of the weight loss, moisture content and decay area. The longer the tuber stored the higher percentage of sprouted and rotten tubers. The color of Porang tubers changed and the odour in the part of the rotten tubers increased during the storage period in each storage place. Porang tubers should be stored than two weeks and placed on a rack to minimize the damage of the tubers.

Keywords: *Amorphophallus oncophyllus*, Iles-iles, Physical change, Yellow konjac

INTRODUCTION

Porang (*Amorphophallus muelleri* Blume) is a plant commodity that belongs to the Araceae family and an indigenous plant with stump tubers in the soil. Porang tubers have high economic value due to their glucomannan content which has many benefits such as for the industries of foods, cosmetics, pharmacies and manufactures. Besides that, porang tubers have been often processed into flour which has frequently used as a thickening agent. As the increases utilization of Porang tubers, the demand for Porang tubers rapidly rises year to year [1].

For fullfilling the demand, however, Porang farmers have problems in handling postharvest tubers which are susceptible to decay. The decay can be indicated by the change in shape, colour, texture, and nutritional content [2]. Common characters of Porang tuber damage are wrinkles, porous, split tubers, and blackened tubers. If porang tubers do not handle properly, the quality of tubers decreases, so that the selling price of tubers also decreases [3].

Based on previous studies, storage place and period have affected on the quality of sweet potatoes, potatoes, and some other tubers. Storage of tuber crops using good methods was able to reduce postharvest damage [4]. The length of storage of tubers should be considered because during the storage, water evaporation took place affecting tubers lose a lot of water and the texture of tubers softens. Besides that, there was a compound that are influenced, one of those were starch and sugar [5]. The starch was reduced to sugar catalyzed by the *phosphorylase* enzyme. Consequently, the amount of starch in the tuber decreased resulting soften tuber [6], then this condition ease the tubers to be damaged [7]. The product quality is very important to be concerned as consumers need. Therefore recommendation of storage place and period is required for maintaining tuber quality.

Bantur is one of the Porang centre farm in Indonesia. Based on observations, most farmers keep the crop on the ground or floor without any special treatment. It can accelerate the decomposition of Porang tubers with erratic storage time. The limited tools for processing porang tubers into flour also cause people to process porang tubers without any option for storage. Therefore, this research was aimed to determine the suitable place and time storage of Porang tubers.

MATERIAL AND METHODS

This research was conducted in August to November 2017. Tubers were collected from Porang farm in Rejosari Village, Bantur, Malang. Tubers were treated 1 week after harvest and selected 72 fresh tubers with no physically

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damaged when harvesting with weight 800 – 1.900 grams. Storage of porang tubers was carried out at the Garden Palma Housing in Malang City.

Tubers were stored using three storage methods as follows: placed on dry soil with a little gravel (S), placed on a rack made of bamboo (R), and placed on the tile floor (F). Each storage place consists of 24 tubers divided into three replications so that each replication consists of 8 tubers. Storage time was designed for 14 weeks, with observations every two weeks. Storage space in semi-open space, the condition of the room include temperature and air humidity recorded using the Blynk program http://blynk.ub.ac.id/microclimate.php (Fig. 1).

Research Design

This research was conducted using a randomized block design with three replication as a group. Replication of tuber samples divided into two groups. Replication 1 and 2 were observed in the first week, while replication 3 was observed in the second week. Due to the gradual harvest, the tuber sample from farmers cannot be taken all together.

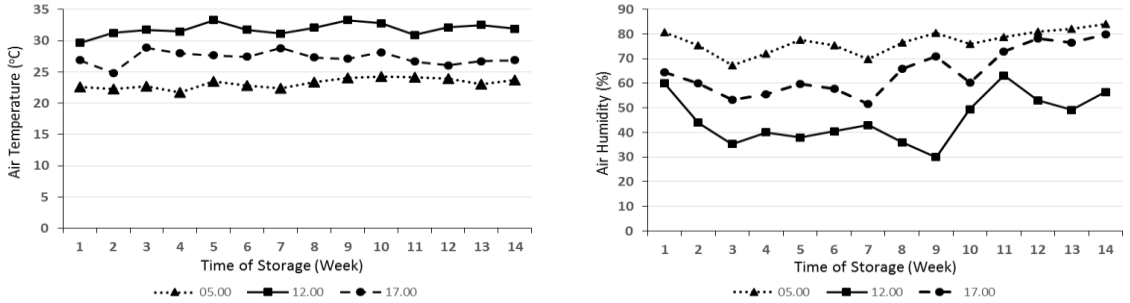

Figure 1. Temperature (°C) and humidity (%) in storage time

Observation on Physical Characteristics of Rotten Tuber Symptoms

The first observation of the morphological characters of tuber rotten symptoms was the colour change of the parts of tubers that experienced rotten tuber (black, brown, white, etc.). Colour changes were determined using a colour table (the *Munsell Color System)*. Odour changes were observed then categorized as (A) very sting odour, (B) sting odour, and (C) not sting odour.

Hardness was observed by checking the level of tuber softness or hardness using a penetrometer [8], the tubers were placed below the penetrometer piercing needle. The stabbing was done on the tubers 10 times in ten places, the results of each stab are indicated by numbers on the penetrometer scale. The rotten area of tubers measured by using a millimeter paper attached to the tuber area, then marked using a pen and measured using a Leaf Area Meter apparatus.

Percentage of Rotten Tubers and Tubers Sprout

Percentage of decayed tubers and sprout tubers were calculated in every storage period. The percentage of tubers is counted every two weeks during storage using the formula:

$$Rotten\ tuber\ presentation\ (\%) = \frac{Number\ of\ rotten\ tubers}{\text{Total tubers}} \text{ x } 100\%$$

The percentage of sprouting tuber is counted every two weeks during the storage period:

$$\text{Sprouts tuber presentation } (\%) = \frac{Number\ of\ sprout\ tubers}{\text{Total tubers}} \text{ x } 100\%$$

Weight Loss of Tubers

The mass of all sample tuber was weighted. Percentages of weight loss were determined by the formula [5]:

$$\text{Weight loss } (\%) = \frac{\text{Tuber weight msp 0} - \text{Tuber weight msp} - \text{n}}{\text{Tuber weight msp 0}} \text{ x } 100\%$$

Water Content Analysis

Tuber was 10 g, then it was dried in the oven at 105°C for 24 hours, and then it was dessicated for 15 minutes until the weight was constant. Determination of water content was calculated by the following formula [8].

$$\text{Water content } (\%) = \frac{\text{Mass the tubers before the oven (g)} - \text{Mass the tubers after the oven (g)}}{\text{Mass the tubers before the oven (g)}} \text{ x } 100\%$$

RESULT AND DISCUSSION

Effect of Parameters on Storage Time, Storage Space and Their Correlation

There was significant influence between parameters, storage period, storage area and only hardness have interaction between storage

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time (TS) and place (PS) (Table 1). Based on the correlation test between parameters, there were correlations that influence each other (Table 2).

Morphological Character Changes (Colour and Stink)

Damage symptoms occured in Porang tubers stored for fourteen weeks of storage on the ground, rack, or floors. The surface of tuber damage was performed as wrinkled and porous. The damage was followed by varying color changes during storage followed by the appearance of concomitant white fungus and maggots. These color changes include dry brown (5YR3/4), wet brown, and wet black. The defective part of the tuber has an unpleasant odor, the longer storage time the odor is more stinging (Fig. 2).

The mechanism of porang tuber decay is thought to be related to its existence *Polyphenol oxidases* (PPO) enzymes and *polyphenolic* compounds, include tannins, causes brownish colour in the tubers if there is direct contact with oxygen in the air [9,10]. The enzyme comes out if there is a wound on the tuber [11]. PPO catalyses the oxidation reaction of phenolic compounds to quinones which are further polymerized into dark-coloured melanin pigments [12].

When the tubers are injured, the *parenchymal* tissue in the tubers thickens the cell wall. It is also affected by the presence of bacterial *pectolytic* enzymes which are capable of destroying the binding material between cells. The presence of microorganisms that come in direct contact with storage or in the air can affect changes in commodity quality during storage, as explained by Purwanti [13] in potato tubers, discoloration occurs due to the presence of pathogenic bacteria such as *Citrobacter*, *Phytophthora infestans*, and *Erwinia carotovora*. Citrobacter bacteria are able to produce PCDE (*Plant Cell Wall Degrading Enzymes)* which can degrade cell walls. It causes damage to the cell wall and organelles to come out, then mucus appears. The appearance of a musty odour by fat oxidation produces carbonyl compounds that react with oxygen in the air [14].

Table 1. Test of interactions between parameters, time storage and place storage

Parameter	Time storage (TS)			Place Storage (PS)			TS vs PS		
	d.f	F	Sig.	d.f	F	Sig.	d.f	F	Sig.
Hardness	7	29.343**	.000	2	23.338**	.000	14	1.991*	.039
Decay area	7	6.442**	.000	2	1.737(ns)	.187	14	.717(ns)	.747
Shrink Weight	7	13.356**	.000	2	.375(ns)	.690	14	1.452(ns)	.167
Water content	7	5.627**	.000	2	1.922(ns)	.157	14	1.054(ns)	.421

Notes: (Sig > 0.05 = not significant), * < 0.05 ; ** < 0.01

Table 2. The correlation between parameters

Parameter	Hardness	Rotten area	% rotten	% sprout	Shrink Weight	Water content
Hardness		-502**	-594**	-595**	- 659**	.285*
Rotten area	-502**		.687**	.679**	.463**	-345**
Percentage of rotten tuber	-596**	.689**		.994**	.589**	-158
Percentage of sprouted tuber	-595	.679**	.994**		.581**	-163
Shrink Weight	-659**	.463**	.591**	.581**		-384**
Water content	.297*	-354**	-158	-163	-384**	

Notes: (Sig > 0.05 = not significant), * < 0.05; ** < 0.01

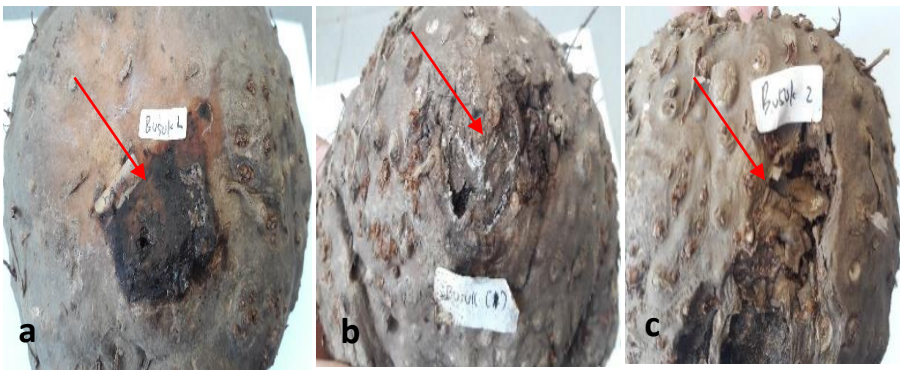

Figure 2. Rotten tubers: (a) Aqueous rot, (b) A runny rot performing white fungus around the rotten area, (c) Dry rot.

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The highest damage of tubers occurred in the area that is directly contacted with the storage place due to less air circulation, such as on the floor and soil. The presence of pathogens in the soil serves as a source that starts the rot. Poor air circulation causes heat buildup and increases moisture as a result of respiration therefore inducing spores and pathogenic growth. Storage on the rack was the best storage because the rotten in tuber is slower because there is sufficient air circulation that can reduce heat buildup and high humidity levels [15].

Hardness of Tubers (mm.g^{-1}.seconds^{-1})

The hardness of tubers during storage decreased gradually (Fig. 3). The decrease in hardness was first seen in the fourth week (W-4). The highest decrease was on the floor, then on the soil and rack. The variation in rotten tuber during storage in the following weeks tend to be harder. It may be affected by activation of respiration overhauled starch to glucose.

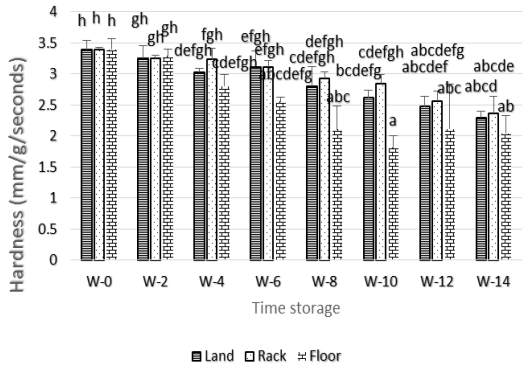

Figure 3. Tuber hardness values during storage times at three storage places. Different letters indicate differences among storage times within storage places. Vertical lines represent the Standard Deviation (SD) of the means across three replicates.

The glucose coud be consumed by pathogens, then remodeled into ethanol and water (H_2O). This process decreases the hardness of tubers. However, the hardness of the tuber stored on the floor increased in W-12 and W-14 because transpiration process reduces the water content in the tuber, then tubers became harder.

Hardness is caused by starch granules that are tightly arranged in the tubers, if the granules in the tubers are high, the penetrometer needle is rather difficult to penetrate into the tubers. The longer the storage, the lower of hardness [8]. Hardness changes can be caused by the transpiration process which causes water loss during storage and effects wrinkling. It made the products less attractive because of the decreased quality [16].

Changes in hardness during the storage period may be also affected by changes in cell wall compounds. The cell wall became simpler that weaken the cell wall and bond cohesion between tissues. In normal tubers, starch granules are tightly arranged so that the texture of the tubers is hard (normal) [5]. Based on the correlation test, hardness of porang tubers were also influenced by several parameters, namely the size of rotten area, the percentage of decayed tubers, the percentage of germinated tuber, the weight loss and the water content of tuber (Table 2).

Spots of tubers rotten area

The area size of tuber rot during storage iincreased (Fig. 4). The tubers softened gradually and the rotten area size was wider. However, there was a decrease in rotten area at the tenth week (W-10) and twelfth week (W-12) due to variation in rotten symptoms in each week of observation. At the first tubers showed runny rot but some weeks after storage the tubers became dry (shrinking) so the rotten area decreased. Increasing the size of rotten areas could facilitate greater water loss, causing shrinkage of the tubers during storage.

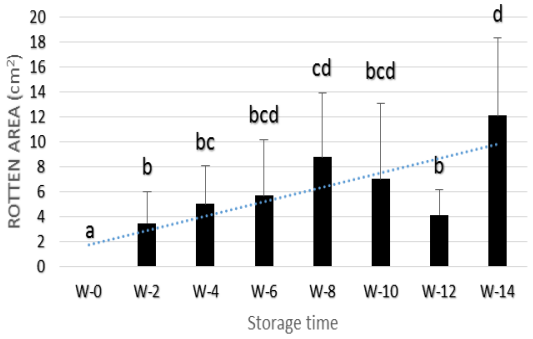

Figure 4. Size of rotten area (cm^2) of porang tubers during storage. Different letters indicate differences among storage times. Vertical lines represent the Standard Deviation (SD) of the means across nine replicates.

The size of rotted area of tuber increased during storage. Based on the correlation test, the symptom had negative correlation with hardness and water content. But, it had positive correlation with the percentage of rotten tuber, percentage of sprouted tuber, and weight loss (Table 2). Damage of the porang tubers appeared firstly on their small part of tuber skin. The physical damage is commonly found to tuber commodities indicating the decrement of tuber

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quality. The tuber skin functions as a barrier or protector from bacterial or fungal attacks. If the skin is damaged, infection by pathogenic microorganisms will expand [16]. Fresh porang tubers were naturally starting to demage shorter after storage.

Percentage of Rotten and Sprout Tubers

The percentage of rotten tuber and sprouted tubers during storage increased in this study (Fig. 5). In the second week (W-2), the tubers stored on the ground, the rack and floors started to be rotten and sprouted. In the fourth week (W-4) until the sixth week (W-6), the percentage of rotten and germinated tubers increased, then at the eighth week (W-8), all tubers in the three storage places were rotten and sprouted.

The tuber rotten evidence could be triggered by activity of respiration process degrading organic material so that the tubers lose the organic compounds and be rotted. As the length of storage time, an increament of the percentage of rotten tubers was also followed by the percentage of sprouting tubers. Sprouted tubers occured when the rotten area appeared in the partial side of the tuber. Physiological processes took place as the respiration process conducted then produced energy used for sprouting.

Sprout growth faster at room temperature than cold temperatures, because the respiration process is increasing in the higher temperature [17]. Rotten in the tubers can occur due to the natural conditions of the tuber, handling during growth and postharvest. Several factors causing penetration microorganisms could be a) before harvesting, through pores that exist naturally in parts of plants that allow air, carbon dioxide, and water vapour to enter and exit the commodity network; b) Postharvest damage due to wrong handling, so that the wound can cause microorganisms ~~to~~ penetrate into the tuber; c) The entry of microorganisms directly due to contact with tuber skin tissue, where these conditions are influenced by the type of commodity, the level of maturity and the type of microbe's direct contact. This can occur either before or after harvesting, which may not appear directly but can occur at any time either during storage or when it reaches the consumer [18].

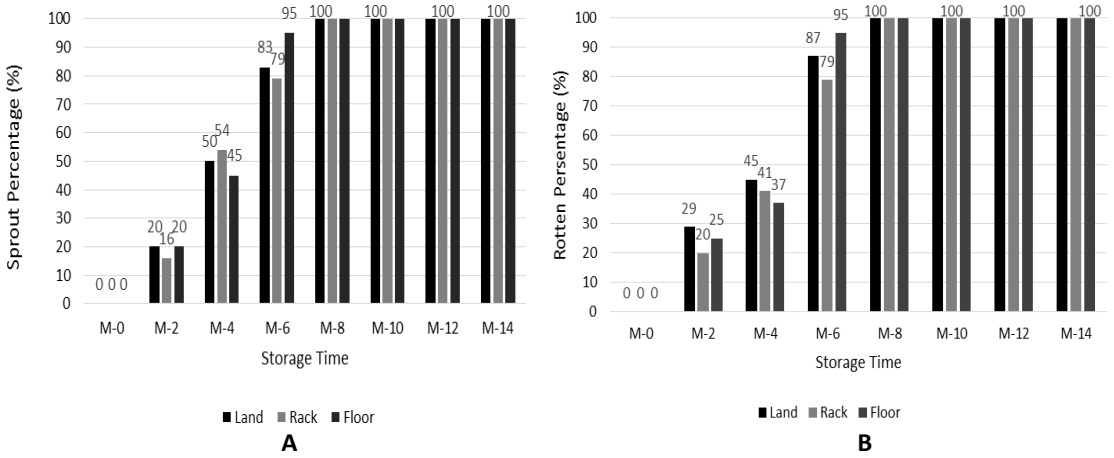

Figure 5. (A) Sprout percentage (%) and (B) Rotten percentage (%) during the storage period for each storage place

As comparation, in the case of white yam (*Dioscorea rotundata*), their growth were increasing on three storage methods (tubers stacked on the floor, traditional barns and open storage side) but they did not show significant differences [15]. Rot on the white yams began to appear at the twelfth week in all three storage areas, storage on the open side was the lowest rot rate (10%). The storage method with tubers stacked on the floor had the highest level of rot rate (26.67%). However, the white yam stored in traditional barns did not differ from the two storage methods.

Weight loss and water content (%)

The length of storage caused weight loss of the tuber. The water content of the tuber fluctuated, but overall the moisture content of the tubers during storage tended to decrease (Fig. 6). The phenomenon could be initiated by increasing respiration rate. For the tubers stored in the soil, the rotten tuber might be accelareted by cell degradation faster into the soil. During storage, the rotten tuber became dry as the water content of Porang tubers decreases.

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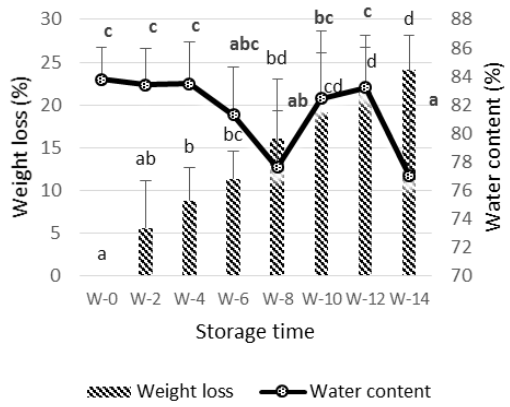

Figure 6. Weight loss and water content during storage.
Description: Lowercase notation to compare between storage periods. The line on the beam shows the Standard Deviation (SD) at α 0.05.

The weight loss might be caused by loss of water in the transpiration process. If the tubers stored for a long time, it causes a decrease on water content, so it gradually undergoes shrinkage [19,20]. The porang tubers was also shrinkage during storage. The process of transpiration causes water levels ~~to~~ decrease. While the process of respiration that produces water causes an increase in water content this may reason that the water content during storage fluctuates.

Water is the most important component of cells where its function is to carry out the function of cell life which includes metabolic reactions and distribution of nutrients to all parts of the organ. The water contained in tubers is in the form of crystallization of water or colloidal water. Some other water is called free water which is a solvent for the hydrophilic compounds found in the tubers. The water content in fresh tubers is 83.3% wet weight [21,22]. Moisture and weight loss are related to the physiological mechanism of the postharvest rotten tuber. Tubers that are stored on the ground exhibit foul symptoms faster in the form of significant weight shrinkage, decreasing in density and moisture content. The symptoms got faster may be caused the soil contains many microorganisms that can infect the tubers and can reduce the quality of the tubers [23].

Weight loss in tubers causes shrinkage, which is one of the criteria for quality standards for agricultural products. Weight loss is a main indicator in quality decrease including changes in appearance, texture, nutritional value of the product, and affects to decrease the selling price of tubers [8]. The respiration, transpiration, and sprouting are the factors responsible for weight loss and influence the appearance and cause tubers to shrivel [15].

Previous research also showed that the value of water content during storage time was not significantly different, but there was a tendency that the water content was decreasing during the period of storage. The transpiration could increase as the increase of the temperature [5]. The same thing also applies to sweet potato research during storage, the moisture content at the beginning of storage was 65.71% and decreases in a row for treatment in the tarpaulin base space, in a sawdust sprinkling box, in a plastic-straw pile base, and in the soil the pile of straw-sand pile were 58.96%, 59.71%, 61.36% and 61.5% [24].

Sweet potatoes showed shrinkage of weight during storage. the highest shrinkage happened at room temperature compared to cold temperature storage. At eight weeks the weight loss composition of Gisting sweet potatoes reached 16.87% for storage space, while in cold storage shrinkage weight was only 10.12%. The same thing also happened in Marga sweet potatoes in the storage of the room shrinkage reached 19.84% while at cold shrinkage the weight reached 14.66% [17]. There was a striking decrease in the number of amyloplast cells observed during the initial stages of shoot formation. This symptom of weight loss in tubers during storage is most likely also related to the occurrence of sprouts [5, 25].

CONCLUSION

The porang tuber have begun to show a decline in quality since two weeks stored. This decrease in quality was performed by symptoms of rotten indicated by the appearance of odor, decreasing in tuber weight, followed by a decrease in water content, an increase of rotten tuber area, and a percentage increment in rotten area and sprout of tuber. The storage place that slows decay most was on the shelf compared to those on the soil and the floor.

REFERENCES

[1] Wootton, N.A., M. Luker-Brown., R.J. Westcott. 1993. The extraction of a glucomannan polysaccharide from konjac corms (elephant yam, *Amorphophallus rivierii*). *J. Sci. Food Agric*. 61. 429-433.
[2] Indonesian Research and Development Center of Porang (P4I). 2013. Modul diseminasi: budidaya dan pengembangan

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E-ISSN. 2338-1655

porang (*Amorphophallus muelleri* Blume) sebagai salah satu potensi bahan baku lokal. University of Brawijaya. Malang.

[3] Indonesian National Standar (SNI) 7938: 2013. Umbi Porang. Badan Standardisasi Nasional.

[4] Amponsah, S.K., J.O. Akowuah., E.A. Kwarteng., E. Bessah. 2015. Design and construction of improved yam storage structure using locally-available materials. *Int. J. Res. Agr. For*. 2. 1-11.

[5] Asgar, A., S.T. Rahayu. 2014. Pengaruh suhu penyimpanan dan waktu pengkondisian untuk mempertahankan kualitas kentang kultivar margahayu. *Berita Biologi.* 13(3). 283-293.

[6] Ali, M.G., S. Houshmand. 2010. Effects of mechanical damage and temperature on potato respiration rate and weight loss. *World Appl. Sci. J.* 8(5). 647-652.

[7] Kibar, H. 2012. Design and management of postharvest potato (*Solanum tubrosum* L.) storage structures. *J. Sci. Tech*. 2(1). 23-48.

[8] Sudarmadji, S., B. Haryono, Suhadi. 1997. Analisa bahan makanan dan pertanian. Liberty. Yogyakarta.

[9] Zhao, J., D. Zhang., G. Srzednicki., S. Kanlayanarat., C. Borompichaichartkul. 2010. Development of a low-cost two-stage technique for production of low-sulphur purified konjac flour. *Int. Food Res. J*. 17. 1113-1124.

[10] Homaida, M.A., S. Yan., H. Yang. 2017. Effects of ethanol treatment on inhibiting fresh-cut sugarcane enzymatic browning and microbial growth. *LWT-Food Sci. Technol*. 77. 8-14.

[11] Richana, N., T.C. Sunarti. 2004. Karakterisasi sifat fisikokimia tepung umbi dan tepung pati dari umbi ganyong, suweg, ubikelapa dan gembili. *Jurnal Pascapanen.* 1(1). 29-37.

[12] Friedman, M. 1996. Food browning and its prevention: an overview. *J. Agric. Food Chem.* 44(3). 631-653.

[13] Purwantisari, S., R.S. Ferniah., B. Raharjo. 2008. Pengendalian hayati penyakit lodoh (busuk umbi kentang) dengan agens hayati jamur-jamur antagonis isolat lokal. *Bioma* 2. 13-19.

[14] Handayani, A., Sriyanto., I. Sulistyawati. 2013. Evaluasi mutu beras dan tingkat kesesuaian penanganannya (studi kasus di kabupaten Karanganyar). *Jurnal Litbang Provinsi Jawa Tengah.* 11(1). 113-124.

[15] Maalekuu, B.K., J.K. Saajah., A.K. Addae. 2014. Effect of three storage methods on the quality and shelf-life of white yam (*Dioscorea rotundata)* cultivars pona and tela. *J. Agr. Sci.* 6(7). 221-230.

[16] Ray, R.C. 2000. Microorganism associated with post harvest spoilage of yams. *Ann. Trop. Res*. 22. 31-40.

[17] Narulita, A., S. Waluyo, D.D. Novita. 2013. Sifat fisik ubi jalar (jalar gisting kabupaten Tanggamus dan jati agung kabupaten Lampung Selatan) pada dua metode penyimpanan. *Jurnal Teknik Pertanian Lampung*. 2(3). 133-146.

[18] Estiasih, T., W.D.R. Putri., E. Waziiroh. 2017. Umbi-umbian dan pengolahannya. University of Brawijaya Press. Malang.

[19] Osunde, Z.D., B.A. Orhevba. 2009. Effects of storage conditions and storage period on nutritional and other qualities of stored yam (*Discorea spp*) tubers. *Af. J. Food Agr. Nutr. Dev.* 9(2). 679-690.

[20] Senkumba, J., A. Kaaya., A. Atukwase., A. Wasukira. 2017. Technical report: effect of storage conditions on the processing quality of different potato varieties grown in Eastern Uganda. Research program on root, tubers, and bananas. Uganda.

[21] Lisinska, G., W. Leszczynski. 1989. Potato science and technology. Elsevier Publication. Essex.

[22] Arifin, A.M. 2001. Pengeringan umbi iles-iles secara mekanik untuk meningkatkan mutu keripik iles. Master Thesis. Postgraduate School, Bogor Agricultural University. Bogor.

[23] Ogunleye, A.O., O.T. Ayansola. 2014. Studies of Some Isolated Rot-Causing Mycoflora of Yams (*Dioscorea* spp.). *Am. J. Microbiol. Biotech.* 1(1). 9-20.

[24] Kafiya, M., Sutrisno., R. Syarief. 2016. Perubahan kadar air dan pati ubi jalar (*Ipomea batatas* L.) segar pada sistem penyimpanan sederhana. *Jurnal Penelitian Pascapanen Pertanian*. 3(3). 136-145.

[25] Chua, M. 2011. An investigation of the biology and chemistry of the chinese medical plant, *Amorphophallus konjac*. Ph.D Thesis. University Of Wolverhampton.

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Molecular Identification and Genetic Characteristics of Genus *Mystacoleucus* Based on Gene Cytochrome Oxidase C Subunit I (COI) in Sengguruh Dam

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Abstract

In general, the process of naming several species of fish including species from the genus *Mystacoleucus* is still carried out based on morphological characters, which are often found in almost the same morphological characters in different fish species. Therefore, it is necessary to apply a more accurate identification method, which is a DNA-based identification method called DNA barcoding. The purpose of this study was to identify the species of the genus *Mystacoleucus* on the Sengguruh Dam molecularly based on the Cytochrome Oxidase C Subunit I (COI) gene. A caudal fin was taken on the test fish and preserved in 95% ethanol solution for molecular identification. The results of the identification showed that the sample belonged to the *Mystacoleucus marginatus* species with Identity values between 99-100% and E-value 0.0. The data obtained showed that from the calculation of genetic distance presented in the form of data matrix and phylogenetic tree reconstruction, there were 2 species that had a far genetic distance from the *M. marginatus* sample from the Sengguruh Dam namely *M. atridorsalis* with the furthest genetic distance of 0.1932-0.2595 and *M. lepturus* with genetic distance between 0.1117-0.1193. However, there is one species that has the closest distance, *M. padangensis* with genetic distance between 0.0019-0.0038 and identity values up to 99%.

Keywords: DNA barcode, Molecular identification, *Mystacoleucus*, Phylogenetic COI.

INTRODUCTION

Indonesia has a very large diversity of freshwater fish. Found as many as 1218 species from 84 families including 1172 native species of 79 families and 630 species are endemic [1]. One of the endemic fish that lives in Indonesia is the Mystacoleucus genus. The genus Mystacoleucus is a family of Cyprinidae [2-5] with specific characteristics that have horizontal spines in front of the dorsal fin [1]. These fish live in tropical rivers [6,7] and are spread throughout most of Asia [8-24].

Search results in FishBase and Catalog of Fishes found eleven species from the *Mystacoleucus* genus, and two of them were found in Indonesia, namely *Mystacoleucus marginatus* Valenciennes 1842 [25,26,27] and *Mystacoleucus padangensis* Bleeker 1852 [1,24]. Apart from Indonesia, *Mystacoleucus marginatus* is also found in Malaysia [28], Thailand [5] and China [29,30].

In general, the naming of several fish species including the genus *Mystacoleucus* is still based on morphological characters [1]. It is quite difficult to ensure the naming of species because there are so many fish that look visually similar but are genetically different, and vice versa. For this reason, it is necessary to apply a method that is more accurate in identifying species and names. Along with the development of molecular biology, a new method has been found for identification of DNA-based species known as DNA Barcoding [31].

DNA barcoding provides speed and accuracy in species identification with a focus on analysis in small segments of mtDNA [32,33]. DNA barcoding can be a solution to the current taxonomic crisis [34] and has been developed to identify species because it is relatively easy to do compared to other techniques [35]. The use of DNA Barcoding can be practically applied [36] or as a tool to support other studies [37] by selecting one or several loci that can be routinely sequenced and can be relied upon to identify large numbers of samples and diverse and easy compared between species.

The current study showed that the partial COI gene sequence of fin clips and muscle tissue can be used as a diagnostic molecular marker for identification and resolution of taxonomic ambiguity of Ompok species [38]. Other study showed that the genes of cytochrome oxidase

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subunit I (COI) contained in mitochondria as DNA Barcoding globally have been chosen as standard tools for molecular taxonomy and animal identification [31]. The COI gene is a region that encodes a protein with a base length of up to 1551 starting from the start codon "GTG" and ending with the stop codon "TAA" in line 5489-7039 in mitochondrial DNA (mtDNA) [39].

This study aims to identify species and determine the genetic characteristics of the genus *Mystacoleucus* found in Sengguruh Dam, Malang. with a genetic approach based on the cytochrome oxidase c subunit I (COI) gene, so that it can confirm further molecular naming.

MATERIALS AND METHODS

Sample Collection

A total of five samples were collected from the Sengguruh Dam, Malang, East Java Province, from June to August 2018. For the purposes of molecular identification, a caudal fin was taken on the test fish and preserved in 95% ethanol solution. Whereas as a reference for identification, a total of nine genus *Mystacoleucus* from various regions were downloaded from GenBank with accession numbers which can be seen in Table 1.

Table 1. DNA sequense of Genus *Mystacoleuscus* from Genbank

Spesies	Location	Accession Number
M. marginatus	Mojokerto	KU692639 [27]
M. marginatus	Jawa barat	KU692643 [27]
M. marginatus	Lumajang	KU692636 [27]
M. marginatus	Malaysia	KT001017 [28]
M. marginatus	Laos	JQ346160 [40]
M. marginatus	China	KJ994653 [30]
M. atridorsalis	Singapura	JF915630.1 [41]
M. lepturus	China	KJ994654.1 [30]
M. padangensis	Medan	MF062176.1 [42]

DNA Extraction, Isolation, and Amplification

Genomic DNA extraction from all samples was carried out using the KIT method: Genomic DNA Mini Tissue Animal Kit (GENEAID). The Geneaid™ DNA Isolation Kit offers a simple and gentle reagent DNA precipitation method for isolating high molecular weight genomic, mitochondrial or viral DNA suitable for archiving or sensitive downstream applications. This highly versatile solution based system can be scaled proportionately in order to satisfy larger sample volumes providing a convenient sample-storage procedure with minimal hands on time.

Initially, cells are lysed in the presence of detergents and a proprietary DNA stabilization solution followed by RNase A treatment. Once proteins and other contaminants are removed DNA is precipitated then rehydrated. The high quality extracted DNA is ready for use in a variety of downstream applications. Amplification (PCR) of the mitochondrial locus cytochrome c oxidase subunit I (COI) gene was carried out using the GO TAQ Green PCR Mix method with universal primary pairs LCO1490: 5'-ggtcaacaaatcataaagatattgg-3' and HCO2198: 5' taaacttcagggtgaccaaaaaatca-3' [43]. Making mastermix (Go Taq Green) was carried out by adding 14 µL ddH2O, forward and reverse primers of 2.5 µL, DMSO 1 µL, Go Taq Green 25 µL and DNA extraction of 5 µL. Amplification is carried out at a 50µl final volume.

The PCR process includes pre-denaturation at 94°C for three minutes, followed by 35 cycles consisting of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds and extention stage at 72°C for 45 seconds. Furthermore, the PCR results were carried out by an electrophoresis process to separate, identify and purify DNA fragments, using 1% agarose gel with 50 mLTris Borate EDTA (TBE). The PCR results that were successfully amplified were then sent to First Base CO (Malaysia) using Big Dye© terminator chemistry (Perkin Elmer), to obtain base arrangements that form DNA or nucleotide sequences.

Data Analysis

Sequencing results are aligned using the Clustal W method found in MEGA 6.06 software [44]. The sequential data is then matched with the data available in the NCBI GenBank (National Center for Biotechnology Information) online using the BLAST (Basic Local Alignment Search Tool-nucleotide) method. Genetic distance analysis of COI gene sequences was calculated using the pairwise distance method found in the MEGA 6.06 program [44]. The results of the calculation of genetic distance are presented in the form of a data matrix that can be used to analyze kinship relationships between species based on the tree of phylogeny. Phylogenetic reconstruction using the Maximum Likelihood Trees method [45], Kimura-2 parameter model and 10000 × bootstrap value using MEGA 6.06 software [44].

RESULTS AND DISCUSSION

Species Identification

The length of the sequence results of the COI gene genus *Mystacoleucus* sequencing from the Sengguruh Dam using primers LCO1490 and HCO2198 is 683-686 bp (base pairs). The primary use of LCO1490 and HCO2198 [43]. The results

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show that the primary pairs of LCO1490 and HCO2198 consistently strengthen the fragments of approximately 700 bp. This result is also in accordance with the research conducted before [27-30,40,41] on the genus *Mystacoleucus* using the mitochondrial COI gene resulting in a sequence length of 549-859 bp. Some length differences in DNA sequences amplified due to the type of primer used, primary base composition, primary length, DNA quality found, food, offspring and environment [46]. A previous study suggested that 658 bp fragments using the COI gene could be used as a differentiating basis between animals [31].

Samples from the Sengguruh Dam were then identified in Genbank using the BLAST method. Samples were identified as *Mystacoleucus marginatus* with query cover values between 95-98%, Identity values between 99-100% and E-value 0.0. Based on the results of the BLAST analysis, it can be concluded that the sample DNA sequences have a very high level of similarity with the DNA sequences in GenBank. Wahyuningsih [47] suggested that with a similarity rate of 99-100% it could be said that species were identical and could be identified as that species. Claverie and Notredame [48] suggest that DNA sequences can be said to have homology if the E-value is smaller than e-0.4. The results of the identification are presented in Table 2.

Genetic Characterisics

Nucleotide analysis is very much needed in research because it is a compiler of DNA sequences. Nucleotides are DNA monomers containing three different parts, namely pentose sugar, nitrogen bases (A, T, G, C) and phosphate groups [49]. The results of the analysis of nucleotide composition showed that the average amount of adenine and thymine was found to be the highest. The average nucleotide composition (Table 4) found in the COI *M. marginatus* gene in Sengguruh Dam is C (Cytosine) of 28.6%, T (Thymine) of 27.0%, A (Adenine) of 27.6% and G (Guanine) of 16.8%. The G + C content of all samples is 45.4% and has a lower number than the number of A + T which amounts to 54.6%.

Based on the nucleotide base composition, the nucleotide base of *M. marginatus* is dominated by alkaline A (adenine) and T (thymine) bonds so that the COI gene of both species is categorized as a rich A-T (A-T rich) group. The hydrogen bond A-T consists of 2 hydrogen bonds which are weaker than the G-C hydrogen bonds which have 3 hydrogen bonds. The nucleotide base composition of Mystacoleucus marginatus is an easily separated bond so the possibility of mutation of species is higher. The composition of the nucleotide bases is presented in Table 3.

Genetic distance and phylogenetic analysis

The genetic distance of the COI gene fragment between *Mystacoleucus* spp. based on research results and GenBank is presented in the form of a data matrix (Table 4). The genetic distance of interspecific *M. marginatus* ranges from 0.0000 - 0.0625. While the highest genetic distance of intraspecies *Mystacoleucus* spp was found in *M. atridorsalis* of 0.2595, then *M. lepturus* and *M. padangensis* for 0.1193 and 0.0038 respectively.

Data from the genetic distance matrix on COI gene fragments were used to analyze kinship relationships based on the phylogeny tree. The phylogenetic reconstruction based on the COI gene using the Maximum Likelihood Trees method of the Kimura-2 parameter and the bootstrap value 10000 is shown in Figure 1.

Table 2. The results of the identification of the Genus *Mystacoleucus* sequences in the Sengguruh Dam through BLAST Analysis

Sample code	Species outcome	BLAST			
		Access code of NCBI	Query Cover(%)	E-value	Identity (%)
BS[1]	*M. marginatus*	KU692642.1	95	0.0	100
BS[2]	*M. marginatus*	KU692641.1	95	0.0	100
BS[3]	*M. marginatus*	KT001063.1	98	0.0	99

Table 3. Composition of Nucleutide bases *Mystacoleucus marginatus* in the Sengguruh Dam

Sample code	Nucleotides			
	T (Thymine)	C (Cytosin)	A (Adenin)	G (Guanine)
BS[01]	27.0	28.7	27.6	16.7
BS[02]	26.8	28.7	27.7	16.8
BS[03]	27.3	28.5	27.5	16.7
Average	27.0	28.6	27.6	16.8

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Table 4. Genetic distance of *Mystacoleucus* spp.

	1	2	3	4	5	6	7	8	9	10	11
BS^{01}											
BS^{02}	0.0000										
BS^{03}	0.0019	0.0019									
Mojokerto	0.0000	0.0000	0.0019								
Lumajang	0.0000	0.0000	0.0019	0.0000							
Jawa_Barat	0.0019	0.0019	0.0057	0.0019	0.0019						
Malaysia	0.0057	0.0057	0.0133	0.0057	0.0057	0.0057					
China	0.0625	0.0625	0.0795	0.0625	0.0625	0.0436	0.0473				
Laos	0.0625	0.0625	0.0795	0.0625	0.0625	0.0436	0.0473	0.0000			
M. padangensis	0.0038	0.0038	0.0095	0.0038	0.0038	0.0019	0.0019	0.0398	0.0398		
M. lepturus	0.1193	0.1193	0.1420	0.1193	0.1193	0.1117	0.0758	0.0852	0.0852	0.0852	
M. atridorsalis	0.2595	0.2595	0.2973	0.2595	0.2595	0.2367	0.1932	0.1269	0.1269	0.2027	0.0417

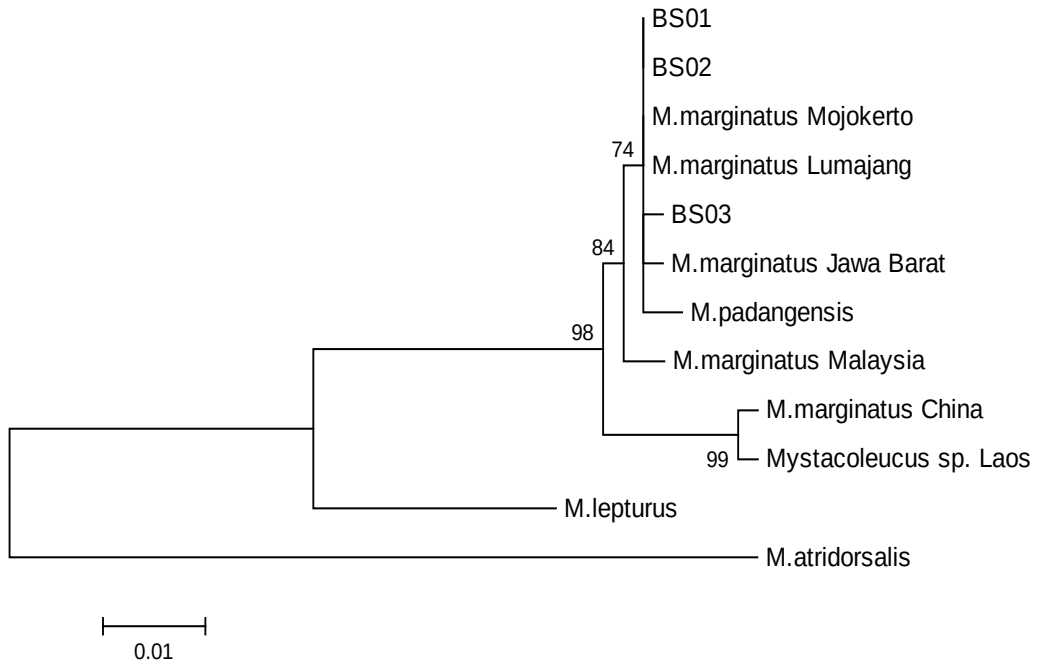

Figure 1. Phylogenetic reconstruction of *Mystacoleucus* spp. based on cytochrome oxidase c subunit I (COI) Gene

The construction of the phylogeny tree based on the COI gene above shows that the species in the *M. marginatus* group are significantly different from the *M. atridorsalis* species with genetic distances of 0.1932-0.2595 and *M. lepturus* with a genetic distance between 0.1117-0.1193. Genetically *M. marginatus* is very close to *M. padangensis*, the genetic distance of these two species ranges from 0.0019-0.0038. This is also proven by the results of BLAST, where the samples identified as *M. marginatus* from the Sengguruh Dam have an Identity value of 99% and E-value 0.0. with *M. padangensis*, so it can be concluded that the DNA sequences of *M. marginatus* and *M. padangensis* have very high genetic similarities. Wahyuningsih [47] suggested that with a 99-100% similarity rate it could be said that the species was identical and DNA sequences had homology if the E-value was smaller than e-0.4 [48].

Based on the phylogenetic tree reconstruction, it was also seen that by ingroup species, individuals of different *M. marginatus* distribution regions had very close relationships with genetic distances between 0.0625-0.0000, where 0.000 is the closest distance and this value indicates that from 1000 base pairs, no one has a different base pair. The closest distance is obtained by the *M. marginatus* species from Sengguruh Dam, Mojokerto and Lumajang. Mojokerto has a genetic closeness with the Sengguruh Dam because it is still in one water source, namely Brantas waters, from Mount Arjuno springs, Brantas source village, Bumi Aji District, Batu City which then flows to Malang, Blitar, Kediri, Mojokerto, and Sidoarjo. This

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pattern of currents results in gene flow or gene exchange between locations resulting in homogeneous genes.

It is also known that the spread of *M. marginatus* is very broad covering the territory of Indonesia (East Java and West Java) to Malaysia, Laos, and China. This spread is possible due to the influence of Sundaland, where the Sunda region has a shallow continental shelf and changes in the eustatic sea repeatedly linking large islands in this region (Sundanese and Southeast Asian exposures) form Sundaland [16,50]. This allows the migration of freshwater fish into and outside Java which may have occurred 10-70 thousand years ago [51].

CONCLUSION

The length of fragments from the amplification of the COI gene of the genus *Mystacoleucus* from the Sengguruh Dam using primary LCO1490 and HCO2198 is 681-686 bp (base pairs), which shows that the test sample belongs to the *M. marginatus* species with an identity value of 99-100% and E-value 0.0. The average nucleotide composition in the COI *M. marginatus* gene in Sengguruh Dam is C (Cytosine) of 28.6%, T (Thymine) of 27.0%, A (Adenine) of 27.6% and G (Guanine) of 16.8%. The G + C content of all samples is 45.4% and has a lower number than the number of A + T which amounts to 54.6%.

REFERENCES

[1] Kottelat, M., A.J. Whitten, S.R. Kartikasari, S. Wirjoatmodjo. 1993. Freshwater fishes of western Indonesia and Sulawesi. Periplus Editions. Singapore.

[2] Roberts, T.R. 1993. The freshwater fishes of Java, as observed by Kuhl and van Hasselt in 1820-23. *Zoologische Verhandelingen (Leiden)*. 285. 1-94.

[3] Vidthayanon, C. 2012. *Mystacoleucus argenteus*. The IUCN Red List of Threatened Species 2012. IUCN.

[4] Rainboth, W. 2012. *Mystacoleucus ectypus*. The IUCN Red List of Threatened Species 2012. IUCN.

[5] Kottelat, M. 2013. The fishes of the inland waters of Southeast Asia: a catalogue and core bibiography of the fishes known to occur in freshwaters, mangroves and estuaries. *Raffles Bull. Zool.* 27. 1-663.

[6] Nelson, J.S. 2006. Fishes of the world, 4th Ed. John Wiley and Sons, Inc.

[7] Froese, R., D. Pauly (Eds). 2014. FishBase. World Wide Web electronic publication. www.fishbase.org, version (08/2013).

[8] Bertin, L., R. Estève. 1948. Catalogue des types de poissons du Muséum National d'Histoire Naturelle. 4e partie. Ostariophysaires (Cypriniformes). *Imp. Nationale, Paris.* 4e. 1-117.

[9] Monkolprasit, S.P. 1969. Type specimens of Thai cyprinoid fishes. *Kasetsart Univ. Fish. Res. Bull.* 5. 1-16.

[10] Whitehead, P.J.P., P.K. Talwar. 1976. Francis Day (1829-1889) and his collections of Indian fishes. Bull. Br. Mus. Nat. Hist. Hist. 5(1). 1-189, Pls. 1-4.

[11] Böhlke, E.B. 1984. Catalog of type specimens in the ichthyological collection of the Academy of Natural Sciences of Philadelphia. Special Publication, *Acad. Nat. Sci. Phila.* 14(i-viii). 1-216.

[12] Kottelat, M. 1984. A review of the species of Indochinese fresh-water fishes described by H.E. Sauvage. *Bull. Mus. Natl. Hist. Nat. Ser. 4: Section A: Zoologie, Biologie et Écologie Animales.* 6(4). 791-822.

[13] Roberts, T.R. 1989. The freshwater fishes of western Borneo (Kalimantan Barat, Indonesia). *Mem. Calif. Acad. Sci.* 14(i-xii). 1-210.

[14] Lim, K.K.P., P.K.L. Ng, M. Kottelat. 1990. On a collection of freshwater fishes from Endau-Rompin, Pahang-Johore, Peninsular Malaysia. *Bull. Raffles Mus.* 38(1). 31-54.

[15] Kottelat, M., K.K.P. Lim. 1995. Freshwater fishes of Sarawak and Brunei Darussalam: a preliminary annotated check-list. *Sarawak Mus. J. (New Series)*. 48(69). 227-256.

[16] Rainboth, W.J. 1996. The taxonomy, systematics and zoogeography of *Hypsibarbus*, a new genus of large barbs (Pisces, Cyprinidae) from the rivers of Southeastern Asia. University of California Press, La Jolla.

[17] Doi, A. 1997. A review of taxonomic studies of cypriniform fishes in Southeast Asia. *Jpn. J. Ichthyol.* 44(1). 1-33.

[18] Kottelat, M. 1998. Fishes of the Nam Theun and Xe Bangfai basins, Laos, with diagnoses of twenty-two new species (Teleostei: Cyprinidae, Balitoridae, Cobitidae, Coiidae and Odontobutidae). *Ichthyol. Explor. Fres.* 9(1). 1-128.

[19] Ng, H.H., H.H. Tan. 1999. The fishes of the Endau drainage, Peninsular Malaysia with descriptions of two new species of catfishes

ISSN. 2087-2852
E-ISSN. 2338-1655

(Teleostei: Akysidae, Bagridae). *Zool. Stud.* 38(3). 350-366.

[20] Kottelat, M. 2000. Diagnoses of a new genus and 64 new species of fishes from Laos (Teleostei: Cyprinidae, Balitoridae, Bagridae, Syngnathidae, Chaudhuriidae and Tetraodontidae). *J. South Asian Nat. Hist.* 5(1). 37-82.

[21] Le, P.Q., Y.Y. Chen. 2000. Fauna sinica, osteichthyes, cypriniformes III. Science Press. Beijing.

[22] Kottelat, M. 2001. Fishes of Laos. WHT Publications, Colombo. 1-198, Pls. 1-48.

[23] Kottelat, M. 2015. The fishes of the Nam Theun and Xe Bangfai drainages, Laos. *Hydroécol. Appl. (Paris).* 19. [1-51]. 271-320.

[24] Nofrita., Dahelmi., H. Syandri, D.H. Tjong. 2015. Morphological differentiation between Bilih Fish (Cyprinidae : *Mystacoleucus padangensis*, Blekeer) in Singkarak Lake and Anai River, West Sumatra , Indonesia. *J. Entomol. Cool. Stud.* 3(5). 171–175.

[25] Freyhof, J., D.V. Serov, T.N. Nguyen. 2000. A preliminary checklist of the freshwater fishes of the River Dong Nai, South Vietnam. *Bonner Zoologische Beiträge.* 49(1-4). 93-99.

[26] Kottelat, M., E. Widjanarti. 2005. The fishes of Danau Sentarum National Park and the Kapuas Lakes area, Kalimantan Barat, Indonesia. *Raffles Bulle. Zool.* 13. 139-173.

[27] Dahruddin, H., A. Hutama, F. Busson, S. Sauri, R. Hanner, P. Keith, R. Hadiaty, N. Hubert. 2017. Revisiting the ichthyodiversity of Java and Bali through DNA barcodes: taxonomic coverage, identification accuracy, cryptic diversity and identification of exotic species. *Mol. Ecol. Resour.* 17(2). 288-299.

[28] Meganathan, P., C.M. Austin, S.M. Tam, P.C. Chew, R. Siow, Z. Abdul Rashid, B.K. Song. 2015. An application of DNA barcoding to the Malaysian freshwater fish fauna: mtDNA COI sequences reveal novel haplotypes, cryptic species and field-based misidentification. Monash University Malaysia. Selangor, Malaysia.

[29] Yang L., T. Sado, M.V. Hirt, E.P. Viel, M. Arunachalam, X. Wang, J. Freyhof, K. Saitoh, A.M. Simons, M. Miya, R.L. Mayden, J. Li, S. He, R.L. Mayden. 2015. Phylogeny and polyploidy: Resolving the classification of cyprinine fishes (Teleostei: Cypriniformes). *Mol. Phylogenetics Evol.* 85. 97-116.

[30] Zheng, L.P., J.X. Yang, X.Y. Chen. 2016. State Key Laboratory of Genetic Resources and Evolution, Kunming Institut of Zoology, Chinese Academi of Sciences, 32 Jiaochang Donglu, Yunan 650223, China. *Biochem. Syst. Ecol.* 68. 250-259.

[31] Hebert, P.D.N., A. Cywinska, S.L. Ball. 2003. Biological identifications through DNA barcodes. *Proc. Royal Soc. London. Series B: Biol. Sci.* 270(151). 313-321.

[32] Muchlisin, Z.A., Z. Thomy, N. Fadli, M.A. Sarong, M.N. Siti-Azizah. 2013. DNA barcoding of freswater fishes from Lake Laut Tawar, Aceh Province, Indonesia. *Acta Ichtyiol. Piscat.* 43(1). 21-29.

[33] Karim, A., A. Iqbal, R. Akhtar, M. Rizwan, A. Amar, U. Qamar, S. Jahan. 2015. Barcoding of fresh water fishes from Pakistan. *Mitochondrial DNA.* 1-4.

[34] Meier, R., K. Shiyang, G. Vaidya, K.L.N. Peter. 2006. DNA barcoding and taxonomy in Diptera. A tale of high intraspesific variability and low identification success. *System Bio.* 55. 715-728.

[35] Woong, L.L., Peatman., J. Lu, H. Kucuktas, S. He, Ch. Zhou, U. Nanacorn. Z. Liu. 2011. DNA barcoding of catfish: species authentication and phylogenetic assessment. *PloS ONE.* 6. e17812.

[36] Stocle, M.Y., C.C. Gamble, R. Kirpekar, G. Young, S. Ahmed, D.P. Little. 2011. Comercials teas highlight plant DNA Barcode identification succeces and obstaclesses and obstacles. *Sci. Rep.* I(42). 1-7.

[37] Ebihara, A., J.H. Nitta, M. Ito. 2010. Molecular species identification with rich floristic sampling: DNA barcoding the pteridophyte flora of Japan. *Plos ONE.* 5(12). e15136.

[38] Malakar, A.K., Lakra, W.S. Goswami, M. Singh, R.M. Mishra. 2012. Molecular identification of threeOmpokspecies using mitochondrial COI gene. *Mitochondrial DNA.* 23(1). 20-24.

[39] Liu, S., Yang, Z., Wang, D. dan Liu, M. 2014. The mitochondrial genome of Mystacoleucus marginatus (Cypriniformes, Cyprinidae). *Mitochondrial DNA.*

[40] Pasco-Viel, E., M. Veran, L. Viriot. 2012. Revision of the genus *Cyclocheilichthys* (Bleeker 1859) and resurrection of the genus *Anematichthys* (Bleeker 1859),based on morphological and molecular data of

ISSN. 2087-2852
E-ISSN. 2338-1655

Southeast Asian Cyprininae (Teleostei, Cypriniformes). *Zootaxa.* 3586. 41-54.
[41] Collins, R.A., Y. Yi, R. Meier, K. Armstrong. 2016. Barbs, barcodes and biosecurity: identifying ctprinid fishes in the ornamental trade. Unpublished
[42] Wibowo, A., S. Kaban. 2017 Genetic diagnosis and reproductive biology of translocated *Mystacoleucus padangensis* in the Toba Lake, North Sumatra. Unpublished.
[43] Folmer O., Black M., Hoeh W., Lutz R., and Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase sub unit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3(5) : 294-299.
[44] Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei, S. Kumar. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likehood, evolutionary distance and maximum parsimony method. *Mol. Biol. Evol.* 28(10). 2731-2739.
[45] Lemey, P., M. Salemi, A.M. Vandamme (Eds). The Phylogenetic handbook: a practical approach to phylogenetic analysis and hypothesis testing. Cambridge University Press. New York. 419-490.
[46] Shizuka, D., B.E. Lyon. 2008. Improving the reliability of molecular sexing using a Wspecific marker. *Mol. Ecol. Resour.* 8. 1249-1253.
[47] Wahyuningsih. 2013. Parameter populasi ikan kakap merah (*Lutjanus malabaricus*) di perairan laut Jawa bagian timur. *Balai Perikanan Laut Jakarta.* 5(3). 175-179.
[48] Claverie, J., C. Notredame. 2003. Bioinformatics for dummies. Wiley Publishing Inc. New York. 325.
[49] Blanco, A., B. Gustavo. 2017. Chapter 6 - medical biochemistry. Academic Press. 121-140.
[50] Voris, H.K. 2000. Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. *J. Biogeogr.* 27. 1153-1167.
[51] Kusuma, W.E., S. Ratmuangkhwang, Y. Kumazawa. 2016. Department of Information and Basic Science and Research Center for Biological Diversity, Department of Aquaculture, Faculty of Fisheries and Marine Science, University of Brawijaya, Andaman Coastal Research Station for Development, Faculty of Fisheries, KU.

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Sensitivity and Stability Analysis of a SEIR Epidemic Model with Information

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Abstract

In this paper, the construction and stability analysis of a SEIR epidemic model with information are discussed. This model contains information about how to prevent the spread of infectious diseases which is transmitted by infected individuals to susceptible individuals. Furthermore, the dynamical analysis of the model which includes determination of equilibrium points terms of existence, stability analysis of the equilibrium points and sensitivity analysis are observed. Local stability of the equilibrium point is determined by linearizing the system around the equilibrium point and checking for the eigenvalue sign of Jacobian matrix at each equilibrium point. Sensitivity analysis is performed by using a sensitivity index to measure the relative change of basic reproduction number on each parameter. Based on the analysis result, there are two equilibrium points namely disease-free equilibrium point and endemic equilibrium point. The disease-free equilibrium point always exists and is locally asymptotically stable if the basic reproduction number is less than one. Moreover, the endemic equilibrium point exists and is locally asymptotically stable under certain conditions. From sensitivity analysis, it is found that the rate of mortality is the most sensitive parameter and the least sensitive parameter is the rate of exposed individual becomes infected individual. Finally, numerical simulation is conducted to support the analysis result.

Keywords: Epidemic, information, sensitivity analysis, *SEIR*, stability analysis.

INTRODUCTION

A disease is defined as a condition where the function of one of the parts of the body gets disrupted so that it cannot work normally. In medical scope, a disease is categorized into two types, namely infectious disease and noninfectious disease. The disease caused by microbes such as virus, bacterium, parasite or fungus is categorized as infectious disease. There are many factors which cause infectious disease spreads extensively. For instance, dislocation of people, lifestyle, sexual practice, traveling abroad, and lack of information about the threat of the disease.

One of the strategies in reducing the risk of an individual to be infected is by using information [1]. Information, as a form of non-medical intervention, is able to publish an outbreak of disease so that prevention of disease can be anticipated as soon as possible. Practically, as people know more about prevention knowledge, the better they can protect themselves by adopting the suitable action derived from the spreading information [2].

The dynamical of the infectious disease transmission can be illustrated by a mathematical model. In 1927, Kermack and Mc Kendrick introduced a simple transmission model of disease that consists of three sub-populations: susceptible (S), infected (I) and recovered (R). Susceptible is the susceptive sub-population, while infected is the infected sub-population and recovered is the healed sub-population. This model is known as a SIR classic model [3].

The mathematical models related to the disease transmissions have been developed especially those which included information effect in disease infection prevention. In 2008, Buonomo et al. modeled and analyzed the stability of the disease transmission model using information. In this research, the disease transmission model was divided into three compartments: susceptive individual compartment (*S*), infected individual compartment (*I*), and information media compartment (*Z*). The relation among those compartments explained the disease transmission model which was stated in the non-linear differential equation system. The disease was transmitted through interaction between a susceptible individual (*S*) and infected individual (*I*) [4].

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Some mathematical models that include latent period, infected individual sub-population who could not spread the disease, have been studied by many researchers. For example, in 2008, Cui et al. investigated the stability of disease transmission epidemic model by involving exposed individual (*E*). The infected individual sub-population was the individual sub-population who received medical treatment in a hospital once they were identified as infected. When this sub-population recovered, they did not spread the disease to the susceptible individual. The effect of media coverage toward infectious disease transmissions was explored. The model in the research aimed to analyze the impact of media on disease transmission [5].

Furthermore, in 2017, Kumar et al. explained the effect of information toward the infectious disease transmission using a SIR epidemic model and added information compartment (*Z*). This information induced the behavior change in the susceptible individual to protect themselves from infection of the disease. The process of infectious disease transmission happened through interaction between a susceptible individual and infected individual. Kumar et al. added the optimum control problem in the model by selecting treatment combination control (pharmacy control) and information effect (non – pharmacy control) [6].

In 2017, Njankou and Nyabadza constructed and analyzed the stability of Ebola disease model using information. The model of disease transmission was divided into seven compartments which were susceptible individual compartment (*S*), exposed individual (*E*), infected individual with no symptom (I_a), infected individual with symptom (I_s), recovered individual (*R*), died individual (*D*), and information media (*Z*). In contrast with Kumar et al. in this model susceptible and exposed sub-population are affected by the information [7].

In this paper, we construct a *SEIRZ* disease transmission model. In contrast with Kumar et al. in this model, it is assumed that information affect susceptible and exposed sub-populations as in Njankou and Nyabadza research. Moreover, this model is also different from the research of Njankou and Nyabadza because in this study, we use saturation rate in the information compartment. A dynamical analysis is carried out by determining the equilibrium point and the conditions of its existence and analyzing the stability of the equilibrium point and its basic reproduction number. Then, sensitivity analysis is performed using a sensitivity index to measure the relative changes of R_0 to each parameter. The results of the stability analysis of the equilibrium point are illustrated through numerical simulations using several parameter values.

MATERIAL AND METHOD

Construction Model

We develop a *SEIRZ* disease transmission model by combining the model used in Kumar et al. [6] and Njankou and Nyabadza[7]. This research adopts the information change rate model from Kumar et al. [6] and information affecting susceptible or exposed sub-population stated in Njankou and Nyabadza [7]. The infectious disease transmission model in this research is stated in five compartments: susceptible individual (*S*), exposed individual (*E*), infected individual (*I*), recovered individual (*R*) and information compartment (*Z*).

Stability Analysis of Equilibrium Point

Local stability analysis can be determined by linearizing of the model around its equilibrium points using the Taylor series. The equilibrium point is stable if the real part of the characteristic roots are negative or equal to zero [8].

Basic Reproduction Number

In disease transmission epidemic model, basic reproduction number is used as a parameter to identify the occurrence of disease outbreaks transmission in a population. The basic reproduction number (R_0) states the average number of individuals infected by others who have been infected before.

Disease transmission occurs when $R_0 > 1$. It is because on average, an infected individual causes more than one new infected individual. If $R_0 < 1$, the disease transmission doesn't occur due to the average infected individual causes less than one new infected individual [9].

Sensitivity Analysis

A sensitivity analysis of *SEIR* (Susceptible, Exposed, Infectious and Recovered) epidemic model is conducted to determine the influential of parameters on the basic reproduction number [10]. Normalized sensitivity index which is obtained by differentiating R_0 againts parameters α_i with $i = 1, \ldots, n$ can be formulated as below:

$$\gamma_{\alpha_i}^{R_0} = \frac{\partial R_0}{\partial \alpha_i} \times \frac{\alpha_i}{R_0}$$

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Sensitivity index on all parameters that have a high influence on R_0 can be an indicator of intervention in order to control the spread of disease [11]. Sensitivity analysis is important as it can be used to determine which parameter is the most important in reducing the level of a disease.

Numerical Simulation

Numerical simulation begins with determining the parameter values that meet the conditions of existence and the stability requirements of the equilibrium point.

RESULT AND DISCUSSION

Model Formulation

We formulate a compartmental model which includes the effect of information. The schematic diagram takes the form:

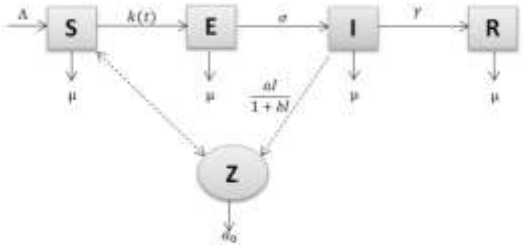

Figure 1. Schematic diagram of $SEIRZ$ model

The SEIRZ epidemic model consists of susceptible (S), infected (I), removed (R), and information (Z) where the recovered individual will not get infected again. In susceptible individual, the population of the individual will increase along the recruitment rate Λ and natural death can cause the decline of susceptible population with the rate μ. The susceptible individual is able to turn into exposed as the effect of interaction with infected population with the rate $\kappa(t)$. The exposed individual is able to decline also when there is natural death with the rate μ and the rate of exposed individual becomes infected individual is represented by σ. The infected individual population will increase when the rate of exposed individual becomes infected individual is represented by σ. This infected individual may also decline when there is either a natural death with the rate μ or death caused by disease with the rate δ. Meanwhile, recovered individual can increase when infected individual gets recovered with the recovery rate γ. In contrast, the degradation of recovered individual happens because of natural death. The information can increase proportionally with the infected population density as $\frac{aI}{1+bI}$, where the growth rate of information is represented by a and b represents saturation constant. The decreasing of information is affected by degradation of the information which is represented by a_0. This degradation can happen due to expired information. The effect of information $Z(t)$ is a reduction factor notated by $f(t) = \big(1 - Z(t)\big), 0 < Z(t) < 1, \forall t \geq 0$.

The following is the rate of infected disease infection toward time $\kappa(t) = c\beta\,(1 - Z(t))I(t)$. Based on that explanation, the model of infectious disease transmission with information can be formulated as below:

$$\begin{aligned}
\frac{dS(t)}{dt} &= \Lambda - \big(\mu + \kappa(t)\big)S(t),\\
\frac{dE(t)}{dt} &= \kappa(t)S(t) - (\mu + \sigma)E(t),\\
\frac{dI(t)}{dt} &= \sigma E(t) - (\,\mu + \delta + \gamma)I(t), \qquad (1)\\
\frac{dR(t)}{dt} &= \gamma I(t) - \mu R(t),\\
\frac{dZ(t)}{dt} &= \frac{aI(t)}{(1 + bI(t))} - a_0 Z(t),
\end{aligned}$$

with initial condition:

$$S(0) > 0, E(0) > 0, I(0) > 0, R(0) > 0, Z(0) > 0.$$

Existence of the Positive Equilibrium

The system (1) has following equilibrium:

1. The disease-free equilibrium point $E_1\,(\frac{\Lambda}{\mu}, 0,0,0,0)$ always exists.
   The basic reproduction number R_0, which represents the expected average number of new disease infections produced by a single infected individual when in contact with a completely susceptible population. The basic reproduction number for system (1) is easily computed by van den Driessche [12]. The formula is given by:
   $$R_0 = \frac{\Lambda\sigma c\beta}{\mu(\mu + \sigma)(\mu + \delta + \gamma)}$$
2. The unique equilibrium point $E_2\,(S^*, E^*, I^*, R^*, Z^*)$ exists if $a < a_0 b$, where $S^* = \frac{\Lambda}{(\mu + c\beta(1 - Z^*)I^*)}$, $E^* = \frac{(\mu+\delta+\gamma)I^*}{\sigma}$, $R^* = \frac{\gamma I^*}{\mu}$, $Z^* = \frac{aI^*}{a_0(1+bI^*)}$ and I^* is the positive root of the following equation:
   $$g(I) = AI^2 + BI + C = 0,$$
   with
   $$A = \frac{1}{R_0}(a - a_0)\frac{\sigma\Lambda\, c^2\, \beta^2}{\mu},$$

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$$B = -\sigma\Lambda a_0 bc\beta\left(\frac{1}{R_0}-1\right) - c\beta a\sigma\Lambda - a_0\big((\mu+\sigma)(\mu+\delta+\gamma)\big),$$

$$C = -\sigma\Lambda\, a_0\, c\beta\left(\frac{1}{R_0}-1\right).$$

Note that $C > 0$ and $A < 0$ if $R_0 > 1$ and hence $g(I) = 0$ has a unique positive root if $R_0 > 1$. Thus the system (1) has a unique infected equilibrium E_2 if and only if $R_0 > 1$.

Stability of the Equilibrium

In this section, we investigate the local stability analysis of the system of nonlinear ordinary differential. The variational matrix J of the system (1) at any point (S, E, I, R, Z) is given by,

$$J(E) = \begin{pmatrix} -(\mu + c\beta(1-Z)I) & 0 & -c\beta(1-Z)S & 0 & c\beta IS \\ c\beta(1-Z)I & -(\mu+\sigma) & c\beta(1-Z)S & 0 & -c\beta IS \\ 0 & \sigma & -(\mu+\delta+\gamma) & 0 & 0 \\ 0 & 0 & \gamma & -\mu & 0 \\ 0 & 0 & \frac{a}{(1+bI)^2} & 0 & -a_0 \end{pmatrix}$$

The stability conditions of equilibrium E_1 and E_2 are stated in the following theorems.

Theorem 1. *The equilibrium point* $E_1(\frac{\Lambda}{\mu}, 0,0,0,0)$ *of system* (1) *is locally asymptotically stable if* $R_0 < 1$.

Proof. The stability of the disease-free equilibrium point can be determined by substituting equilibrium point E_1 in the Jacobian matrix of system (1), so that it is obtained

$$J(E_1) = \begin{pmatrix} -\mu & 0 & -\frac{\Lambda c\beta}{\mu} & 0 & 0 \\ 0 & -(\mu+\sigma) & \frac{\Lambda c\beta}{\mu} & 0 & 0 \\ 0 & \sigma & -(\mu+\delta+\gamma) & 0 & 0 \\ 0 & 0 & \gamma & -\mu & 0 \\ 0 & 0 & a & 0 & -a_0 \end{pmatrix}$$

The eigenvalues of $J(E_1)$ are $\lambda_1 = \lambda_2 = -\mu$, $\lambda_3 = -a_0$ and the other two eigenvalues $\lambda_{4,5}$are the roots of the following equation

$$\lambda^2 + \zeta_1\,\lambda + \zeta_2 = 0,$$

where $\zeta_1 = -(2\mu+\delta+\sigma+\gamma)$ and $\zeta_2 = (\mu+\sigma)(\mu+\delta+\gamma) - \frac{\Lambda c\beta\sigma}{\mu}$. E_1 will be locally asymptotically stable if $\lambda_{4,5} < 0$, which is $(\mu+\sigma)(\mu+\delta+\gamma) - \frac{\Lambda c\beta\sigma}{\mu} > 0$ that gives $\frac{\Lambda c\beta\sigma}{\mu(\mu+\sigma)(\mu+\delta+\gamma)} < 1$, where $\frac{\Lambda c\beta\sigma}{\mu(\mu+\sigma)(\mu+\delta+\gamma)}$ is basic reproduction number (R_0).

Theorem 2. *The equilibrium point* $E_2(S^*, E^*, I^*, R^*, Z^*)$ *of system* (1) *is locally asymptotically stable if* $\frac{\Lambda}{\mu} > S^*$, $\varphi_1\varphi_2 - \varphi_3 > 0$, *and* $(\varphi_1\varphi_2 - \varphi_3)\varphi_3 - \varphi_1(\varphi_1\varphi_4) > 0$.

Proof. The stability of the endemic equilibrium point can be determined by substituting the equilibrium point E_2 in the Jacobian matrix of system (1) as follows

$$J(E_2) = \begin{pmatrix} -(\mu + c\beta(1-Z^*)I^*) & 0 & -c\beta(1-Z^*)S^* & 0 & c\beta I^*S^* \\ c\beta(1-Z^*)I^* & -(\mu+\sigma) & c\beta(1-Z^*)S^* & 0 & -c\beta I^*S^* \\ 0 & \sigma & -(\mu+\delta+\gamma) & 0 & 0 \\ 0 & 0 & \gamma & -\mu & 0 \\ 0 & 0 & \frac{a}{(1+bI)^2} & 0 & -a_0 \end{pmatrix}$$

The eigenvalue of $J(E_2)$ is $\lambda_1 = -\mu$, and the other eigenvalues are roots of the following characteristic equation

$$\lambda^4 + \varphi_1\lambda^3 + \varphi_2\lambda^2 + \varphi_3\lambda + \varphi_4 \qquad (2)$$

where

$$\varphi_1 = (\mu+\sigma) + \frac{\Lambda}{S^*} + (\mu+\delta+\gamma) + a_0$$

$$\varphi_2 = \frac{\Lambda}{S^*}\big((\mu+\sigma) + (\mu+\delta+\gamma) + a_0\big) + (\mu+\sigma)(\mu+\delta+\gamma) + a_0\,(\mu+\sigma) + (\mu+\delta+\gamma)a_0 + \frac{\sigma}{I^*}(\Lambda - \mu\, S^*)$$

$$\varphi_3 = \left(\frac{\Lambda}{S^*}\right)((\mu+\sigma) + (\mu+\delta+\gamma) + a_0(\mu+\sigma) + (\mu+\delta+\gamma)a_0) + (\mu+\sigma)(\mu+\delta+\gamma)a_0 + \frac{a_0\sigma\Lambda}{I^*} + \frac{a\sigma c\beta\, I^*\, S^*}{(1+bI^*)^2} + \frac{\mu\sigma}{I^*}(\Lambda - a_0\, S^* - \mu\, S^*)$$

$$\varphi_4 = \left(\frac{\Lambda}{S^*}\right)(\mu+\sigma)(\mu+\delta+\gamma)a_0 + \frac{a\sigma\mu\xi_1}{(1+bI^*)^2} + \frac{a_0\sigma\mu}{I^*}(\Lambda - \mu S^*)$$

Clearly that $\varphi_1 > 0$, and $\varphi_4 > 0$ if $\frac{\Lambda}{\mu} > S^*$. By using Routh- Hurwitz criterion, the roots of equation (2) have negative real part if only if $\frac{\Lambda}{\mu} > S^*$, $\varphi_1\varphi_2 - \varphi_3 > 0$, and $(\varphi_1\varphi_2 - \varphi_3)\varphi_3 - \varphi_1(\varphi_1\varphi_4) > 0$ [13].

Sensitivity Analysis

The sensitivity index R_0 depends on the differentiation of the parameters $\Lambda, \sigma, c, \beta, \mu, \delta$ and γ. Suppose that the parameter values are given in Table 1.

Table 1. Parameters Value

No	Point	Description
1	Λ	0.5
2	σ	0.005
3	c	1
4	β	0.0005
5	μ	0.004
6	δ	0.1
7	γ	0.005

Based on the parameter values in Table 1, this value produces $R_0 = 5.787$ and a sensitivity index of R_0 is obtained as shown in the Table 2.

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Table 2. Index Sensitivity Parameters

No	Point	Description
1	μ	-1.474
2	Λ	1
3	β	1
4	c	1
5	γ	-0.499
6	δ	-0.1
7	σ	0.074

Table 2 shows the sensitivity level of the most sensitive parameters to less sensitive parameters namely mortality rate (μ), recruitment rate (Λ), transmission rate (β), number of contacts with infected individuals (c), recovery rate (γ), rate of death due to disease (δ), and the rate of exposed individual becomes infected individual (σ). The effect of changing the parameter value on the R_0 is presented in Table 3.

Table 3. Basic Reproduction Number

Parameter	R_0 Value	
	$P+10\%$	$P-10\%$
μ	6.747	5.021
Λ	6.365	5.208
β	6.365	5.208
c	6.365	5.739
γ	6.091	5.511
δ	5.729	5.845
σ	5.826	0.074

Table 3 shows that if one of the parameter values is increased or decreased by 10%, then the value of R_0 also increase or decrease. Therefore the endemicity of infectious diseases will increase or decrease.

Numerical Simulation

Numerical Simulation for $R_0 < 1$.

In this simulation, we use the parameter values Λ = 0.2, β = 0.0001, c = 10, δ = 0.01, γ = 0.5, μ = 0.005, σ = 0.08, a_0 = 0.06, a = 0.01, b= 1. Based on these parameter values, we have $R_0 = 0.0003655054255 < 1$ and $E_1\left(\frac{\Lambda}{\mu}, 0.0,0,0\right)$ = (40,0,0,0,0). According to analysis result, E_1 is a locally asymptotically stable since $R_0 < 1$. In Figure 2, it can be seen that with initial value (S(0),E(0),I(0),R(0),Z(0)) = 95,55,45,40,0.4 the numerical solution is convergent to the equilibrium point E_1. It indicates that there is no exposed and infected individuals for a long time.

Figure 3 shows a portrait phase of the solution in the SEI space with three different initial values (*S*(0), *E*(0), *I*(0), *R*(0), *Z*(0)) = (50,23,10,1,2), (*S*(0), *E*(0), *I*(0), *R*(0), *Z*(0)) = (15,20,1,0,0), and (*S*(0), *E*(0), *I*(0), *R*(0), *Z*(0)) = (30,12,10,10,1). The simulation results show that by using the three initial values, the orbits in the SEI space is stable to the disease-free equilibrium point (E_1).This is in accordance with the result of the analysis.

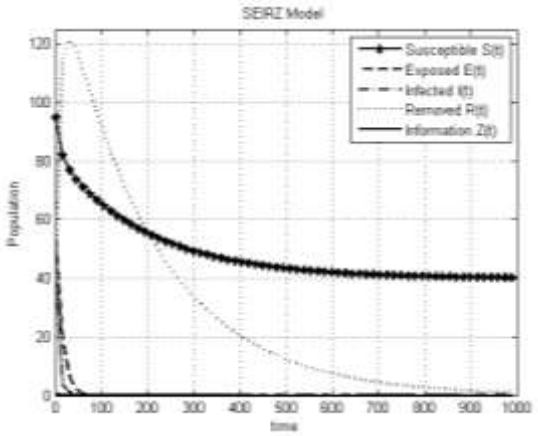

Figure 2. Solution of system (1) when $R_0 < 1$.

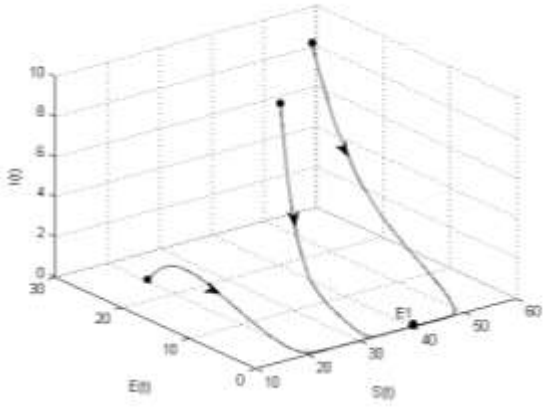

Figure 3. Portrait phase of the SEI space in disease-free equilibrium point

Numerical Simulation $R_0 > 1$.

In this simulation,we use the parameter values $\Lambda = 0.5, \beta = 0.0005, c = 1, \delta = 0.001, \gamma = 0.005, \mu = 0.004, \sigma = 0.05, a_0 = 0.0006, a = 0.000001, b = 1$,so that $R_0 = 5.787 > 1$.

Based on these parameter values, a disease-free equilibrium point and an endemic equilibrium point are obtained $E_1\left(\frac{\Lambda}{\mu}, 0.0,0,0\right) = (125,0.0,0,0)$ and $E_2(S^*, E^*, I^*, R^*, Z^*) = (21.635,7.656,38.283,47.854,0.0016)$. Furthermore, we have $\varphi_1\,\varphi_2 - \varphi_3 = 0.0002128670467 > 0$ and $(\varphi_1\varphi_2 - \varphi_3)\varphi_3 - \varphi_1(\varphi_1\varphi_4) = 3.3756 \times 10^{-9} > 0$, $\frac{\Lambda}{\mu} - S^* = 103.635 > 0$, that satisfy the stability condition. In Figure 4, it can be seen that numerical solution with an initial value $(100,50,60,30,0.3)$ is stable to the equilibrium point E_2.

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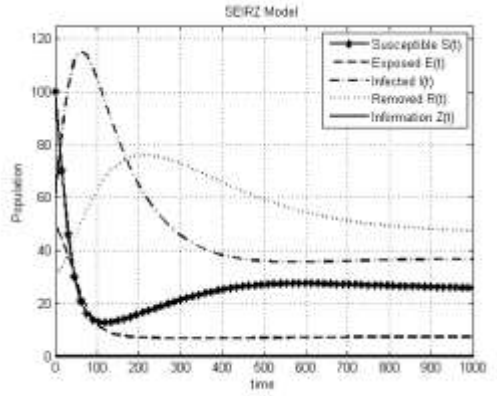

Figure 4. Solution of system (1) when $R_0 > 1$.

Figure 5 shows a portrait phase of the solution in the SEI space with three different initial values (*S*(0),*E*(0),*I*(0),*R*(0),*Z*(0)) = (40,2,5,50,0.5), (*S*(0),*E*(0),*I*(0),*R*(0),*Z*(0)) = (1,40,3,10,0), and (*S*(0),*E*(0),*I*(0),*R*(0),*Z*(0)) = (3,35,75,1000,0.5). The simulation results show that by using several different initial values, the orbits in the SEI space is stable to E_2. This shows that when $R_0 > 1$ the equilibrium point E_2 is locally asymptotically stable. However, a disease-free equilibrium points (E_1) it is unstable.

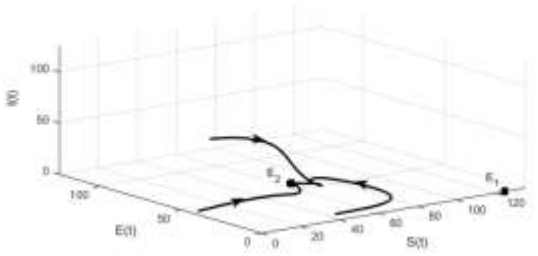

Figure 5. Portrait phase of the SEI space in endemic equilibrium point.

Numerical Simulation of Sensitivity Analysis

In conducting a sensitivity analysis, it is done by increasing or decreasing the one parameter value while the other parameter value is constant. Numerical simulation of parameters that influence the spread of disease to the population is presented in Figure 6 and 7.

In Figure 6, it is shown that by increasing the parameter value of μ by $10\,\%$ while the other parameters are fixed, the number of infected individuals will decrease. Conversely, by reducing the value of μ by $10\,\%$ the number of infected individuals will increase. Figure 7 shows that by increasing the parameter value of σ by $10\,\%$ while the other parameters are fixed, the number of infected individuals will also increase. Conversely, by decreasing the value of σ by $10\,\%$ the number of infected individuals will also decrease. The changing of these parameters will cause the changing of the value of the basic reproduction number. It means that the endemicity of infectious diseases is greatly affected by the rate of mortality and the rate of exposed individual becomes infected individual.

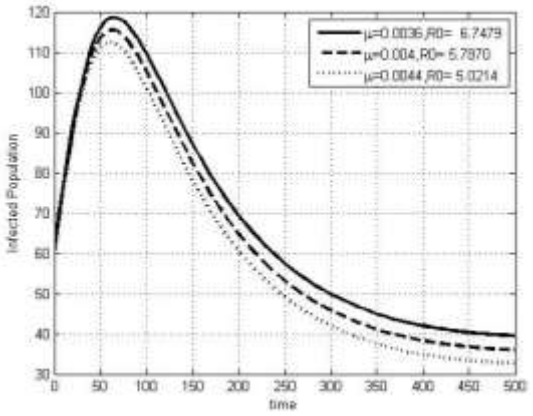

Figure 6. Graphs showing the effect of varying μ on infected population.

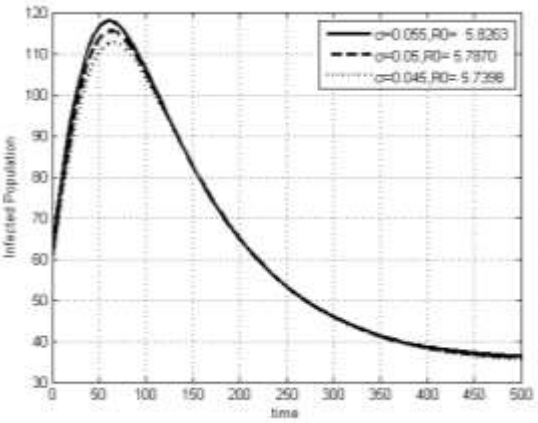

Figure 7. Graphs showing the effect of varying σ on infected population.

CONCLUSION

In this paper, we have studied a modified *SEIR* epidemic model with information. The model has two equilibrium, i.e. the disease-free equilibrium point and the endemic equilibrium point. The existence of the disease-free equilibrium point always exists. The endemic equilibrium point exists under certain condition. From the sensitivity analysis, it is obtained that the most sensitive parameters are the rate of mortality and the least sensitive parameter is the rate of an exposed individual becomes an infected individual. Numerical simulation agrees with the analysis result.

REFERENCES

[1] Buonomo, B., A. d'Onofrio, D. Lacitignola. 2008. Globally stable endemicity for infectious diseases with information-related

changes in contact patterns. *Appl. Math. Lett.* 25(7). 1056-1060.
[2] Cui, J., Y. Sun, H. Zhu. 2008. An SIS infection model incorporating media coverage. *Rocky Mountain J. Math.* 38(5).1323-1334.
[3] Kermack, W.O., A.G. Mc Kencdrick. 1972. A contribution to the mathematical theory of epidemics. Proceedings of the Royal Society of London. Series A, Containing Papers of a Mathematical and Physical Character. 115(772).
[4] Buonomo, B., A. d'Onofrio, D. Lacitignola. 2008. Global stability of an SIR epidemic model with information dependent vaccination, *Math. Biosci*. 216(1). 9-16.
[5] Cui, J., Y. Sun, H. Zhu. 2008. The impact of media on the control of infectious diseases. *J. Dyn. Differ. Equ.* 20(1). 31-53.
[6] Kumar, A., P.K. Srivastava, Y. Takeuchi. 2017. Modeling the role of information and limited optimal treatment on disease prevalence. *J. Theor. Biol.* 414. 103-119.
[7] Njankou, S.D.D., F. Nyabadza. 2017. Modelling the potential role of media campaigns in ebola transmission dynamics, *Int. J. Differ. Equ*. 1-13.
[8] Boyce, W.E., R.C. DiPrima. 2010. Elementary differential equations and boundary value problem, 10th Ed. John Wiley and Sons, Inc. USA.
[9] Braurer, F. 2004. Backward bifurcation in simple vaccination model. *J. Math. Anal. Appl.* 418-431.
[10] Hurint, R.U., Z.N. Meksianis, M. Lobo. 2017. Analisis sensitivitas model epidemi SEIR (in Indonesian). *J. Nat. Sci*. 6(1. 22-28.
[11] Chitnis, N., J.M. Hyman, J.M. Cushing. 2008. Determining important parameters in the spread of malaria through the sensitivity analysis of a mathematical model. *Bull. Math. Biol*. 70(5). 1272-1296.
[12] Van den Driessche, P., J. Watmough. 2002. Reproduction numbers and subthreshold endemic equilibrium for compartmental models of disease transmission, *Math. Biosci.* 180(1-2). 29-48.
[13] Murray, J.D. 2002. Mathematical biology I. an introduction, 3rd Ed. Springer-Verlag, Berlin Heidelberg.

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Phytoremediation of Lead-Contaminated Soil by Using Vetiver Grass (*Vetiveria zizanioides* L.)

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Abstract

Lead becomes a frequent problem in the environment, especially in the soil. However, there are plenty of plant species that can be used to overcome that problem. This study was aimed to analyze the effectiveness of Vetiver grass to reduce hyper-accumulate heavy metal such as lead (Pb) in the soil. Moreover, this study focused on the determination of heavy metals in the soil before plantation and after plantation of Vetiver. The method used was by planting the Vetiver grass in soil that was already treated by lead nitrate, lead chloride, and lead sulfate. Samples were collected from these treated soil every two weeks and samples of the vetiver grass were taken after one week for ten weeks. The result shows that Vetiver grasses are able to remove hyper-accumulate lead from the treated soil.

Key words: Heavy metals, hyper accumulator, Vetiver grass.

INTRODUCTION

Environmental pollution is an issued topic since 50 years ago. Water and soil are already polluted in some places. It is getting polluted in several ways. There is urgency in controlling soil pollution to maintain soil fertility and increase productivity. Pollution can be defined as undesirable changes in physical, chemical and biological parameters of water, air and soil quality affecting human life, the life of plants and other useful living animals, industrial process, the living conditions, and cultural assets. Pollutants are something that interferes with the health, comfort, property or community environment. Generally, most pollutants are introduced in the environment by waste, accidental disposal or by-products or residues from the production of something useful. Some valuable natural resources such as air, water, and soil are increasingly polluted [1,2].

Heavy metal pollution is now a main environmental concern because metal ions are not easily degraded in the environment due to their non-degradable properties. The tendency of heavy metal toxicity and bioaccumulation in the environment creates a chronic health problem of living organisms. Compared to organic contaminants, heavy metals are not possible to be diminished by chemical or biological processes. Therefore, converting into less toxic species is the only possible solution [3]. At low concentration, most heavy metals are toxic and can infiltrate the food chain, thereby accumulate in and inflict in hazard to living species. Every metal has a potential to indicate harmful impacts at higher concentrations and the toxicity of each metal relies on the quantity exposed to the organism, the absorbed dose, the route and the duration of exposure [4].

Each heavy metal has a 5 $g.cm^{-3}$ molecular mass which is higher than average particle density of soil (2.65 $g.cm^{-3}$). Some heavy metals, for example, cobalt, iron, zinc, manganese, copper, or molybdenum are necessary for the organism growth. Meanwhile, other heavy metal s function in peroxidase and nitrogenation by vanadium (V) or in hydrogenation by nickel (Ni). The rest of the heavy metals remain toxic to organisms including Lead, cadmium, Uranium, Chromium, Thallium, Mercury and Silver. Otherwise, Selenium (Se) and Arsenic (As) are not classified as heavy metals. However, since they partially possess toxicity characteristics similar to heavy metals, they are commonly called as metalloid in publications [5].

All soils contain heavy metals. No heavy metals, Pb, Zn, Cu, Ni, Cd, and Cr concentrations are between 0.0001 and 0.065%. Meanwhile, Manganese (Mn) and Iron (Fe) can range from 0.002% to 10.0%, respectively. With the exception of iron, in the soil, all heavy metals with the

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concentration of more than 0.1% are toxic to vegetation thereby altering the structure of plant communities in contaminated habitats. Nonetheless, every plant organism has a certain threshold level for every heavy metal which causes toxicity. Specifically, plants that are able to adapt to living on heavy metal soil are known as metallophytes. The zinc-rich soil (0.1-10.0% Zn) often contains high Pb content, but almost no Cd, whereas the soil with a high concentration of copper (Cu) ranging from 0.1 to 3.2% has other heavy metals in higher concentrations such as Pb, Zn, Ni, Co, and Cd [5].

Mining gives negative impacts on the environment, causing ecosystem damage and changes, including loss of biodiversity and accumulation of environmental pollutants. Processing of ore is a major source of heavy metal pollution in the soil, and the restoration of the ecosystem from mining activities can take several decades. This activity creates a huge quantity of irons and dumps, which are often left neglected. Abandoned mines pollute water from the release of chemicals and particulates accumulated in water sources thus creating the need to treat contaminated heavy metal wastes, prior to discharge into the environment [6].

Other factors that affect the existence and levels of heavy metals in soils include the degree of complications with the ligand, density and type of charge in the soil colloids, and the relative surface area of the soil. The large interfaces and specific surface areas provided by the soil colloids help control heavy metal concentrations in natural soils. In addition, the soluble concentration of metals in contaminated soils can be reduced by soil particles with high specific surface area, although this may be metal specific. For example, McBride and Martinez reported that the addition of an amendment consisting of hydroxides with a highly reactive surface area reduces the solubility of Cu, Mo, As, Cd, and Pb, meanwhile Ni and Zn remained insoluble. Soil aeration, microbial activity, and mineral composition have been reported to affect the level of heavy metals in the land [7].

The contamination of heavy metal in soil has become common throughout the world due to the increasing geological and anthropogenic activity. Plants growing on this soil show a decline in performance, growth, and harvest. Bioremediation becomes a suitable method to treat contaminated soils of heavy metals. This is a widely accepted method mostly done in situ. It is particularly suitable for the establishment/re-establishment of plants on the treated soil. Microorganisms and plants use different mechanisms for bioremediation of contaminated soil. Using plants for the treatment of contaminated soil is a more general approach in bioremediation of contaminated soils of heavy metals [8,9].

Combining both microorganisms and crops is an approach to bioremediation that ensures more efficient cleaning of contaminated soils of heavy metals [10]. However, the success of this approach is highly dependent on the species of organisms involved in the process [7].

Phytoremediation is a technology that applies plants to clean pollutants in the environment. Benefits of phytoremediation include being an economical, energy-efficient and environmentally-friendly method, having been able to be used in a large area, and being useful to resolve various contaminants (metals, organic substances or radionuclides) and the media of the planting activities such as mud, soil, water or sediment). Phytoremediation can be applied into many implementation methods which include phytoextraction, in which the soil is decontaminated by the plants to the air part through the process of heavy metal uptake and then the plants are harvested and dislocated from the site. Phytostabilization is a process of minimizing the mobility of heavy metals in contaminated soil by the plants. And Phytovolatilization, in which plants extract volatile metals from the soil and vaporize them from the leaves [11,12].

A hyper accumulator means a plant that has the ability to grow on soil contaminated with heavy metals. Not only able to grow, the plant can also absorb heavy metals without occurring symptoms of phytotoxicity. The base type for the active hyper accumulator plant is in taking a substantial quantity of heavy metals from the soil. Moreover, heavy metals are translocated but are translocated to shoots and accumulated above the soil organ, especially the leaves without storing in the roots. Despite the different features, hyper accumulation also depends on hyper-tolerance which is an essential key property preventing heavy metal poisoning in plants. Around 450 species of

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angiosperm have been identified as far as heavy mortgage accumulators [13].

Vetiver comes from India and has been used traditionally since ancient times due to perfume obtained from roots. This planting effort of this plant species to rehabilitate the land using vetiver was started in 1956 by the National Botanical Garden, Lucknow (it is known as the National Botanical Research Institute) which shows that vetiver grass has an amazing ability. Those plant could help improving soil fertility and facilitate groundwater filling. Since then these have been made to implement vetiver plant in land reclamation and conservation by the support of state government in various parts of India. The World Bank also initiated several projects in India in the 1980s for the systematic development of Vetiver Grass Technology (VGT), now known as Vetiver System (VS) for watershed management, soil conservation and slope stabilization [14]. This research is aimed to analyze the effectiveness of Vetiver grass to reduce hyper-accumulate heavy metal such as lead (Pb) in the soil from Batu City.

MATERIALS AND METHODS

This research used a descriptive quantitative approach with experimental method since descriptive research relies on observation as a means of collecting data. It attempts to examine situations in order to establish what the norm is, i.e. what happen again under the same circumstances [15]. The descriptive research tries to systematically describe a problem, service, situation, phenomenon or program, or gives fix information or describes attitudes towards an issue. Based on Hyman [16], generally, in descriptive surveys, the aim is to study a large and heterogeneous population. Moreover, quantitative research is conducted according to the quantity or amount of measurement. It can be implemented to phenomena which are possible to be described in quantity [17].

Materials

The experiment was conducted from September to November 2017. The soil sample was taken from Batu City. The materials used in research were soil, vetiver grass, tap water, distilled water, and lead chloride. The types of equipment used in this study are Sieve, a standard flask with a capacity of 1000 mL, sensitive balance, and nomination paper. Plastic bags were also used in the research.

Research Procedure

Three mol of lead chloride (Pb Cl) solution and six kilograms of soil were mixed and then were distributed into three plastic bags. The 45 gram of vetiver grass were planted in each plastic bag. Then about 45 grams vetiver grass planted per basin. The soil samples were taken and analyzed every two weeks. In the same time, samples of the vetiver grass were taken after a week, five weeks and ten weeks. The overall duration of this experiment was ten weeks.

The artificial treatment of heavy metals was prepared based on the range of heavy metal concentrations submitted by the Council of Canadian Ministry of the Environment [18] and the Department of Environment, Malaysia [19].

RESULTS AND DISCUSSION

Lead Compound in Soil

Figure 1 shows that the difference in observation times influence or has different effects on the Pb Cl contain in the Soil. The effect of vetiver grass is starting to shown at the observation times where the heavy metal-induced soil has decreased Pb Cl after 2 weeks. However, at the last observation time (4 weeks) no further decreasing Pb Cl in the Soil, compared to the 2 weeks observation. We assumed that it shows the ability of vetiver grass to reach its maximum level to remediate the heavy metal. This result was similar to the results of the previous study whereby the end of four days, most of the heavy metals had already been taken by plants [20].

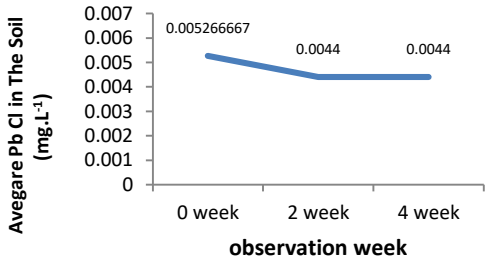

Figure 1. Concentration of Lead Compound in the Soil

Another research result showed heavy metals in soil, even at the level above critical value to plant growth, had no negative effect on vetiver growth [21,22]. Another study showed that some metals

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such as lead are largely immobile in soil and their extraction rate is by solubility and diffusion through the root surface [23].

Lead Compound in Vetiver Grass

Along observation times, different concentration of Pb Cl in the Vetiver grass was measured. The changes in the Pb Cl concentration started to appear after 5 weeks. After 1 week dap, the Pb Cl in the vetiver increased, showing the absorption of Pb Cl by the vetiver. Similar to the concentration of Pb Cl in the soil, Pb Cl in the vetiver grass for the observation in 10^{th} week is at the same concentration, i.e. 0.0044 $mg.L^{-1}$, which means no more decreasing of Pb Cl concentration.

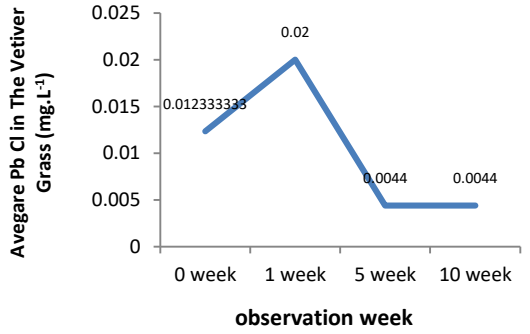

Figure 2. Concentration of Lead Compound in the Vetiver Grass

Truong [24] presented a similar result on his research that the vetiver grass will take Pb slowly and continuously for long periods. Similar findings were also observed previously [25], which reported that vetiver grass showed high tolerance to a very adverse condition, thereby making it possible to be employed for mine rehabilitation, tailings industrial waste dumps and garbage landfills that usually contain high acid or alkaline levels and possess high degree of heavy metals, while having low plant nutrients. Another study explained the most conspicuous characters of vetiver grass includes its fast growth, large biomass, strong root system, and high level of metal tolerance [26]. Therefore, vetiver grass is an important candidate for stabilization of metal-contaminated soils. Truong [21] also demonstrated that vetiver is highly tolerant to many heavy metals.

Limitation and Recommendation

This research was expected to give some parts of recommendation for phytoremediation study in the future, which meant to focus more on analyzing the effects of heavy metals on human health and the use of plants for the treatment of these biomaterials. The government is expected to take steps to reduce soil and environmental pollution from harmful contaminants such as non-dumping plant remnants to the ground.

The complexity of factors that control the efficiency of these techniques, such as soil properties, plant species and climatic conditions, courage the fact that more research needs to be done. More species that have remediation capabilities need to be identified, especially crops that can contribute to the social and economic development of local people, such as industrial species. Also, in the future, research should focus on developing agricultural techniques to improve phytoremediation efficiency and reduce the time and cost of heavy metal removal from the soil. The success of some industrial residues to increase heavy metal phytoavailability can be investigated. An important advantage of fragrant roots (such as in the vetiver grass) is that it is not considered to be a hazardous waste that can be safely used for bioenergy production, compost or even as a craft material.

CONCLUSION

Phytoremediation is a promising green technology that can be used to remediate heavy metal-contaminated soil. In developing countries, this technology can provide low-cost solutions to recover contaminated areas, especially abandoned industrial sites (mines and garbage dumps). The results of experiments show that the vetiver of the ecotype has a high potential for absorbing heavy metals contaminated in the soil. Although vetiver is not an accumulator, it has proven to be extremely tolerant of very adverse land conditions. Therefore, vetiver is possible to be applied for mine rehabilitation, tailings mining and industrial waste that is often very acidic or alkaline in heavy and low metals in plant nutrients. According to these experimental results, the lead (Pb Cl) remaining on the soil gradually begins to decrease as the increased contact time of the experiments proves the efficiency of the plant in phytoremediation even in high concentrations of heavy metals.

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REFERENCES

[1] Ashraf, M.A., M.J. Maah, I. Yusoff. 2014. Soil contamination, risk assessment and remediation. In: Soriano, M.C.H. (Ed). Environmental Risk Assessment of Soil Contamination. 3-56. DOI: 10.5772/57287.

[2] Leksono, A.S., B. Yanuwiadi. 2014. The Effect of bio and liquid organic fertilizer on weight and quality of apple. *Int. J. Agron. Agr. Res*. 5(5). 53-58.

[3] Ayangbenro, A.S., O.O. Babalola. 2017. A new strategy for heavy metal polluted environments: a review of microbial bio-sorbents. *Environ. Res. Public. Health*. 14(1). E94.

[4] Mani, D., C. Kumar. 2014. Biotechnological advances in bioremediation of heavy metals contaminated Ecosystems: An overview with special reference to phytoremediation. *Int. J. Environ. Sci. Technol.* 11. 843–872.

[5] Bothe, H. 2011. Plants in heavy metal soils. In: Detoxification of heavy metals Springer Berlin Heidelberg. 35-57.

[6] Adler, R.A., M. Claassen, L. Godfrey, A.R. Turton. 200. Water, mining and waste: An historical and Economic perspective on conflict management in South Africa. *Econom. Peace Secur. J.* 2. 32-41.

[7] Chibuike, G.U., S.C. Obiora. 2014. Heavy metal polluted soils: effect on plants and bioremediation methods. *Appl. Environ. Soil Sci.* 1-12.

[8] Oktaviani, R., B. Yanuwiadi. 2016. Analisis vegetasi riparian di tepi Sungai Porong, Kabupaten Sidoarjo. *Jurnal Biotropika*. 4(1). 25-31.

[9] Kurniawan, H., B. Yanuwiadi, S. Soemarno. 2016. Persepsi masyarakat malam (studi kasus di Dusun Rembang II Desa Rembang Kec. Rembang Kab. Pasuruan). *Jurnal Pembangunan dan Alam Lestari*. 7(1). 76-85.

[10] Oktaviani, R., B. Yanuwiadi. 2016. Persepsi Masyarakat Terhadap Konservasi Spesies Riparian di Tepi Sungai Porong, Kabupaten Sidoarjo. *Jurnal Biotropika*. 4 (3): 81-87.

[11] Cunningham, S.D., W.R. Berti, J.W.W. Huang. 1995. Phytoremediation of contaminated soils. *Trends Biotechnol.* 13. 393-397.

[12] Wantouw, S., Antariksa, B. Yanuwiadi, Z. Tamod. 2014. Perception and participation on co-management of green open space in coastal reclamation area Manado. *Int. J. Appl. Sociol*. 4(4). 108-113.

[13] Rascio, N., F.N. Izzo. 2011. Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? *Plant Sci.* 169-181.

[14] Lavania, U.C. 2008. Vetiver in India: historical perspective and prospective for development of specific genotypes for environmental or industrial application. In: Truong, P. (Ed). Proceeding of 1st Indian Vetiver Workshop – Vetiver System for Environment Protection and National Disaster Management, Cochin, India. 40-47.

[15] Walliman, N.S.R. 2001. Your research project: a step-by-step guide for the first-time researcher. Sage Publications, Ltd. London.

[16] Hyman, H.H. 1955. Survey design and analysis: principles, cases, and procedures. Free press.

[17] Kothari, C.R. 2004. Research methodology: methods and techniques, 2nd Ed. New Age International Publishers.

[18] CCME. 1999. Canadian environmental quality guidelines. Canadian Ministry of the Environment. Canada.

[19] DOE. 2009. Guidance document on Health Impact Assessment (HIA) in Environmental Impact Assessment (EIA). Department of Environment, Malaysia.

[20] Lim-Suelee, A., S.N.M. Syed-Hasan, F. Mohd-Kusin, F. Mohamat-Yusuff, Z. Zaiton-Ibrahim. 2017. Phytoremediation potential of vetiver grass (*Vetiveria zizanioides*) for treatment of metal-contaminated water. *Water Air Soil Pollut.* 228(4). 158.

[21] Truong, P.N.V. 1999. Vetiver grass technology for mine rehabilitation. PRVN Technical Bulletin No. 1999/2. Office of the Royal Development Projects Board, Bangkok, Thailand.

[22] Roongtanakiat, N., P. Chairoj. 2001. Uptake potential of some heavy metals by vetiver grass. *Kasetsart. J. Nat.Sci.* 35.46-50.

[23] Lombi, E., F.J. Zhao, S.J. Dunham, S.P. McGrath. 2001. Phytoremediation of heavy metal-contaminated soils: natural hyper-accumulation versus chemically enhanced

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E-ISSN. 2338-1655

phytoextraction. *J. Environ. Qual.* 30. 1919-1926.

[24] Truong, P.N.V. 2000. The global impact of vetiver grass technology on the environment. In: Proceedings of 2nd international Vetiver Conference, Thailand. 46-57.

[25] Roongtanakiat, N., P. Chairoj. 2001. Vetiver grass for the remediation of soil contaminated with heavy metals. *Kasetsart J. Nat. Sci.* 35. 433-440.

[26] Raskin, I., B.D. Ensley. 2000. Phytoremediation of toxic metals. John Wiley and Sons, Inc. New York.

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Protein Structure and Function Analysis Method of Aminoacyl-tRNA Synthetase Cofactor and Biotinylation Effect: Journal Review

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Abstract

Protein has been known as an important macromolecule which has a vital role among the living organism. One of the most interesting protein is Arc1p, which is a yeast-specific tRNA-binding protein. Arc1p is a unique protein that has the ability to form a ternary complex with glutamyl-tRNA synthetase ($GluRS_c$) and methionyl-tRNA synthetase (MetRS) in the cytoplasm. This complex can significantly enhance the aminoacylation efficiency of these two aaRSs to their respective cognate tRNAs. Recently, it was found that Arc1p can be biotinylated via post-translational modification at Lys86 (K86) in the N-domain. Here, we try to figure it out what kind of method that will help to create some clear information both in structure and function of this protein, when mutations occur inside of the K86 site within SSKD motifs of Arc1p. Several methods to better understanding obviously about protein characteristics comprises protein structural analysis; such as gel mobility shift assay, CD Spectroscopy, and limited proteolysis; protein functional analysis, and *in silico modeling*.

Keywords: Arc1p, biotinylation, function, *in silico*, structure.

INTRODUCTION

Protein is an important macromolecule which has an indispensable role in the widely living organism. Analysis of protein became a very important step in biochemistry and biomolecular study. Extensive application from this analysis will be more helpful to understand both structure and function. For example, is protein Arc1. Arc1p (Aminoacyl-tRNA Synthetase Cofactor 1) is a yeast-specific tRNA-binding protein that has the ability to make a ternary complex with glutamyl-tRNA synthetase ($GluRS_c$) and methionyl-tRNA synthetase (MetRS) in the cytoplasm. This complex can significantly enhance the aminoacylation efficiency of these two aaRSs to its cognate tRNAs. Recently, Arc1p can be biotinylated via post-translational modification at Lys86 (K86) in the N-domain. Lysine itself commonly plays an important role in the structure [1]. Identification of Arc1p as a novel biotinylated yeast protein became very intriguing to find since Arc1p is not known to be involved in any carboxylation reaction (carboxylation, decarboxylation, or transcarboxylation reactions) [2].

Furthermore, in Arc1p a lack of sequence similarity was reviewed between the biotin binding site and the highly conserved biotin binding consensus sequence of known carboxylases, AMKM [3]. Mostly biotinylated lysine residues are positioned within this consensus. However, Arc1p may still be biotinylated in vivo. One interesting thing is that biotinylation of Arc1p was performed by the same enzyme which also catalyzes some yeast carboxylases, Bpl1p. SSKD in Arc1p may represent a secondary biotinylation site for Bpl1p [3]. In this review, we try to figure it out what kind of method that will help to create some clear information both in structure and function of this protein, when mutations occur inside of the K86 site within SSKD motifs of Arc1p.

PROTEIN STRUCTURE ANALYSIS METHOD

Streptavidin-based Gel Mobility Shift Assay

Gel mobility shift assay is a usual and common tool in molecular biology to check characteristic of the protein. In this case, Arc1p known has biotinylation activity [3]. Thus, in order to measure the relative biotinylation levels of Arc1p variants from WT and yeast mutants, a streptavidin-based gel mobility shift assay was determined [4]. This phenomenon was based on due to the almost irreversible binding of biotin with streptavidin ($K_d = 10^{-14}$ M) [5]. This binding is one of the strongest interactions between noncovalent molecules. Highest known affinity in nature between these two molecules has been largely applied as a powerful tool for diagnostic purposes [6].

Besides streptavidin, avidin also has the ability to binding with biotin. Moreover, there

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was research of 3D structures of both avidin and streptavidin to compare their binding properties. This research exhibit that both proteins have a conserved tyrosine-containing stretch (Fig. 1) [6,7] The difference between avidin and streptavidin is the lengths of the loops that happened when binding of biotin, 36-44 in avidin and 45-50 in streptavidin, respectively. As consequences, the longer loop of avidin causes tighter closure of the occupied binding site [6]. Recently reported that avidin has a tighter binding of biotin than streptavidin (K_d =6 x 10^{-16} and 4 x 10^{-14} M, respectively) [8].

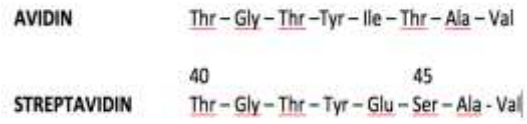


Figure 1. Conserved sequences of avidin and streptavidin tyrosine-containing stretches [7]

Circular Dichroism (CD) Spectroscopy

Circular Dichroism (CD) Spectroscopy is well known as a common method in order to study and analyze the secondary structure or conformation changes of macromolecules, in which sensitive to its environment, temperature, pH, or interaction with other molecules. An important concept of CD spectroscopy was that this is a result of the interaction of polarized light with chiral molecules since most biological molecules are chiral (Fig. 2). The best example is 19 of the 20 common amino acids that form proteins are themselves chiral, along with the higher structures of proteins, like DNA and RNA [9]. Recently, most widely applied of circular dichroism are for study the secondary structure of proteins, such as the α-helix and the β sheet (Fig. 3).

Moreover, this method allows us to explore deeper the higher order structures of chiral macromolecules including proteins and DNA. Importantly, in CD spectrum, the structure of a DNA molecule or protein is not a total of the CD spectra from the individual residues or bases but is extremely influenced by the 3-dimension structure of the macromolecule itself. This could be because each structure from a macromolecule has a specific circular dichroism characteristic, and allow to identify structural elements at once the structure changes of chiral macromolecules. Principally, the CD is an inference from the interaction among polarized light with chiral molecules [9].

In this case, CD Spectroscopy was used in order to investigate whether biotinylation affects the secondary structure of Arc1p variants. Using purified Arc1p, the secondary structure changes of Arc1p, eventually revealed. Protein structure can be determined by CD Spectroscopy in the far UV, which is an α-helix has the negative band at 222 and 208 nm, and β-sheet has a negative band at 218 nm. Also, CD Spectroscopy conducted to compare the thermal stabilities of Arc1p variants [4].

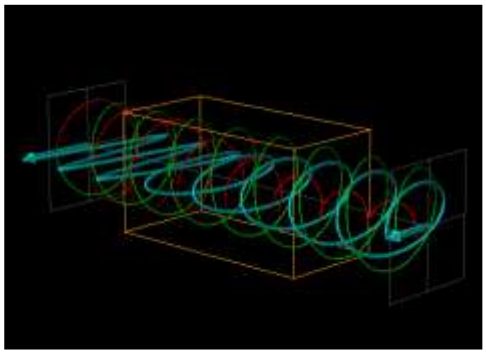
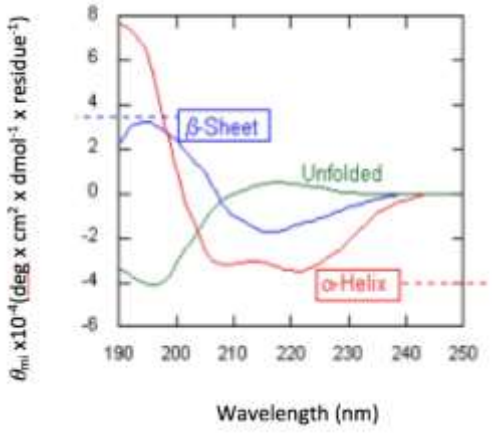


Figure 2. The principle of circular dichroism (orange cuboid represents the sample) [9].



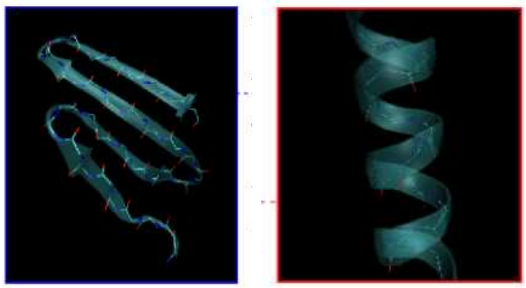


Figure 3. The secondary structure conformation and the CD spectra of protein structural elements. Upper is the associated CD spectra. Bottom left (blue outline) is a β-sheet and the bottom right (red outline) is an α-helix [9].

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Limited Proteolysis

Limited Proteolysis was used to check and analyze structure conformational changes of the protein [10,11]. The main concept of this method is the incubation of protein with one or several types of protease at various time. Subsequently, this enzyme will be cut at recognition sites inside the protein (Fig. 4) [12,13]. Proteolysis of a protein substrate specifically occur only if the polypeptide chain has the ability to bind and adapt to the specific stereochemistry of protease's active site [14]. We can change some variables, such as type of protease, dilution of protease, temperature and time of incubation. After digestion by protease, identification of cleavage products could be identifying by SDS-PAGE. Digestion of full-length protein can be detected by the lower molecular weight bands, together with intensity and appearance of bands can be observed [13].

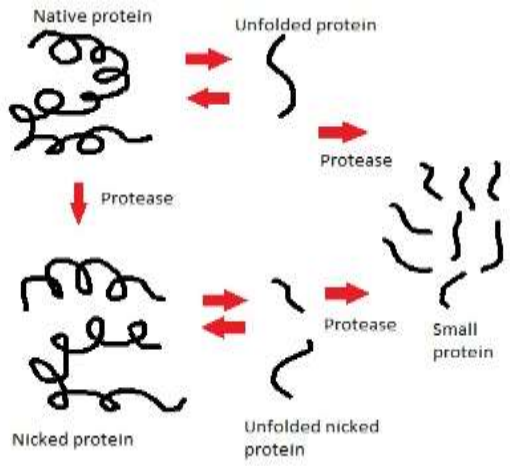

Figure 4. Principle of Limited Proteolysis [17]

Various application of limited proteolysis has been discovered. This method was very useful in protein study such as crystallization studies, or in situ proteolysis for protein crystallization and structure determination; like to expect the presence of stable sub-domains [12]. Limited proteolysis also can be used as a surface probe for membrane proteins [15]. Besides, limited proteolysis also able to detect binding between one protein to another, in a way by running two identical sets of proteolysis experiments, with and without the putative target. The expected result will exhibit by the rate of digestion of the protein. When it is slowed by the addition of the putative target, one can take finding that they interact with each other [12,16].

In the case of Arc1p, limited proteolysis can be used in order to test whether biotinylation will alter the structural flexibility of Arc1p. Limited proteolysis with Arc1p/trypsin using is a ratio of 1,000:1 and conducted at 30 and 37°C. They were using 0, 8, 16, and 16 minutes of time [3]. After the treatment of trypsin, the result will be obtained which Arc1p is more flexible in term of structure than the others.

PROTEIN FUNCTION ANALYSIS METHOD

Complementation Assay

Arc1p has become one of the most intriguing research topics in recent years. Arc1p is an auxiliary protein of the MARS complex, which also consists of two aaRSs, GluRS and MetRS. This protein is also known as a homolog of human p43. Arc1p has a key role in some cellular activities, such as in multiple cellular pathways, tRNA channel, and nucleocytoplasmic transport [2,18].

Complementation assay generally used to test the rescue activity of knock out strain. This method could be conducted to analyze mitochondrial or cytoplasm activity. Selective medium for cytoplasm and mitochondrial activity were using 5-fluoroorotic acid (5-FOA) and YPG medium, respectively [19]. In the case of Arc1p, complementation assay was held in order to investigate whether biotinylation of Arc1p will affect the activity in vivo. Genes encoding the *wild type* and mutant of Arc1p were respectively cloned in pADH vector (high-copy number yeast shuttle vector with a constitutive *ADH* promoter and a *LEU2* marker). Subsequently, the resulting constructs were tested in an *arc1*$^{-}$ yeast strain at 20ºC, 30ºC, and 37ºC respectively in SD/-Leu plates [3]. Using this method, we can conclude whether biotinylation is needed for rescue activity of Arc1p.

***IN SILICO* MODELING**

There was some method related to structure analysis of protein. One kind of method is *in silico* modeling. *In silico* modeling methods are computational-based approaches to analyze and simulate the macromolecules, include protein. In this method we also able to predict and visualize molecule. Recently, *in silico* modeling is often used in combination in vivo analysis [20]. Moreover, *in silico* tool also widely used in pharmacology, toxicology, and drugs. For example, this method has been developed to predict all drugs activity and reaction once they get in into the human body or certain organism [21].

In this case, Arc1p can be analyzed by interaction with another molecule, such as GluRS

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and MetRS. As we know, Arc1p is an auxiliary protein of the MARS complex, which also consists of two aaRSs, GluRS and MetRS [2]. Based on a recent experiment, the catalytic efficiency of Arc1p-GluRS increase by 100-fold for its cognate $tRNA^{Glu}$ [22]. Using *in silico* modeling, we allowed to analyzing chemical and biological properties in Arc1p to represents within software tools to predict.

CONCLUSION

Arc1p defined as yeast-specific tRNA-binding protein and has the primary ability to make a ternary complex with glutamyl-tRNA synthetase ($GluRS_c$) and methionyl-tRNA synthetase (MetRS) in the cytoplasm [3]. This complex's main function is to enhance the aminoacylation efficiency of both, $GluRS_c$ and MetRS, for their cognate tRNAs. Specifically, Arc1p acts as a tRNA-attracting molecule thus enhancing tRNA availability for these two enzymes [5,23]. Although Arc1p lacks a highly conserve biotinylation consensus and does not engage in any biotin-dependent carboxylases reaction, Arc1p still has biotinylation activity, even when modified by Bpl1p in yeast [3,24]. One another interesting thing is that Arc1p is only biotinylated by Bpl1p, while other biotin protein ligases, such as HCS in mammals and BirA in *E. coli*, do not act on Arc1p as a substrate. It has recently been known that a high degree of similarity among biotin binding domains makes broad substrate specificity. Since it is known that biotin protein ligase has the ability not only to biotinylate different in the apoenzyme in the same organism but even came from different organisms, this finding is remarkable. Like in previous reports, the SSKD motif may represent a secondary biotinylation site for yeast Bpl1p [25]. Despite having very vital functions, *arc1Δ* mutants are not lethal. This is caused by the operation of a second, exportin Los1p which is required by aminoacylation-independent nuclear tRNA export pathway in yeast [26]. Lethality in yeast only occurred when inactivation of these two genes, *ARC1* and *LOS1* occurred simultaneously, resulting in no additional tRNA export pathway existing in yeast [18,23].

In this review, we could see some method which usually uses in the study of the structure and function of a protein. Based on the description above, Arc1p is likely to remain one of the interesting research objects in the biomolecular field. Some of the methods described above will open up new knowledge regarding the characteristics of this protein. Subsequently, from the results of the structure and function analysis, may be able to draw conclusions, why Arc1p has very unique roles and characteristics. This may lead to a better understanding of several methods of a complete view related to protein attributes. Thus, our review about some methods in the study of protein characteristics, especially in Arc1p, may only represent a little in fact that nowadays technology and molecular techniques are absolutely developing rapidly.

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REFERENCES

[1] Betts, M.J., B.R. Robert. 2003. Amino acid properties and consequences of substitutions. In: Barnes, M.R., I.C. Gray (Eds). Bioinformatics for Geneticists. John Wiley and Sons, Ltd.

[2] Edvardson, S., A. Shaag, O. Kolesnikova, J.M. Gomori, I. Tarassov, T. Einbinder, O. Elpeleg. 2007. Deleterious mutation in the mitochondrial arginyl-transfer RNA synthetase gene is associated with pontocerebellar hypoplasia. *Am. J. Hum. Genet.* 81. 857-862.

[3] Yao, C.C., C. Chia-Pei, C. Shruti, W. Shao-Win, T. Yi-Kuan, W. Chien-Chia. 2016. Modulating the structure and function of an aminoacyl-tRNA synthetase cofactor by biotinylation. *J. Biol. Chem.* 291(33), 17102-17111.

[4] Nathanson, L., M.P. Deutscher. 2000. Active aminoacyl-tRNA synthetases are present in nuclei as a high molecular weight multienzyme complex. *J. Biol. Chem.* 275. 31559-31562

[5] Frechin, M., K. Daniel, P.M. Robert, D.B. Hubert, S. Bruno. 2010. Arc1p: Anchoring, routing, coordinating. *FEBS Lett.* 584. 427-433.

[6] Livnah, O., A.B. Edward, W. Meir, L.S. Joel. 1993. Three-dimensional structures of avidin and the streptavidin-biotin complex. *Proc. Natl. Acad. Sci*. *USA.* 90. 5076-5080.

[7] Gitlin, G., A.B. Edward, W. Meir. 1990. Studies on the biotin-binding sites of avidin and streptavidin. *Biochem. J.* 269. 527-530.

[8] Green, N.M. 1990. Avidin and streptavidin. *Methods Enzymol*. 184. 51-67.

ISSN. 2087-2852
E-ISSN. 2338-1655

[9] Dmitry. 1999. An introduction to circular dichroism spectroscopy. Available at: https://www.chem.uci.edu/.
[10] Fontana, A., P.P. de Laureto, V. De Filippis, E. Scaramella, M. Zambonin. 1997. Probing the partly folded states of proteins by limited proteolysis. *Folding Design.* 2(2). R17-R26.
[11] Hubbard, S.J. 1998. The structural aspects of limited proteolysis of negative proteins. Biochim. Biophys. *Acta.* 1382(2). 191-206.
[12] Moldoveanu, T., C.M. Hosfield, Z. Jia, J.S. Elce., P.L. Davies. 2001. Ca^{2+}-induced structural changes in rat *m*-calpain revealed by partial proteolysis. *Biochim. Biophys. Acta.* 1545(1-2). 245-254.
[13] Quevillon-Cheruel, S., B. Collinet, L. Trésaugues, P. Minard, G. Henckes, R. Aufrère, K. Blondeau, C.Z. Zhou, D. Liger, N. Bettache, A. Poupon, I. Aboulfath, N. Leulliot, J. Janin, H. van Tilbeurgh. 2007. Cloning, production, and purification of proteins for a medium-scale structural genomics project. *Methods Mol. Biol*. 363. 21-37.
[14] Schechter, I., A. Berger. 1967. On the size of the active site in proteases. I. Papain. *Biochem. Biophys. Res. Commun.* 27. 157-162.
[15] Hargrave, P.A., S.L. Fong, J.H. McDowell, M.T. Mas, D.R. Curtis, J.K. Wang, E. Juszczak, D.P. Smith. 1980. The partial primary structure of bovine rhodopsin and its topography in the retinal rod cell disc membrane. *Neurochem. Int.* 1. 231-244.
[16] Chazin Lab. 2002. Chazin Lab protocol for limited proteolysis. Available at: http://structbio.vanderbilt.edu/chazin/wisdom/labpro/proteolysis.html
[17] Fontana, A., P. de L. Patrizia, S. Barbara, F. Erica, P. Paola, M. Zambonin. 2004. Probing protein structure by limited proteolysis. CRIBI Biotechnology Centre, University of Padua, Padua, Italy.
[18] Ibba, M., D. Soll. 2000. Aminoacyl-tRNA synthesis. *Annu. Rev. Biochem*. 69. 617-650.
[19] Chang CY., C.I. Chien, C.P. Chang, B.C. Lin, C.C. Wang. 2016. WHEP domain regulates the dynamic structure and activity of *Caenorhabditis elegans* glycyl-tRNA synthetase. *J. Biol. Chem.* 291(32). 16567-16575
[20] Myatta, Glenn J. et al. 2018. In silico toxicology protocols. *Regul. Toxicol. Pharmacol.* 96. 1-17.
[21] Pinero, J., I.F. Laura, S. Ferran. 2018. In silico models in drug development: where we are. *Curr. Opin. Pharmacol.* 42.111-121.
[22] Graindorge, J.S., B. Senger, D. Tritch, G. Simos, F. Fasiolo. 2005. Role of Arc1p in the modulation of yeast glutamyl-tRNA synthetase activity. *Biochemistry.* 44. 1344-1352.
[23] Simos, G., A. Sauer, F. Fasiolo, E.C. Hurt. 1998. A conserved domain within Arc1p delivers tRNA to aminoacyl-tRNA synthetases. *Mol. Cell.* 1. 235-242.
[24] Beckett, D. 2007. Biotin sensing: universal influence of biotin status on transcripstion. *Annu. Rev. Genet.* 41. 443-464.
[25] Copley, R.R., G.J. Barton. 1994. A structural analysis of phosphate and sulphate binding sites in proteins. Estimation of propensities for binding and conservation of phosphate binding sites. *J. Mol. Biol.* 242. 321-329.
[26] Sarkar, S., A.K. Hopper. 1998. tRNA Nuclear Export in Saccharomyces cerevisiae: In Situ Hybridization Analysis. *Mol. Biol. Cell*. 9(11). 3041-3055.

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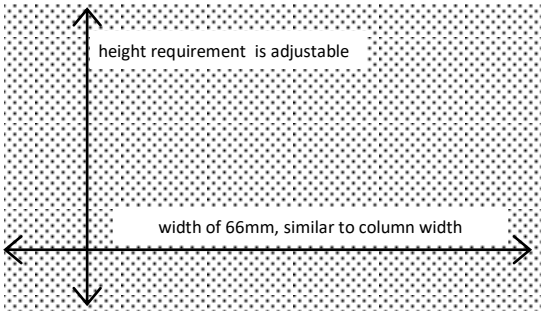

Figure 1. Illustration of Dimensional Figure of one column width. Figure dimension adjusted to the width of one column. Name the figure (diagram) written below the image. (Calibri 8.5 Justify)

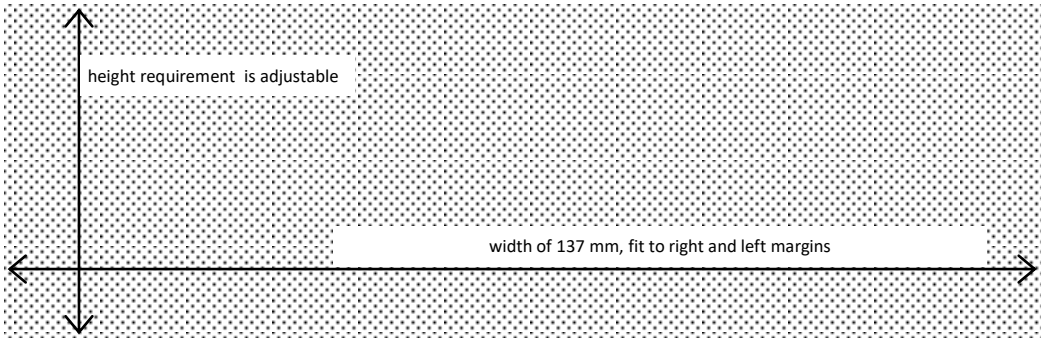

Figure 2. . Illustration of Dimensional Figure of two column width. Figure dimension adjusted to the width of two columns (137 mm). Figure were align top or bottom of the page. (Calibri 8.5 Justify)

References

1. Primary references include journal, patent, dissertation, thesis, paper in proceeding and text book.
2. Avoid self citation.
3. Author should avoid reference in reference, popular book, and internet reference except journal and private ana state institution.
4. Author was not allowed to use abstract as references.
5. References should been published (book, research journal or proceeding). Unpublished references or not displayed data can not be used as references.
6. References typed in numbering list (format number 1,2,3,…), ordered sequentially as they appear in the text (system of Vancouver or author-number style).
7. Citation in the manuscript typed only the references number (not the author and year), example: Obesity is an accumulation of fat in large quantities which would cause excessive body weight (overweight) [1]. Obesity is a risk factor of diabetic, hypertension dan atherosclerosis [2].

CONCLUSION (Calibri 10 Bold, Left, Capslock)

Conclusion of the study's findings are written in brief, concise and solid, without more additional new interpretation. This section can also be written on research novelty, advantages and disadvantages of the research, as well as recommendations for future research.(Calibri 10 Justify)

ACKNOWLEDGEMENT (Calibri 10 Bold, Left, Capslock)

This section describes gratitude to those who have helped in substance as well as financially. (Calibri 10 Justify)

REFERENCES (Calibri 10 Bold, Left, Capslock)

[1]. (Calibri 10 Justify, citation labelling by references numbering)
[2]. Vander, A., J. Sherman., D. Luciano. 2001. Human Physiology: The Mecanisms of Body Function. McGraw-Hill Higher Education. New York. (Book)
[3]. Shi, Z., M. Rifa'i, Y. Lee, K. Isobe, H. Suzuki. 2007. Importance of CD80/CD86-CD28 interaction in the recognation of target cells by $CD8^{+}CD122^{+}$ regulatory T cells. *Journal Immunology.* 124. 1:121-128. (Article in Journal)
[4]. Syafi'i, M., Hakim, L., dan Yanuwiyadi, B. 2010. Potential Analysis of Indigenous Knowledge (IK) in Ngadas Village as Tourism Attraction. pp. 217-234. In: Widodo, Y. Noviantari (eds.) Proceed-ing *Basic Science National Seminar 7* Vol.4. Universitas Brawijaya, Malang. (Article within conference proceeding)
[5]. Dean, R.G. 1990. Freak waves: A possible explanation. p. 1-65. *In* Torum, A., O.T. Gudmestad (eds). Water wave kinetics. CRC Press. New York. (Chapter in a Book)
[6]. Astuti, A.M. 2008. The Effect of Water Fraction of *Stellaria* sp. on the Content of TNF-α in Mice (*Mus musculus* BALB-C). Thesis. Department of Biology. University of Brawijaya. Malang. (Thesis)

Cover Images:

3D Structure of EGCG (*Epigallocatechin-3-Gallate*)
Green Tea Component

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