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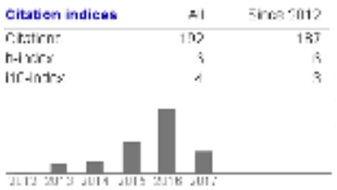

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Characterization of the Interaction between RIN13 (RPM1-Interacting13) and Sumo Proteins in *Arabidopsis thaliana*

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Abstract

Small Ubiquitin-related MOdifier (SUMO) proteins can be found in many organisms, including *A. thaliana*, which possesses 9 SUMO genes. SUMO binds to various target proteins in a reversible reaction called SUMOylation. SUMOylation participates in transcription, chromosome organization, proteins localizations and stress responses. Our study showed that RIN13 (RPM1-Interacting13/At2g20310) is a target of SUMOylation, which was initially found by interaction between this protein and AtSCE1a (E2). Recent report showed that overexpression of RIN13 enhanced the resistance to pathogen without inducing hypersensitive response. However, the molecular interaction between RIN13 and SUMO proteins and its significance have not been studied yet. Thus, our study aimed to characterize the Interaction between RIN13 and SUMO proteins in *A. thaliana*. The result showed an isoform-specific SUMOylation between RIN13 and SUMO proteins. RIN13 is SUMOylated by SUMO1, 2, 3, and 5. Though expressed ubiquitously in *A.thaliana*, fluorescence microscopy showed that RIN13 localizes subcellularly in the nuclear body. Moreover, complete abolishment of SUMOylation with inactive E2 suggests the exclusion of RIN13 from nuclear body. These results showed that SUMOylation affected RIN13 localization, and indirectly influenced its interaction to other proteins and putative function. This paper presents evidence of RIN13 SUMOylation. Furthermore, RIN13 function in pathogenic resistance is shown to be supported by SUMOylation. Thus, this study enhanced the understanding of SUMO in plants and served as reference to molecular studies concerning post-translational modification of SUMO.

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INTRODUCTION

Posttranslational modifications are important integral mechanisms for eukaryote signaling cascades. Ubiquitin is the most familiar polypeptide modifier, and the enzymology of its activation and transfer has been extensively studied. Recently, a family of ubiquitin-like proteins (Ubls) has been identified to attach to target proteins through enzymatic processes similar to that of ubiquitination (Geiss-friedlander & Melchior, 2007; Meulmeester & Melchior, 2008). Small Ubiquitin-related MOdifier (SUMO) is one of the most intriguing Ubls and is the most widely studied to date. SUMO becomes attached to targets through a multistep process that requires an activating (E1-activating enzyme), a conjugating (E2-conjugating enzyme) and a ligating (E3-ligase) enzyme (Park et al., 2011). The E1 exists as one common enzyme for all SUMO substrates. This enzyme links to SUMO in ATP-dependent fashion and subsequently passes active SUMO to E2. SUMO covalently binds to E2 via thioester bond, similar to ubiquitination. The binding of SUMO to targets may be assisted directly by E2 or E3. Interestingly, E3 was proven to be an unessential requirement for SUMO conjugation to target proteins, displayed by the fact that SUMOylation can occur in reconstituted system (Okada et al., 2009).Although SUMOylation occurs only in a small portion of the total pool of the protein, SUMO has been shown to play important roles in diverse processes such as nucleo-cytoplasmic transporter, chromosome segregation, gene expression, chromatin structure, signal transduction, and genome maintenance (Geiss-Friedlander & Melchior, 2007). Unlike ubiquitination, SUMOylation is not known to target proteins for degradation, but rather is thought to regulate protein-protein interactions, alter the subcellular localization and/or activity of targets, and antagonize ubiquitin-dependent degradations (Martin, Wilkinson, Nishimune, & Henley, 2007; Miller & Vierstra, 2011; Xu & Yang, 2013).

Many of the core components of SUMOylation have been identified in *Arabidopsis thaliana* (Lois, Lima, & Chua, 2003; Novatchkova, Tomanov, Hofmann, Stuible, & Bachmair, 2012) such as ubiquitin, has emerged as a common and important mechanism for regulating protein function. Small ubiquitin-like modifier (SUMO. The *Arabidopsis* genome contains eight full-length SUMO genes (*AtSUMOs*), a single gene for the larger subunit of SUMO activating enzyme (SAE), SAE2 (*At2g21470*) and 2 genes for SAE smaller subunit, SAE1a (*At4g24940*) and SAE1b (*At5g50580*), one active gene SUMO-conjugating enzyme homolog to Ubc9 (AtSCE1a, for *A. thaliana* SUMO-conjugating enzyme, *At3g57870*), and 12 genes for putative SUMO protease Ulp-type (ubiquitin like protein) deconjugating enzymes. However, an understanding of SUMOylation in plants is still in rudimentary stage.

One of the found target protein for SUMO in *A. thaliana* was At2g20310. This protein is termed RIN13 (RPM1-Interacting Protein13) and was suggested to function as a positive regulator of RPM1 (Resistance to *P. syringae PV maculicola* 1). RPM1 belongs to the family of R (Resistance) protein, specialized in conferring immune response to avirulence (avr) protein of *Pseudomonas malicola* (Ashfield et al., 2014; Russell, Ashfield, & Innes, 2015)*the Pseudomonas syringae effector proteins AvrB and AvrRpm1 are both detected by the RESISTANCE TO PSEUDOMONAS MACULICOLA1 (RPM1*. The avr protein effectors (especially AvrRpm1) entered the host plants through type III secretion system (TTSS) and activated resistance pathways. RIN4, other cofactor of RPM1 became phosphorylated by AvrRpm1 and in turn activated RPM1 (Kim et al., 2005; Lee, Bourdais, Yu, Robatzek, & Coaker, 2015; Liu, Elmore, & Coaker, 2009; Selote & Kachroo, 2010; Takemoto & Jones, 2005)where they function to manipulate host defense and metabolism to benefit the extracellular bacterial colony. The activity of these virulence factors can be monitored by plant disease resistance proteins deployed to \"guard\" the targeted host proteins. The Arabidopsis RIN4 protein is targeted by three different type III effectors. Specific manipulation of RIN4 by each of them leads to activation of either the RPM1 or RPS2 disease resistance proteins. The type III effector AvrRpt2 is a cysteine protease that is autoprocessed inside the host cell where it activates RPS2 by causing RIN4 disappearance. RIN4 contains two sites related to the AvrRpt2 cleavage site (RCS1 and RCS2. RPM1 changes conformation, allowing RIN13 to bind to NB-ARC (nucleotide-binding-site-domain found in Apaf-1, R proteins, CED-4) and participate in race-specific normal defense signaling processes, resulted in hypersensitive response. Interestingly, ectopic expression of RIN13 conferred enhanced resistance to AvrRpm1 and AvrB in the absence of hypersensitive cell death, possibly by occupation of binding sites that activate bacterial restriction mechanisms (Al-Daoude, de Torres Zabala, Ko, & Grant, 2005).

Thus, this study aims to characterize the Interaction between RIN13 and SUMO proteins and its significance in *A. thaliana*, by assessing

the isoform-specificity of SUMOylation and RIN13 localization observation using fluorescence microscopy. As SUMO proteins are highly conserved in eukaryotes, the result signifies the importance of SUMO modulation in influencing the function of other proteins. This study presents the evidence of SUMO modulation of RIN13, a protein partially conserved in plants. Thus, this study enhanced the rudimentary understanding of plant SUMOs and may serve as a reference to molecular studies concerning post-translational modification by SUMO proteins in plants specifically and eukaryotes in general.

METHODS

Plant materials and growth conditions

The wild type used was of Columbia ecotype. Surface sterilized seeds were sown on germination medium containing 1% sucrose and were stratified for 2d at 4°C. Seeds were then incubated under 16 h light/8 h dark photoperiod at 22°C.

Yeast two-hybrid experiments

The basic procedure for the yeast two-hybrid system was based to the Clontech Yeast Protocols Handbook. The GAL4 DNA-binding domain (GBD) was fused to the *AtSUMO1*, *-2, -3, -5*, and *AtSCE1a* cDNAs as follows: the *AtSUMO1*, *AtSUMO2*, *AtSUMO3*, *AtSUMO5*, and *AtSCE1a* cDNAs were amplified by PCR from MATCHMAKER *A. thaliana* cDNA library. DNA extraction was done according to (Fibriana & Hadiyanti, 2016). *Nco*I-*Sal*I digested of each cDNA was inserted into pAS404 (Nakashima, Noguchi, & Nishimoto, 1999) to fuse in frame to the 3' end of the coding sequences of GBD. pAS404-AtSUMO1, AtSUMO2, AtSUMO3, AtSUMO5, and AtSCE1a were integrated into the *TRP* locus of yeast Y190 strain (Harper, Adami, Wei, Keyomarsi, & Elledge, 1993) respectively (Supplemental Table 1). Putative AtSCE1a-interacting clones were screened by the growth on the synthetic medium lacking His but containing 30 mM 3-amino-1,2,4-triazol (3-AT) and confirmed by blue color on medium containing the 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal). Transformation of *S. cerevisiae* was carried out by the lithium acetate method.

In vitro SUMO conjugation assay

The plasmid pET28a (Novagen, Madison, WI) was used for the expression of T7-His_6-recombinant proteins in *E. coli* BL21(DE3). The *At2g20310* cDNA was cloned into the *Nco*I/*Xho*I sites of pET28a vector and *Not*I/*Xho*I sites of pET28c vector to fuse in frame to the 3' end of the coding sequences of T7-His_6. This system contains E1 enzyme (50 ng of His_6-Fub2/Rad31), E2 enzyme (100ng of His_6-Hus5), E3 enzyme (200ng of His_6-Pli1), Pmt3-GG (1μg), 40 mM ATP and T7-His_6-At2g20310 protein substrate (100 ng) in SUMOylation buffer (50 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 1 mM dithiothreitol, 5 mM ATP) (Okada et al., 2009). The products were separated and detected using anti-T7 monoclonal antibody (Novagen).

E.coli in vivo reconstituted SUMOylation assay

E.coli BL21(DE3) competent cells containing pACYCDuet-AtSAE1a/b-AtSAE2 was cotransformed with pET28a-RIN13 and pCDFDuet-AtSUMO1/2/3/5 (AA or GG)-AtSCE1a. Transformed cells were grown on Lactose selection plate containing appropriate antibiotics and then grown in Lactose Broth medium until OD_{600} was 1.0. Protein was induced by IPTG (isopropyl β-D-1-thiogalactopyranoside) of final concentration of 0.1 mM. After 12-15 h incubation on 25°C, the protein was isolated and subjected to SDS-PAGE immunoblotting.

Western blotting analysis

The protein was extracted from *E. coli* using SDS buffer. Proteins were separated on 8-12.5 % SDS-PAGE gel and transferred onto PVDF membrane (Immobilon-P Millipore). Immunodetection was carried out using appropriate antibody, such as His polyclonal (Qiagen), T7 monoclonal (Novagen), or myc polyclonal antibody, followed by appropriate 2nd antibody (Promega). The proteins were then visualized by Immobilon Western Chemiluminescent HRP Substrate (Millipore) or Super Signal West Femto Maximum Sensitivity Substrate (Pierce).

Transient expression assay

Fluorescent proteins were expressed in *Allium* stem cells using particle-mediated DNA delivery. Coating of gold particle with desired plasmid was conducted as follows: gold particles were washed with 70% ethanol, followed by sterile water, and stored in 50% glycerol to the final concentration of 120 mg/ml. To 15 μl of well-mixed gold particle, 6 μg DNA was added, followed by 15 μl 2.5 M $CaCl_2$ and 7.5 μl 0.1 M spermidine. The supernatant was removed and washed by 70% ethanol and 100% ethanol subsequently. The particles were then stored in 20 μl 100% ethanol and spread equally onto the surface of 2 sterile microcarriers. Onion (*Allium cepa*) stem epidermal strips were placed on 2% agar plate and the vectors were introduced by gold particle

bombardment with a Biolistic Particle Delivery System-100/He (Bio-Rad). Bombardment was done twice for each sample under the following delivery conditions: gold particle diameter, 1.0 μm; helium pressure, 1.100 p.s.i.; target distance, ±9 cm; and chamber vacuum pressure, 550 mm Hg. Samples were incubated in the dark, 24-48 h before observation using Olympus DP71 camera on Olympus BX51 microscope. Nucleus position was confirmed with DAPI (4,6'-diamidino-2-phenylindole).

RESULTS AND DISCUSSION

Identification of *Arabidopsis* SUMO E2 enzyme interacting proteins

Recognition of SUMO targets is partly mediated by the E2 enzyme. *A. thaliana* has one pseudogene and one active gene (AtSCE1a; At3g57870) for SUMO E2 enzyme (Novatchkova et al., 2012). To identify potential target proteins for SUMOylation in *A. thaliana*, a yeast two-hybrid screening using an *Arabidopsis* SUMO E2 enzyme, AtSCE1a, as the bait was conducted. After the correct expression of GBD-HA-AtSCE1a had been confirmed (data not shown), ~2x10^6 clones have been screened and 16 positive AtSCE1a-interacting clones were obtained. Out of 16 positive clones, 7 clones were found to be derived from the same *Arabidopsis* gene (AtSIZ1; At5g60410) that encodes an SUMO E3 ligase and 4 clones were from the At2g20310 gene that encodes a RIN13 protein of 430 amino acids. The identification of SUMO E3 ligase, AtSIZ1, as an AtSCE1a-interacting protein seems to be quite reasonable. To eliminate the possibility that RIN13 is promiscuous in protein interaction, a specificity test in which its interaction with AtSCE1a was compared with control vector was performed. As shown in Figure 1 (line 1-2, 4-5, last row), the Interaction between RIN13 to AtSCE1a seemed to be specific and failed in interaction with control vector.

Interaction between RIN13 and RIL1 with AtSUMOs

Comparison to the *Arabidopsis* genome revealed that At2g20310 is a single-copy gene with 2 analogs, 448 amino acids hypothetical protein of At4g28690 (36% identity, 48% similarity) and 182 amino acids hypothetical protein of At4g27660 gene (43% identity, 61% similarity) (Figure 2). Amino acids similarity was also found in other organisms, such as with hypothetical protein ACG43586.1 constituting of 570 amino acids from *Zea mays* (33% identity, 48% similarity), 557 amino acids hypothetical protein product of *Os10g0466000* from *Oryza sativa Japonica group* (41% identity, 54% similarity) and 511 amino acids unnamed protein product of CAO40534.1 from *Vitis vinifera* (41% identity, 61% similarity). Due to the high degree of similarity between At2g20310, At4g28690, and At4g27660, the possibility of interaction of both analogs with AtSCE1a were tested. As shown in Figure 1 (line 3 and 6, last row), At4g28690 could also interact with AtSCE1a in the two-hybrid assay, while At4g27660 could not (data not shown).

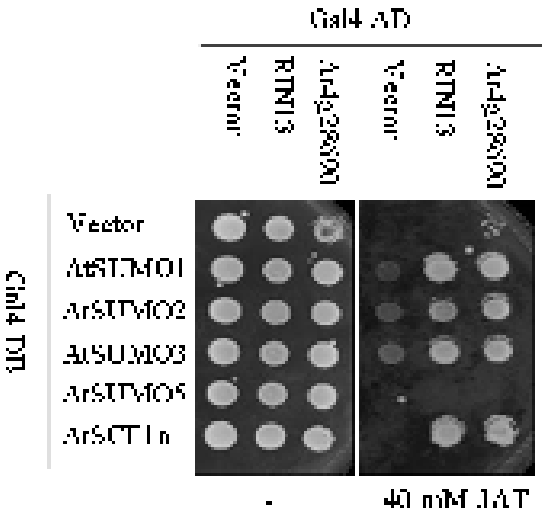

Figure 1. RIN13 and At4g28690 interacted with AtSCE1a and SUMO1, 2, and 3 in yeast two-hybrid. The AtSCE1a-interacting clones were screened on the synthetic medium containing 40 mM 3-amino-1,2,4-triazol (3-AT).

Several proteins that interact directly with SUMO by the yeast two-hybrid system have been demonstrated to be direct targets for SUMOylation (Elrouby & Coupland, 2010). Hence, the interaction of RIN13 and At4g28690 with isoforms of *Arabidopsis* SUMO proteins (AtSUMOs; AtSUMO1, AtSUMO2, AtSUMO3, AtSUMO5) whose transcripts have been detected (Kurepa et al., 2003)location, and/or half-life. Here we show that the SUMO conjugation system operates in plants through a characterization of the Arabidopsis SUMO pathway. An eight-gene family encoding the SUMO tag was discovered as were genes encoding the various enzymes required for SUMO processing, ligation, and release. A diverse array of conjugates could be detected, some of which appear to be SUMO isoform-specific. The levels of SUMO1 and -2 conjugates but not SUMO3 conjugates increased substantially following exposure of seedlings to stress conditions, including heat shock, H(2 were also tested.

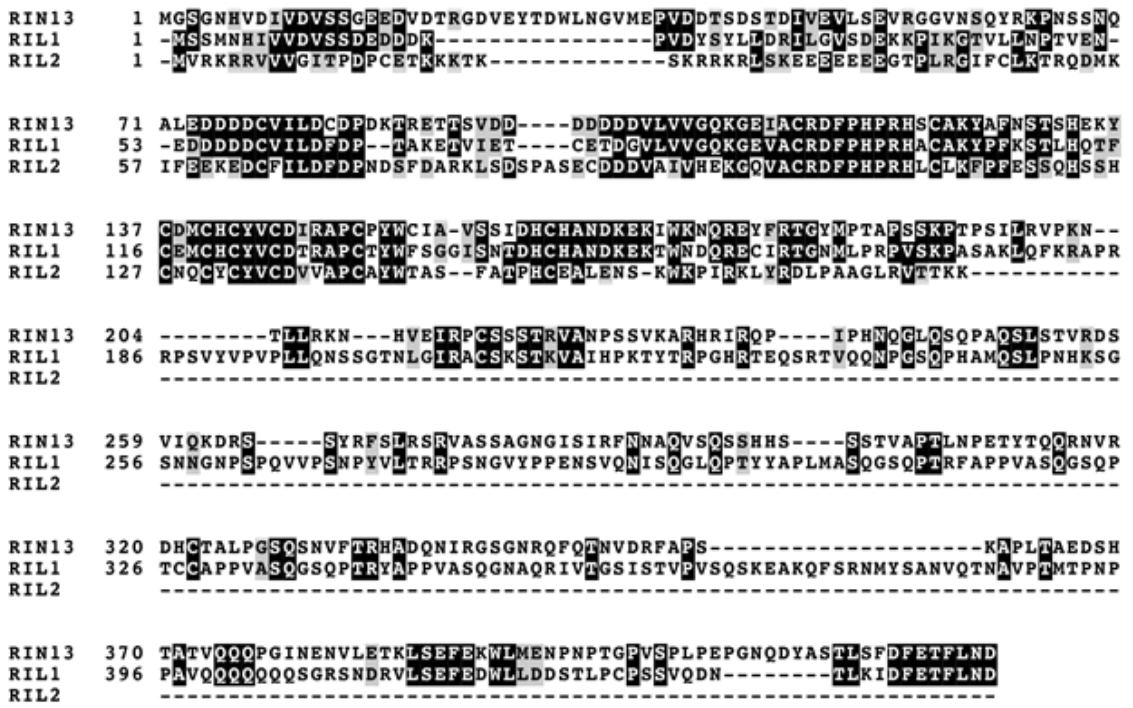

Figure 2. RIN13 Gene Family of *Arabidopsis thaliana***.** Amino acids comparison between RIN13, RIL1, and RIL2. RIN13: *At2g20310*; RIL1:*At4g28690*; RIL2:*At4g27660*. RIL : RIN13-Like.

Interestingly, both RIN13 and At4g28690 could interact with AtSUMO1, 2, and 3, but not with AtSUMO5 (Figure 1), suggesting that RIN13 and At4g28690 are potential targets of AtSUMO1, 2, and 3 modification. At this initial stage, this study focused only to RIN13.

RIN13 was modified by SUMO *in vitro*

Many studies had developed *in vitro* and *in vivo* methods to search for potential targets for SUMOylation (Elrouby, 2015; Elrouby & Coupland, 2010; Vethantham & Manley, 2008)inactivation of genes encoding SUMO or SUMO-conjugation enzymes is lethal, emphasizing the importance of SUMOylation in plant development. Despite this, little is known about SUMO targets in plants. Here we identified 238 Arabidopsis proteins as potential SUMO substrates because they interacted with SUMO-conjugating enzyme and/or SUMO protease (ESD4. To confirm whether RIN13 is a potential target for SUMOylation, *in vitro* modification assay with the purified components were used. Purified components sufficient for Pmt3 modification in fission yeast (Fub2/Rad31 (E1), Hus5 (E2), Pli1 (E3), and the mature form of Pmt3 (Pmt3-GG, SUMO) was employed. The T7-His_6-RIN13 produced by *E.coli* were incubated with the purified components of the fission yeast Pmt3 modification machinery in the presence of ATP and Pmt3-GG.

As shown in Figure 3A, lane 4, *in vitro* modification of T7-His_6- RIN13 gave rise to the appearance of larger forms of T7-His_6- RIN13. These sizes were consistent with those of T7-His_6-RIN13 modified by one or two molecules of Pmt3, respectively. The identities of these modifications of RIN13 was further supported by the fact that it did not occur in the absence of Pmt3-GG or ATP (Figure 3A, lane 1 and 2).

RIN13 was SUMOylated by SUMO1, 2, 3 and 5 in *E. coli* SUMOylation system

To further confirm RIN13 SUMOylation and its isoform specificity, the reconstituted *Arabidopsis* SUMOylation system in *E. coli* (Okada et al., 2009) was used. SUMOAA for each SUMOs was employed as negative control. These SUMOAAs have modified carboxy terminal processing pattern, from Gly-Gly to Ala-Ala (SUMOGG to SUMOAA). This modification eliminates the capability of SUMO conjugation to any substrates.

As shown in Figure 3B, the substrate bands of T7-His-RIN13 were detected at approx. 80 kDa. Conjugation of His-SUMO (detected at ~20 kDa) gave rise to SUMO-T7-His-RIN13 at around 110 kDa. The *in vitro* results are well consistent with the *in vivo* observation (Figure 3B), and thus eliminated the possibility of two-hybrid false positive. Combined, the results suggested

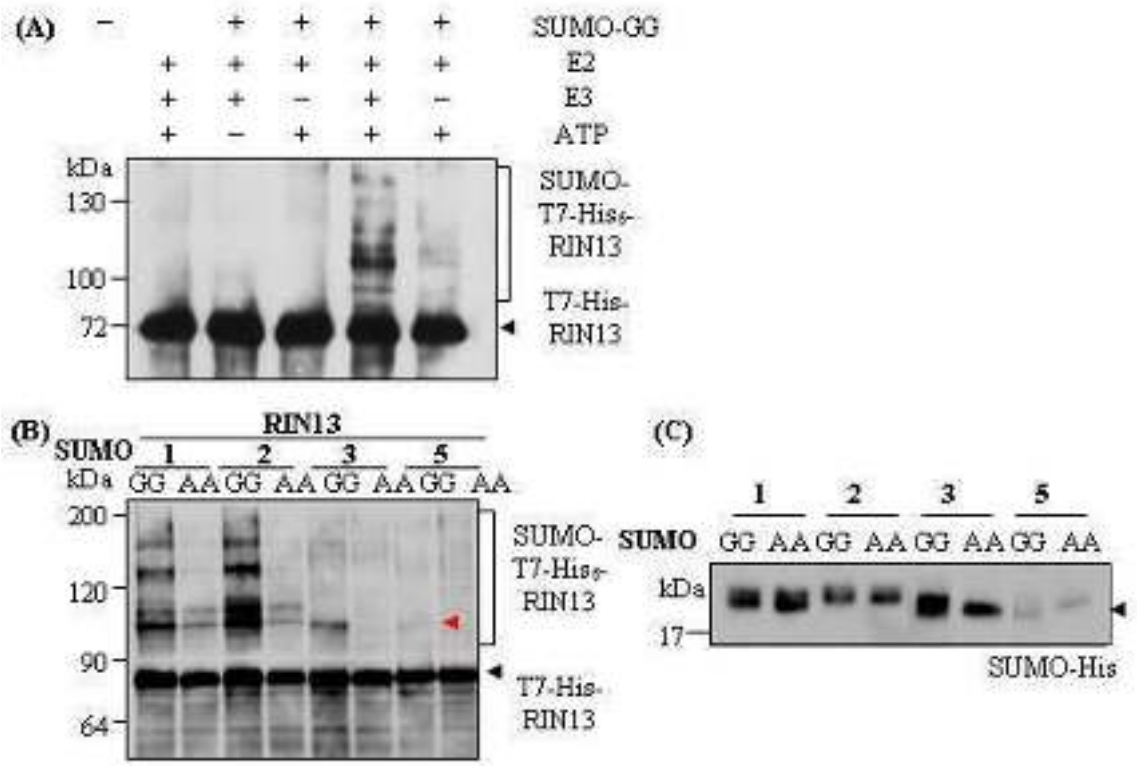

Figure 3. RIN13 was SUMOylated *in vitro* and *in vivo***.** (A) T7-His_6-RIN13 (100 ng) were incubated in *in vitro* SUMOylation system for 90 min. The reaction was terminated by SDS buffer containing 1 mM dithiothreitol (DTT) prior separation and detection. (B) T7-His_6-RIN13 and His-SUMO + E2 were coexpressed in *E.coli* containing E1 enzyme. The expression of T7- His_6-RIN13 was induced by 0.1 mM IPTG addition. Fusion proteins were then detected using appropriate antibodies. SUMOAAs served as negative control. (C) SUMO expression of each SUMO.

that RIN13 is a subject of SUMOylation.

Isoform specific SUMOylation of RIN13 and poly-SUMO chain formation

Using *in vivo* SUMOylation system, RIN13 was conjugated to SUMO1, SUMO2, SUMO3, and SUMO5. However, SUMO5 did not interact with RIN13 (Figure 1) in yeast two-hybrid. Considering the possibility of false negative result of yeast two-hybrid, the emphasis was put to SUMOylation assay *in vivo*. Thus far, it is assumed that the low expression level of SUMO5 caused insufficient expression of histidine to encounter the effect of 3-AT used for sensitivity control.

Poly-SUMO chain formation was observed clearly in SUMO1 and SUMO2 modification of RIN13, at >120 kDa (Figure 3A). Most of SUMO target proteins were known to conjugate to monomeric SUMO. However, similar to ubiquitin, certain SUMO isoforms were able to multimerize and construct poly-SUMO chain (Vertegaal, 2007). In *vitro* experiment showed that yeast SUMO and human SUMO1 was able to form chains using Lys7, Lys16 and Lys 17 (Colby, 2006; Eckhoff & Dohmen, 2015; Park et al., 2011; Yang, Hsu, Ting, Liu, & Hwang, 2006). In addition, human SUMO2 and SUMO3 formed chains in vitro and in vivo, primarily through a conserved acceptor, Lys11 (Park et al., 2011; Tatham et al., 2001). The SUMO polymerization was assisted by conjugating enzyme and SUMO E3 ligase, and diassembled by SUMO proteases. Poly-SUMO chain for SUMO3 was not observed clearly in the experiment, perhaps due to the low level of poly-SUMO chains.

Unfortunately, these isoform specific SUMOylation could not be tested in the complete series of *Arabidopsis* SUMO due to the unavailable EST (expression sequence tag) for the other SUMOs. Thus far, SUMOylation by SUMO1 and SUMO2 were predictable due to their high similarity. SUMO5 was the most different among all isoforms. Moreover, SUMO isoforms are likely to have redundant function and often have overlapping substrates specificity (Chosed et al., 2006). Considering that there is only one gene for the larger subunit of SUMO-E1 (SAE2) and two genes for its smaller subunit (SAE1a/SAE1b) and

one active gene for SUMO-E2 (SCE) in *A. thaliana*, substrate specificity was usually determined by specific E3 ligase or a certain motif within the target proteins. Covalent modification by SUMO requires consensus motif of ψKXD/E (ψ, hydrophobic amino acid; K, lysine; X, any amino acid, D, aspartic acid; E, glutamic acid), while non-covalent binding of SUMO usually determined by SIM (SUMO-interacting Motif) or SBM (SUMO-binding Motif) (Novatchkova et al., 2012; Park et al., 2011).

Gene expression pattern of *RIN13 and RIL1*

RIN13 gene expression was ubiquitous in *A. thaliana*, during all of the developmental stage (Figure 4). The expression was considerably high during germination, boulting, flowering and silique development. This ubiquitous and constitutive expression of RIN13 promotes its binding to SUMO protein, especially AtSUMO1 and AtSUMO2, which also have constitutive expression (Park et al., 2011). Interestingly, the gene expression in etiolated seedlings was considerably lower than in normal seedlings; implying that *RIN13* was partially down-regulated in the dark. It is feasible that ubiquitous expression of *RIN13* might suggest its fundamental roles in the development of *A. thaliana*, rather than served location-specific function. As SUMO has been shown to play crucial roles in a variety of process, it is also possible that RIN13 has broader function and is involved in more than single mechanism.

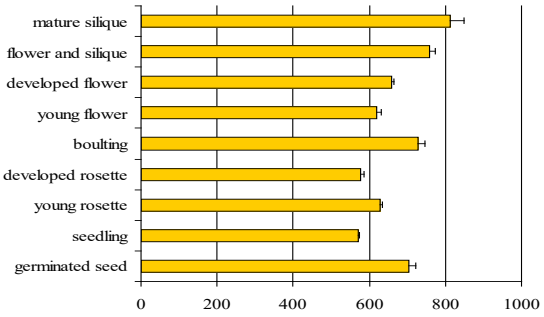

Figure 4. Gene expression profile of *RIN13***.** The data was obtained from www.genevestigator.com.

Subcellular Localization of RIN13

To understand the subcellular localization of RIN13, an expression plasmid containing the native *RIN13* promoter was used to drive the expression of the RIN13::GFP (green fluorescent protein) fusion proteins. The plasmid was transiently expressed in *Allium* stem cell. As clearly shown in Figure 5A(3), RIN13 was recruited into nuclear body.

Although many novel plant nuclear bodies, along with their nuclear domains, had been discovered, their functions are not clear yet. Interestingly, the formation and disruption of nuclear body in many organisms often required PML (pro-myeloid leukemia) SUMOylation. Moreover, the formation of poly-SUMO5 mediates the life cycle of nuclear bodies in human (Liang, Lee, Yao, Lai, & Schmitz, 2016; Percherancier et al., 2009; Shen, Lin, Scaglioni, Yung, & Pandolfi, 2006). However, PML itself does not evolutionarily conserved in *A. thaliana*. PML-nuclear bodies are shown to be involved in various mechanism, such as transcription, defense response, DNA repair, apoptosis, and senescence (Liang et al., 2016). RIN13 localization to nuclear bodies/domains may reflect the interaction sites, the sequestration location, and the site of function or modification, such as splicing (Shaw & Brown, 2004). Alternatively, nuclear bodies may serve as the assembly location prior to transport to the protein functional site (Liang et al., 2016).

To further study RIN13 SUMOylation, RFP fusion proteins of AtSCE1a and AtSCE1a_C94S were employed. This mutant possessed altered amino acid in its catalytic site, Cys-94, and therefore became inactive (Lois et al., 2003). There was no difference between the signal of AtSCE1a and AtSCE1a_C94S. Both proteins

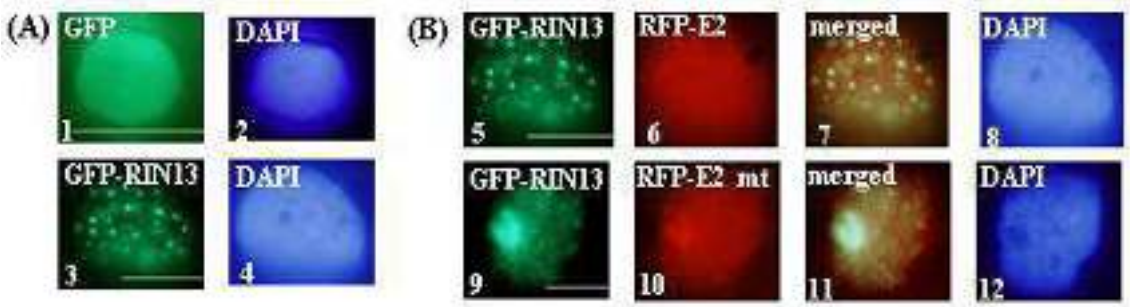

Figure 5. RIN13 localized in nuclear body. (A)-(B) Transformed *Allium* stem cells with transient expression of desired protein(s). For (B), merged figures of first and 2nd respective rows were shown on 3rd rows. DAPI staining confirmed the nucleus position. Bars = 20 μm.

showed uniform signals within the nucleus (Figure 5B(6,10)). Interestingly, when coexpressed with RIN13, this mutation caused striking differences to RIN13 signal. The recruitment of RIN13 to nuclear body became less clear and RIN13 signal were distributed evenly within the nucleus (Figure 5B(9)). Thus, SUMOylation changed the RIN13 localization or disrupt the formation of nuclear body. However, it has not been determined yet whether the direct or the indirect SUMOylation is the cause for this change.

Protein localization correlates directly to its putative function. RIN13 was reported to take part in pathogenic resistance through its interaction to RPM1. Similar to RIN13, RPM1 is a nuclear protein and both proteins may be able to interact due to specific location placement. Thus, the localization change due to the lost SUMOylation may cause a hindrance for RIN13 to function.

Collectively, the results above enhanced the understanding of SUMO processes specifically in plants and eukaryotes in general. The results showed that SUMO proteins functions in the regulation of other protein function. It has been shown that SUMO proteins influence the function of a partially conserved protein in plants. Thus, it is feasible that SUMO proteins are involved in the basal mechanism of eukaryotes. Therefore, this study may serve as reference to other studies focusing on the molecular approach of post-translational modification by SUMO proteins.

CONCLUSIONS

The study showed that RIN13 is a target of SUMOylation, and was shown to interact to SUMO1, SUMO2, SUMO3, and SUMO5. Interaction to other isoforms remains to be confirmed. RIN13 was found to be expressed ubiquitously in *A. thaliana* and localizes subcellularly in nuclear body. This localization to nuclear body is SUMOylation dependent, as the complete abolishment of SUMOylation caused an aberrant localization of RIN13 within the nucleus. It has been known that SUMOylation is involved in stress responses, which may include pathogenic resistance. It is feasible that the interaction and modification of RIN13 by SUMO proteins is involved in the putative role of RIN13 as a positive regulator of RPM1. However, further study is still needed.

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REFERENCES

Al-Daoude, A., de Torres Zabala, M., Ko, J.-H., & Grant, M. (2005). RIN13 is a positive regulator of the plant disease resistance protein RPM1. *The Plant Cell*, *17*(3), 1016–1028.

Ashfield, T., Redditt, T., Russell, A., Kessens, R., Rodibaugh, N., Galloway, L., Kang, Q., Podicheti, R., & Innes, R. W. (2014). Evolutionary relationship of disease resistance genes in soybean and Arabidopsis specific for the Pseudomonas syringae effectors AvrB and AvrRpm1. *Plant Physiol*, *166*(1), 235–251.

Chosed, R., Mukherjee, S., Lois, L. M., & Orth, K. (2006). Evolution of a signalling system that incorporates both redundancy and diversity: Arabidopsis SUMOylation. *Biochemical Journal*, *398*(3), 521–529.

Colby, T. (2006). SUMO-Conjugating and SUMO-Deconjugating Enzymes from Arabidopsis. *Plant Physiology*, *142*(1), 318–332.

Eckhoff, J., & Dohmen, R. J. (2015). In vitro studies reveal a sequential mode of chain processing by the yeast SUMO (Small Ubiquitin-related Modifier)-specific protease Ulp2. *Journal of Biological Chemistry*, *290*(19), 12268–12281.

Elrouby, N. (2015). Analysis of Small Ubiquitin-Like Modifier (SUMO) Targets Reflects the Essential Nature of Protein SUMOylation and Provides Insight to Elucidate the Role of SUMO in Plant Development. *Plant Physiology*, *169*(2), 1006–17.

Elrouby, N., & Coupland, G. (2010). Proteome-wide screens for small ubiquitin-like modifier (SUMO) substrates identify Arabidopsis proteins implicated in diverse biological processes. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(40), 17415–17420.

Fibriana, F., & Hadiyanti, L. N. (2016). Phylogenetic Relationships of Local Durian Species based on Morphological Characteristics and PCR-RFLP Analysis of the Ribosomal Internal Transcribed Spacer (ITS) DNA. *Biosaintifika: Journal of Biology & Biology Education*, *8*(3), 361-369

Geiss-friedlander, R., & Melchior, F. (2007). Concepts in sumoylation: a decade on. *Nature Reviews Molecular Cell Biology*, *8*(12), 947–956.

Harper, J. W., Adami, G. R., Wei, N., Keyomarsi, K., & Elledge, S. J. (1993). The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell*, *75*(4), 805–816.

Kim, H.-S., Desveaux, D., Singer, A. U., Patel, P., Sondek, J., & Dangl, J. L. (2005). The *Pseudomonas syringae* effector AvrRpt2 cleaves its

C-terminally acylated target, RIN4, from*Arabidopsis* membranes to block RPM1 activation. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(18), 6496–501.

Kurepa, J., Walker, J. M., Smalle, J., Gosink, M. M., Davis, S. J., Durham, T. L., Sung, D-Y., & Vierstra, R. D. (2003). The small ubiquitin-like modifier (SUMO) protein modification system in Arabidopsis. Accumulation of sumo1 and -2 conjugates is increased by stress. *Journal of Biological Chemistry*, *278*(9), 6862–6872.

Lee, D., Bourdais, G., Yu, G., Robatzek, S., & Coaker, G. (2015). Phosphorylation of the Plant Immune Regulator RPM1-INTERACTING PROTEIN4 Enhances Plant Plasma Membrane H $^{+}$ -ATPase Activity and Inhibits Flagellin-Triggered Immune Responses in Arabidopsis. *The Plant Cell*, *27*(7), 2042–2056.

Liang, Y., Lee, C., Yao, Y., Lai, C., & Schmitz, M. L. (2016). SUMO5, a Novel Poly-SUMO Isoform, Regulates PML Nuclear Bodies. *Scientific Reports*, *6*(26509), 1–15.

Liu, J., Elmore, J. M., & Coaker, G. (2009). Investigating the functions of the RIN4 protein complex during plant innate immune responses. *Plant Signaling & Behavior*, *4*(12), 1107–1110.

Lois, L. M., Lima, C. D., & Chua, N.-H. (2003). Small ubiquitin-like modifier modulates abscisic acid signaling in Arabidopsis. *The Plant Cell*, *15*(6), 1347–59.

Martin, S., Wilkinson, K. A., Nishimune, A., & Henley, J. M. (2007). Emerging extranuclear roles of protein SUMOylation in neuronal function and dysfunction. *Nature Review Neuroscience*, *8*(12), 948–959.

Meulmeester, E., & Melchior, F. (2008). Cell biology: SUMO. *Nature*, *452*(7188), 709–711.

Miller, M. J., & Vierstra, R. D. (2011). Mass spectrometric identification of SUMO substrates provides insights into heat stress-induced SUMOylation in plants. *Plant Signalling and Behaviour*, *6*(1), 130–133.

Nakashima, N., Noguchi, E., & Nishimoto, T. (1999). Saccharomyces cerevisiae putative G protein, Gtr1p, which forms complexes with itself and a novel protein designated as Gtr2p, negatively regulates the Ran/Gsp1p G protein cycle through Gtr2p. *Genetics*, *152*(3), 853–867.

Novatchkova, M., Tomanov, K., Hofmann, K., Stuible, H. P., & Bachmair, A. (2012). Update on sumoylation: Defining core components of the plant SUMO conjugation system by phylogenetic comparison. *New Phytologist*, *195*(1), 23–31.

Okada, S., Nagabuchi, M., Takamura, Y., Nakagawa, T., Shinmyozu, K., Nakayama, J. I., & Tanaka, K. (2009). Reconstitution of Arabidopsis thaliana SUMO pathways in E. coli: Functional evaluation of SUMO machinery proteins and mapping of SUMOylation sites by mass spectrometry. *Plant and Cell Physiology*, *50*(6), 1049–1061.

Park, H. J., Kim, W. Y., Park, H. C., Lee, S. Y., Bohnert, H. J., & Yun, D. J. (2011). SUMO and SUMOylation in plants. *Molecules and Cells*, *32*(4), 305–316.

Percherancier, Y., Germain-Desprez, D., Galisson, F., Mascle, X. H., Dianoux, L., Estephan, P., Chelbi-Alix, M.K., & Aubry, M. (2009). Role of SUMO in RNF4-mediated promyelocytic leukemia protein (PML) degradation. Sumoylation of PML and phospho-switch control of its SUMO binding domain dissected in living cells. *Journal of Biological Chemistry*, *284*(24), 16595–16608.

Russell, A. R., Ashfield, T., & Innes, R. (2015). *Pseudomonas syringae* effector AvrPphB suppresses AvrB-induced activation of RPM1, but not AvrRpm1-induced activation. *Molecular Plant-Microbe Interactions : MPMI*, *28*(6), http://dx.doi.org/10.1094/MPMI-08-14-0248-R.

Selote, D., & Kachroo, A. (2010). RIN4-like proteins mediate resistance protein-derived soybean defense against Pseudomonas syringae. *Plant Signaling & Behavior*, *5*(11), 1453–6.

Shaw, P. J., & Brown, J. W. (2004). Plant nuclear bodies. *Current Opinion in Plant Biology*, *7*(6), 614–620.

Shen, T. H., Lin, H.-K., Scaglioni, P. P., Yung, T. M., & Pandolfi, P. P. (2006). The mechanism of PML-nuclear body formation. *November*, *24*(3), 331–339.

Takemoto, D., & Jones, D. a. (2005). Membrane release and destabilization of Arabidopsis RIN4 following cleavage by Pseudomonas syringae AvrRpt2. *Molecular Plant-Microbe Interactions : MPMI*, *18*(12), 1258–1268.

Tatham, M. H., Jaffray, E., Vaughan, O. A., Desterro, J. M. P., Botting, C. H., Naismith, J. H., & Hay, R. T. (2001). Polymeric Chains of SUMO-2 and SUMO-3 are Conjugated to Protein Substrates by SAE1/SAE2 and Ubc9. *Journal of Biological Chemistry*, *276*(38), 35368–35374.

Vertegaal, A. C. O. (2007). Small ubiquitin-related modifiers in chains. *Biochemical Society Transactions*, *35*(6), 1422–1423.

Vethantham, V., & Manley, J. L. (2008). In Vitro Sumoylation of Recombinant Proteins and Subsequent Purification for Use in Enzymatic Assay. *Cold Spring Harb Protoc*, *23*(1), 1–7.

Xu, P., & Yang, C. (2013). Emerging role of SUMOylation in plant development. *Plant Signaling & Behavior*, *8*(7), e24727.

Yang, M., Hsu, C. T., Ting, C. Y., Liu, L. F., & Hwang, J. (2006). Assembly of a polymeric chain of SUMO1 on human topoisomerase I in vitro. *Journal of Biological Chemistry*, *281*(12), 8264–8274.

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Characterization of Ethanolic Extract of *Streptomyces* sp. as a Pancreatic Lipase Inhibitors Produced by Endophytic *Streptomyces* sp. AEBg12

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Abstract

Endophytic *Streptomyces* sp. AEBg12 isolated from *Zingiber cassumunar* (Bangle) is known to produce pancreatic lipase inhibitory compound. However, the characteristics of this active compound has not been reported yet. This study aimed to determine the characteristics of pancreatics inhibitory compound produced by *Streptomyces* sp. AEBg12 and to assess the role of endophytic actinobacteria in producing pancreatic lipase inhibitor using endophytic-free bangle tissue culture, wild bangle and compared with the activity of *Streptomyces* sp. AEBg12 endophytes. Supernatant of *Streptomyces* sp. AEBg12 was extracted using ethanol, ethyl acetate, and n-hexane solvents. Toxicity test was performed using larvae of shrimp *Artemia salina*. The results showed that the best solvent to obtain pancreatic lipase inhibitor compounds was ethanol. Phytochemical analysis showed that ethanolic extract of endophytic *Streptomyces* sp. AEBg12 contained flavonoids. IC_{50} value of ethanol extract was 180.83 µg/ml. The result of TLC showed that ethanolic extract of *Streptomyces* AEBg12 had a blue luminescence band indicated that there were either flavone, flavanones, flavonols or isoflavones. Inhibitory activity of *Streptomyces* sp. AEBg12 was higher than wild bangle and bangle tissue culture. The information from this study can be be used as a basic data for further characterization of the active compound, which might be developed as an antiobesity agent through its pancreatic lipase inhibitory activity.

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INTRODUCTION

Obesity is an abnormal excess of gained weight that occurs due to an excessive fat accumulation. It is the result of energy balance disorders where calories that enter the body are more than calories needed. Among all the treatments for obesity, one of the most promising strategies for weight loss is by inhibiting fat absorption using pancreatic lipase. Fat is not directly absorbed by the intestine unless it has been degraded by pancreatic lipase. Many study of this mechanism were performed to determine the effectiveness of natural products as antiobesity agents. Pancreatic lipase is an enzyme secreted by pancreas and contribute in fat digestion (Shin et al., 2003).

Various types of medicinal plants were used to prevent obesity. This knowledge has been passed down from generation to generation based on their custom (Pradono et al., 2011). *Zingiber cassumunar* (Bangle) is one potential medicinal plants as a lipase inhibitor. Iswantini et al. (2011) reported that 100 ppm of ethanolic extract of bangle rhizomes had the highest inhibitory effect on pancreatic lipase activity (29.17%). The inhibitory effect was higher than 100 ppm inhibitory effects of Xenical ® / orlistat as a positive control, with inhibition percentage at 17.53%.

A part of plants, microbes are also able to produce secondary metabolites as a pancreatic lipase inhibitor, it can be used as an antiobesity drug. Endophytic microbes are microbes that can live in plant tissue and are able to form colonies without harming the host. Most of seed plants could associate with several endophytic microbes which are able to produce bioactive compounds. It was suspected that the bioactive compounds was associated with coevolution or genetic transfer from the host plant to endophytic microbes (Tan & Zou, 2001). From the preliminary study, a total of 9 isolates of endophytic actinobacteria from bangle which were capable of producing pancreatic lipase inhibitor was obtained. AEBg12 was the isolate which was able to produce the highest pancreatic lipase inhibitor. Based on morphological, microscopic and molecular observation, it was found that isolates of AEBg12 was *Streptomyces* sp. This study aimed to discover the content of the compound, toxicity values, inhibition values, TLC of ethanolic extract of *Streptomyces* sp. AEBg12 and the role of endophytic actinobacteria in producing pancreatic lipase inhibitor compared to plant tissue culture, and plant from the nature. However, the characterization of pancreatic lipase inhibitor compounds produced by *Streptomyces* sp. AEBg12 has not been studied. This study aimed to determine the characteristics of the ethanolic extract which contained pancreatics inhibitory compound produced by *Streptomyces* sp. AEBg12, and to assess the role of endophytic actinobacteria in producing pancreatic lipase inhibitor using endophytic- free bangle tissue culture plant, bangle plant from nature, and compared with the activity of endophytic *Streptomyces* sp. AEBg12 from bangle plant. It is expected that the output of the study can be used as a basic data for further molecular characterization and development of the active compound as an agent for antiobesity which is obtained from indigenous actinobacteria.

METHODS

Actinobacteria isolate was inoculated into Erlenmeyer flask containing 1000 ml yeast starch broth medium and incubated at 30°C for 10 days on a rotary shaker with 150 (Kekuda et al., 2011). The filtrate was collected by centrifugation at 4000 rpm at 4°C for 25 min to separate supernatant and biomass. Supernatant obtained from selected culture was extracted using various solvents to obtain the active compounds. The solvents tested were ethanol, n-hexane and ethyl acetate. Extraction was performed by adding solvent in to supernatant with a ratio of 1:1, then they were homogenized using magnetic stirrer for 2 hours to form water fraction and solvent fraction. Solvent fraction then separated and concentrated by rotary evaporator to obtain concentrated fraction. The fraction obtained then used to test the activity as an inhibitor of pancreatic lipase.

Inhibitor of pancreatic lipase activity test was carried out by using method of Etoundi et al. (2010) with some modifications. A total of 800µl of triolein mixture was added into a test tube containing 200µl swine lipase and 200µl sample. The solution was mixed and then the absorbance was measured using UV-Vis spectrophotometer at 450 nm wavelength. Then, the solution was incubated for 30 min at 37°C and the absorbance was measured as above. The percentage of pancreatic lipase inhibitory activity was calculated by using the formula:

Inhibition of pancreatic lipase = A - B / A x 100

where A = pancreatic lipase activity, B = pancreatic lipase activity after incubation

The toxicity test was performed using larvae of *Artemia salina*. Determination of IC_{50} values was performed by testing the inhibitory activity of the extracts on a wide range concentrations. After inhibition value of each concentration of the extract was obtained, then the equation

as a function of the extract concentration and the amount of inhibition produced was made.

Separation of pancreatic lipase inhibitor compounds was performed by using several solvents so that the right eluent could be obtained. The selected eluent then was dried and its predicted components were detected using reprostar 3 Camag integrated with WinCATS software. Detection was performed with UV at a lenght of 254 nm and 366 nm. Furthermore, the value Retention Factor (Rf) was determined by using WinCATS software with formula:
Rf = migration distance of the substance/migration distance of the solvent front

To determine the role of endophytic actinobacteria isolates in producing pancreatic lipase inhibitor, plant endophyte-free from tissue culture and bangle derived from nature wer eused. A total of 0.5 g samples plant was crushed aseptically then it was added by 0.5 ml of phosphate buffer 0.1 M (pH 8). Then, pancreatic lipase inhibitory activity of supernatant obtained was tested.

RESULTS AND DISCUSSION

Extraction of pancreatic lipase inhibitor of *Streptomyces* sp. AEBg12

Solvents which were used in this study consisted of ethanol, ethyl acetate and n-hexane. Extraction with various solvents are presented in Table 1. Extraction process with three solvent provided varies result for each solvent. The results showed that extraction with ethanol produced the highest yield (4.39 g) compared to other solvents, followed by ethyl acetate and n-hexane which were 0.48 and 0 g, respectively. The higher amount of pancreatic lipase inhibitor was produced by ethanol extract. It was probably because the ethanol had a high polarity. Ethanol had a low boiling point and it tend to be safe, non-toxic and harmless solvent. N-hexane could cause some negative effects such as disease and air pollution due to the characteristic of hexane which was toxic if it was consumed. In addition, it was a flammable liquid and had a low biodegradability (Azis et al., 2014).

The result showed that the content of polar compounds in supernatant of *Streptomyces* sp. AEBg12 were relatively larger than semi-polar and non-polar extract. The result is in line with the research done by Yuhernita (2011) that performed an extraction using a polar solvent (methanol), semi-polar (ethyl acetate) and non-polar (n-hexane) in which the content in polar compounds was higher than non-polar compounds. Ethanol extracts and ethyl acetate extracts were tested for their ability to inhibit the activity of pancreatic lipase. The content of the compound which would be extracted should be considered for selecting the solvents (Septiana & Asnani, 2012). Ethanol was a polar compound, so that another polar compound would be drag into the extract. The using of ethanol as a solvent in leaves extraction could dissolve alkaloid compounds, polyphenols, and flavonoids (Ayini et al., 2014).

Table 1. Yield of extraction of pancreatic lipase inhibitor of *Streptomyces* sp. AEBg12 with various solvents

Solvent	Yield (g)
n-hexane	0
Ethyl acetate	0.48
Ethanol	4.39

Phytochemical content of *Streptomyces* sp. AEBg12

Culture extracts of *Streptomyces* sp. AEBg12 were chemically analysed for its phytochemical content (Table 2). Major classes of active compounds contained in the extract could obtained through this analysis (Pujiyanto, 2012). Phytochemical analysis results showed that the water extract of *Streptomyces* sp. AEBg12 derived from bangle contained flavonoids, saponins and steroids, while ethanol and ethyl acetate extracts only contained flavonoids. Saponins and steroids in ethanol and ethyl acetate extract which were not detected probably because the compounds were small or they were not exist in the sample (Iswantini et al., 2011). Flavonoids, saponins and steroids allegedly able to act as pancreatic lipase inhibitor. These all compounds allegedly capable as an inhibitor of pancreatic lipase. The study of Iswantini et al. (2011) stated that the phytochemical analysis of bangle showed that they contained flavonoids, saponins, steroids and tannins. Flavonoids have been shown to inhibit the activity of lipase in vitro, including that contained in the rhizome of bangle. Saponins also proven capable to inhibit lipase activity both in vitro and in vivo. The study of Dzomba & Musekiwa (2014) showed that flavonoid extract from roots of *Dioscorea steriscus* could inhibit the activity of pancreatic lipase and α-amylase.

Toxicity test of ethanolic extract of *Streptomyces* sp. AEBg12

Toxicity test of ethanolic extract of *Streptomyces* sp. AEBg12 were conducted on larvae of shrimp *Artemia salina* (Table 3). The table showed that if the concentration is higher, the more sh-

rimp larvae were dead. The highest concentrations used in this study was 1000 µg/ml where the percentage of mortality reaches 100% and the lowest concentrations was 50 µg/ml where the percentage of mortality reaches 26.6 %. The values of LC_{50} of ethanol extract was 231.44 µg/ml,this means that at small concentrations, this extract was able to eliminate half of larvae *A. salina* population. According to Meyer et al. (1982), an extract was considered as a highly toxic when it had LC_{50} values below 30 µg/ml, considered as a toxic if it had LC_{50} values around 30 to 1000 µg/ml, and considered as a nontoxic if it had LC_{50} more than 1000 µg/ml. It showed that ethanolic extract of *Streptomyces* sp. AEBg12 were toxic and indicated that ethanolic extract of *Streptomyces* sp. AEBg12 contained a high bioactive compound.

Table 2. Phytochemical content of *Streptomyces* sp. AEBg12

Compounds	Solvent		
	Water	Ethanol	Ethyl acetate
Alkaloid	-	-	-
Flavonoid	+	++	+
Tannin	-	-	-
Saponin	+	-	-
Quinone	-	-	-
Steroid	+	-	-
Triterpenoid	-	-	-

This study used 48 hours old larvae of *Artemia salina*. Shrimp larvae at the age of 48 hours already had a complete limb so that the testing would be more certain (Muaja et al., 2013). Shrimp larvae toxicity test was performed as a preliminary study to observe the bioactivity potency and toxicity of each sample, so that the concentration of the extract which was safe for the test could be determined (Pradono et al., 2011).

Table 3. LC_{50} value of ethanolic extract of shrimp larvae

Concentration (µg/ml)	LC_{50} (%)
0	0
50	26.6
100	30
500	83.3
1000	100

Several researchers had performed the toxicity test using Brine Shrimp Lethality Test (BSLT). Kekuda et al. (2011) reported that isolate of *Streptomyces* origin from soil in Agumbe, Karnataka, India had LC_{50} values at 42.11 µg/ml with the highest concentration used was 1000 µg/ml with the percentage of mortality reached 100%. Tantithanagorngul et al. (2011) did initial screening of 459 isolates of *Streptomyces* origin from soil in Thailand. A total of 3 isolates were selected, namely 442, 449 and 145 (2010), they had a strong toxicity activity that is 10, 3.5 and 12.5 mg/ml respectively.

Pancreatic lipase inhibitory activity assay of extracts *Streptomyces* sp. AEBg12

Extract of *Streptomyces* sp. AEBg12 were tested for inhibitory activity against lipase. The result showed that ethanolic extract of *Streptomyces* sp. AEBg12 was able to produce the highest inhibition value compared to the ethyl acetate extract of *Streptomyces* sp. AEBg12 (Table 4). At a concentration of 1000 ppm, ethanolic extract of *Streptomyces* sp. AEBg12 was able to inhibit the activity of pancreatic lipase by 92.78%, while ethyl acetate extract of *Streptomyces* sp. AEBg12 inhibit by 65.28%. Ethanolic extract of *Streptomyces* sp. AEBg12 activity was higher compared with orlistat as a positive control that inhibit by 90.28%. This was because the number of secondary metabolites countained in ethanolic extracts of *Streptomyces* sp. AEBg12 were more compared to ethyl acetate extract of *Streptomyces* sp. AEBg12. It was probably due to the number of secondary metabolites of lipase inhibitor in ethanol extract was more compared to ethyl acetate extract. These results were in line with Pradono et al. (2010) which reported that ethanolic extract of tamarind leaves at a concentration of 150 ppm was able to inhibit the activity of pancreatic lipase enzymes to hydrolyze oleic acid by 49.0%. Ethanolic extract of tamarind leaves had the highest inhibitory activity than water extract of tamarind leaves and positive control that was act as an orlistat against human pancreatic lipase activity.

Table 4. Lipase inhibitory activity of various extracts of *Streptomyces* sp. AEBg12 and orlistat

Concentration µg/ml	Extract inhibitory (%)		Orlistat (%)
	Ethyl acetate	Ethanol	
100	18.89±1.73	38.33±3.63	40.56±1.92
250	29.44±1.27	57.5±1.67	47.22±1.27
500	33.33±1.67	66.67±1.67	71.11±2.68
750	52.5±0.83	82.78±2.68	75.56±1.27
1000	65.28±2.10	92.78±1.27	90.28±0.48

Mopuri & Meriga (2014) performed an extraction on *Terminalia paniculata* using various solvents. The results showed that ethanol extracts which was used to produce pancreatic lipase inhibitory activity had the highest content compared with extracts from another solvent with activity at 75%. Hadrich et al. (2014) also reported that ethanol and methanolic extracts of pomegranate skin were able to inhibit the activity of pancreatic lipase. The highest lipase inhibitor activity (100%) was obtained at a concentration of 1 mg/ml after 30 minutes of incubation. The study of Dzomba & Musekiwa (2014) showed that flavonoid produced from ethanolic extract of *Dioscorea steriscus* had a higher lipase inhibitor activity which was 95.88% compared to ethyl acetate and chloroform extract. Yuniarto et al. (2015) reported that ethanolic extract of *kumis kucing* leaves were able to inhibit pancreatic lipase up to 63.92% at 1000 µg/ml.

The results also showed that ethanolic extract of *Streptomyces* sp. AEBg12 at a concentration of 100 ppm could inhibit the pancreatic lipase up to 38.33%, while ethanolic extract of *Zingiber cassumunar* (bangle) at a concentration of 100 ppm could inhibit lipase inhibitor activity up to 29.17% (Iswantini et al., 2011). This result indicated that *Streptomyces* sp. AEBg12 had higher potency than the host plant, but further research was needed using the same substrate and enzyme.

The results also showed that the lower concentration of the extract, the lower its ability to inhibit pancreatic lipase enzyme activity and the higher concentration of the extract, the higher its ability to inhibit pancreatic lipase enzyme activity. These results were in line with other study which stated that ethanolic extract of *kumis kucing* leaves produced a higher inhibition percentage with the increases of concentration which were 38.55, 40, 44.26, 57.5 and 63.92% at concentrations of 0.1, 1, 10, 100 and 1000 ug/ml (Yuniarto et al., 2015). Based on these points, ethanolic extract of *Streptomyces* sp. AEBg12 had a greater effect on inhibition of pancreatic lipase activity, so it was possible to be used as an antiobesity drug.

In this study, IC_{50} values of each extract were determined. It indicated the concentrations of extract and orlistat. The results showed that the lowest IC_{50} values was obtained from ethanolic extract of *Streptomyces* sp. AEBg12 that was 180.83 µg/ml while IC_{50} values of ethyl acetate extract of *Streptomyces* sp. AEBg12 was 676.6µg/ml (Figure 1). IC_{50} value of ethanolic extract of *Streptomyces* sp. AEBg12 obtained was lower than orlistat (195.63µg/ml). The results showed that ethanolic extract of *Streptomyces*sp. AEBg12 had pancreatic lipase inhibitory activity which was higher compared to orlistat, so it was possible forfurther development as an antiobesity drug.

Broussonone A that were isolated from the stem barks of *Broussonetia kanzinoki* showed a noncompetitive inhibitory activity on pancreatic lipase with an IC_{50} of 28.4 µM (Ahn et al., 2012). Buthanol extract of *Streptomyces variabilis* strain of PO-178 produced pancreatic lipase inhibitor with IC_{50} of 44.32 mg/ml (Kekuda & Onkaroppa, 2014).The study of Adnyana et al. (2014) showed that ethanolic extract of pomegranate leaves inhibited pancreatic lipase with IC_{50} 20.64 ug/ml. *Syzygium aromaticum* extracts could inhibit pancreatic lipase with IC_{50} value of 0.015 mg/ml.

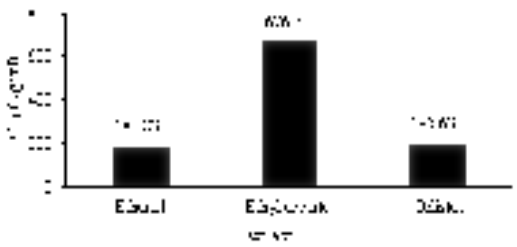

Figure 1. IC_{50} Values of extract ethanol, ethyl acetate of *Streptomyces* sp. AEBg12 and orlistat

Profile of TLC (Thin Layer Chromatography) of ethanolic extract *Streptomyces* sp. AEBg12

Ethanolic extract of *Streptomyces* sp. AEBg12 was further fractionated using thin layer chromatography (TLC). TLC was a method to analyze a mixture by separating the compounds which contained in the mixture. TLC method could be used to determine the number of components in a mixture, the identity and the purity of the compounds (Markham, 1988).

TLC testing on this study was also finding a mixed solvent that was able to separate active compounds contained in ethanol extract. From several experiments which has been conducted, eluent that wasable to separate the active components in the extract was indicated by separate bands formed from the result of chromatographic. The band produced from the elution of each eluent was examined under UV light at a wavelengths of 254 and 366 nm. The best eluent which wasable to separate active components of *Streptomyces* AEBg12 extract was a mixture of methanol:chloroform in the ratio of 9:1. This eluent could separate components contained in the extract into 4 bands with Retention factor (Rf) of 0.03; 0.65; 0.75; 0.76 (Table 5).

In this study, TLC of orlistat was also

performed using a mixture solvent containing methanol:chloroform in the ratio of 9:1. The result showed that *Streptomyces* AEBg12 and orlistat had a different color band. *Streptomyces* sp. AEBg12 had a blue luminescence band while orlistat has a green luminescence band, this indicated that *Streptomyces* sp. AEBg12 had different components compounds compared to orlistat (Figure 2). Detection of the components of ethanolic extract of isolate *Streptomyces* sp. AEBg12 was better observed at a wavelength of 366 nm than at 254 nm.

Table 5. The result of TLC to determine the best solvent

Solvent	Number of spot	Rf Value
Chloroform : n-hexane (9:1)	2	0.03; 0.60
n-hexane : ethyl acetate (2:8)	2	0,04; 0.65
n-hexane : ethyl acetate (3:7)	2	0.03; 0.65
Methanol : chloroform (9:1)	4	0.03; 0.65; 0.75; 0.76
Methanol : chloroform (8:2)	3	0.03; 0.60; 0.75
Methanol : chloroform (5:5)	2	0.04; 0.9
Methanol : chloroform (3:7)	3	0.07; 0,03; 0.9
Methanol : chloroform (2:8)	2	0.03; 0.65
Methanol : chloroform (1:9)	2	0.04; 0.65

According to Markham (1988), blue luminescence band showed on TLC plate when observed at a wavelength of 366 nm indicating several compounds: flavone, flavanones, flavonols and isoflavones. C-glycoside, a flavone found in leaves of *Eremochloa ophiuroides* could inhibit pancreatic lipase inhibitor with IC_{50} values range from 18.5 - 50.5 µM (Lee et al., 2010). Galangin, a flavonols found in *Alpinia galanga* was able to inhibit pancreatic lipase with IC_{50} 48.50 µM (Kumar & Alagawadi, 2013).

ImageJ program would convert the band showed on TLC plate into peaks showed in densitogram (Fereira & Rasband, 2012). A high peak indicating high luminescence color on the band on TLC plate. It could be seen that the peak of band 1 was higher than the other band.

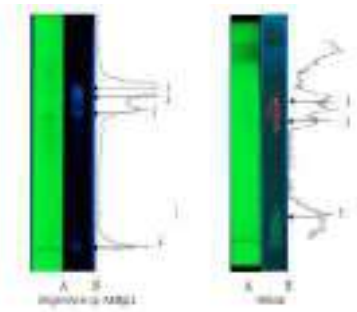

Figure 2. Visualization of thin layer chromatography of ethanolic extract of *Streptomyces* sp. AEBg12 and orlistat at a wavelength of A) 254 nm and B) 366 nm

Lipase inhibitor activity of tissue and host plant of *Streptomyces* sp. AEBg12

In this study, pancreatic lipase inhibitory activity produced by tissue culture endophyte-free bangle, bangle derived from the nature and endophytic actinobacteria were compared (Figure 2). The results were expected to give an overview of the role of the endophytic actinobacteria in producing lipase inhibitor.

The results showed that 2 months old plant tissue culture have a low capability to produce pancreatic lipase inhibitor compound compared to bangle derived from nature and *Streptomyces* sp. AEBg12. Inhibition value of plant tissue culture of bangle was 3.3%. Bangle derived from nature produced higher inhibition activity than the tissue culture bangle (23.9%), but it was lower compared to isolate AEBg12 (95.6%). The existence of endophytic actinobacteria in bangle which had the ability as a lipase inhibitor was in line with Tan & Zou (2001) which stated that endophytic microorganisms could produce certain phytochemicals similar to phytochemicals produced by the host plant, and it might be related to the evolution and gene transfer between the endophytic microorganisms with its host.

This results were in line with Pujiyanto (2012) which stated that endophyte-free plant which was obtained from 3 months old plant tissue culture have a low capability to produce inhibitor compound α-glucosidase (0.06%) compared with *Tinospora crispa* derived from nature (rod, 1.64; roots, leaves 3.39 and leaves, 4.52%). However, the ability of α-glucosidase inhibitor produced by endophytic actinobacteria BWA65 was higher over the host plant activity (10.98%).

Azadirachtin was a biopesticide produced by *Azadirachta indica* that were also found on endophytic fungi (Kusari et al., 2012). Azadirahtin were also detected in induced tissue culture from leaves explants (2.68% DM) at the age of 20 weeks and flower explant (2.48% DM) at the age of 12 weeks (Veeresham et al., 1998). The presence of pancreatic lipase inhibitor activity in plant tissue culture of bangle has not been reported.

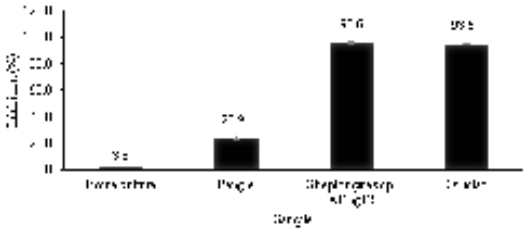

Figure 3. Activity of pancreatic lipase inhibitor produced by tissue culture of bangle, natural plants bangle, and *Streptomyces* sp. AEBg12.

Based on the data from this study, ethanolic extract of *Streptomyces* sp. AEBg12 is possibly a new compound that is able to be used as antiobesity drug through the approach to the pancreatic lipase inhibitor. The result also showed that endophytic actinobacteria from plants could produce the same secondary metabolite compounds as the host plants.

CONCLUSIONS

The results of this study concluded that the water extract of *Streptomyces* sp. AEBg12, endophytic actinobacteria in bangle contained flavonoids, saponins and steroids while ethanol and ethyl acetate extract contained flavonoids. IC_{50} value of ethanol and ethyl acetate extract were 180.9 µg/ml and 655.3 µg/ml respectively. IC_{50} value of ethanol extract was lower than orlistat that was 190 µg/ml. The result of TLC showed that ethanolic extract of *Streptomyces* AEBg12 had blue luminescence band indicated that there were either flavone, flavanones, flavonols or isoflavones. *Streptomyces* sp.AEBg12 produced inhibition value higher than bangle and plant tissue culture of bangle that was 95.6%.

REFERENCES

Adnyana, I. K., Sukandar, E. Y., Yuniarto, A., & Finna, S. (2014). Anti-obesity effect of the pomegaranate leaves ethanol extract (*Punicagranatum* L) in high-fat diet induced mice. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(4), 626-631.

Ahn, J. H., Liu, Q., Lee, C., Ahn M. J., Yoo, H. S., Hwang, B. Y., & Lee M. K. (2012). A new pancreatic lipase inhibitor from *Broussonetia kanzinoki*. *Bioorganic & Medicinal Chemistry Letters*, 22(8), 2760-2763

Arifianti, L., Oktarina, R. D., & Kusumawati, I. (2014). Pengaruh jenis pelarut pengektraksi terhadap kadar sinensetin dalam ekstrak daun *Orthosiphon stamineus* Benth. *E-Journal Planta Husada*, 2(1), 1-4.

Ayini, U., Harnina, B. S., & Dewi, T. C. (2014). Efek antibakteri ekstrak daun mimba (*Azadirachta indica* A. Juss) terhadap bakteri *Vibrio algynoliticus* **s**ecara in vitro. *Biosaintifika: Journal of Biology & Biology Education*, 6(1), 67-75.

Azis, T., Febrizky, S., & Mario, A. D. (2014). Pengaruh jenis pelarut terhadap persen yield alkaloid dari daun salam india (*Murraya koenigii*). *Teknik Kimia*, 2(20), 1-6.

Dzomba, P., & Musekiwa, C. (2014). Anti-obesity and antioxidant activity of dietary flavonoids from *Dioscorea steriscus* tubers. *Journal of Coastal Life Medicine*, 2(6), 465-470.

Etoundi, C. B., Kuaté, D., Ngondi, J. L., & Oben, J. (2010). Anti-amylase, anti-lipase and antioxidant effects of aqueous extracts of some Cameroonian spices. *Journal of Natural Products*, 3, 165-171.

Ferreira, T., & Rasband, W. (2012). ImageJ user guide IJ 1.46r. *National Institute of Health* [internet]. Diambil dari http://www.imagej.nih.gov/ij/docs/guide. diakses 12 Mei, 2016.

Hadrich, F., Cher, S., Gargouri, Y. T., & Adel, S. (2014). Antioxidant and lipase inhibitory activities and essential oil composition of pomegranate peel extracts. *Journal of Oleo Science*, 63(5), 515-525.

Iswantini, D., Silitonga, R. F., Martatilofa, E., & Darusman, L. K. (2011). *Zingiber cassumunar, Guazomaulmifolia*, and *Murraya paniculata* extracts as anti-obesity: in vitro inhibitory effect on pancreatic lipase activity. *Hayati*, 18(1), 6-10.

Kekuda, T. R. P., & Onkarappa, R. (2014). Antioxidant, antihelmintic and enzyme inhibitory potential of *Streptomyces variabilis* Strain PO-178 Isolated form western ghat soil, Agumbe, Karnataka, India. *Journal of Biological Scientific Opinion*, 2(2), 170-176.

Kekuda, T. R. P., Shobha, K. S., & Onkarappa, R. (2011). Pancreatic lipase inhibitory and cytotoxic potential of a *Streptomyces* species isolated from western ghat soil, Agumbe, Karnataka, India. *International Journal of Pharmaceutical and Biological Archives*, 2(3), 932-937.

Kumar, S., & Alagawadi, K. R. (2013). Anti-obesity effects of galangin, a pancreatic lipase inhibitor in cafeteria diet fed female rats. *Pharmaceutical Biology*, 51(5), 607-613

Kusari, S., Verma, V. C., Lamshoeft, M., & Spiteller, M. (2012). An endophytic fungus from *Azadirachta indica* A. Juss.that produces azadirachtin. *World Journal of Microbiology and Biotechnology*, 28(3),1287-1294.

Lee, E. M., Lee, S. S., Chung, B. Y., Cho, J. Y., Lee, I. C., Ahn, S. R., Jang, S. J., & Kim, T. H. (2010). Pancreatic lipase inhibition by C-Glycosidic flavones isolated from *Eremochloa ophiuroides*. *Molecules*, 15(11), 8251-8259.

Markham K. R. (1988). *Cara mengidentifikasi flavonoid*. Bandung: Penerbit ITB.

Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E., & McLaughlin, J. L. (1982). Brine shrimp: a convenient general bioassay for active plant constituents. *Journal of Medicinal Plants Research*, 45(05), 31-34.

Mopuri, R., & Meriga, B. (2014). Anti-Lipase and anti-obesity activities of *Terminalia paniculata* bark in high calorie diet-induced obese rats. *Global Journal of Pharmacology*, 8(1), 114-119.

Muaja, A. D., Koleangan, H. S. J., & Runtuwene, M. R. J. (2013). Uji toksisitas dengan metode BSLT dan analisis kandungan fitokimia ekstrak daun soyogik (*Saurauia bracteosa* DC) dengan metode soxhletasi. *Jurnal Mipa Unsrat*, 2(2), 115-118.

Pradono, D. I., Darusman, L. K.,& Susanti, A. (2011). Inhibisi lipase pankreas secara in vitro oleh ekstrak air dan etanol daun asam jawa (*Tamarindus indica*) dan rimpang kunci pepet (*Kaempferiae rotundae*). *Jurnal Natur Indonesia*, 13(2), 146-154.

Pujiyanto, S. (2012). Kajian inhibitor α-glukosidase aktinomiset endofit asal brotowali (*Tinospora crispa*). *Disertasi*. Bogor: Program Pasca Sarjana. Institut Pertanian Bogor.

Shin, J. E., Han, M. J., & Kim, D. H. (2003). 3-Methylethergalangin isolated from *Alpinia officinarum* inhibits pancreatic lipase. *Biological & Pharmaceutical Bulletin*, 26(6), 854-857.

Septiana, A. T., & Asnani, A. (2012). Kajian sifat fisikokimia ekstrak rumput laut coklat *Sargassum duplicatum* menggunakan berbagai pelarut dan metode ekstraksi. *Agrointek*, 6(1), 22-28.

Tan, R. X., & Zou, W. X. (2001). Endophytes: a rich source of functional metabolites. *Natural Products Reports*, 18(4), 448-459.

Tantithanagorngul, W., Sujitwanit, A., Piluk, J., Tolieng, V., Petsom, A., Sangvanich, P., Palaga, T., Puthong, S., Thamchaipenet, A., & Pinphanichakarn, P. (2011). Screening for brine shrimp larvicidal activity of *Streptomyces* sp. isolated from soil and anti-tumor activity of the active isolates. *Australian Journal of Basic and Applied Sciences*, 5(7), 15-22.

Veeresham, C., Kumar, M. R., Sowjanya, D., Kokate, C. K., & Apte, S. S. (1998). Production of azadirachtin from callus cultures of *Azadirachta indica*. *Fitoterapia*, 69(5), 423-424.

Yuhernita, J. (2011). Analisis senyawa metabolit sekunder dari ekstrak metanol daun surian yang berpotensi sebagai antioksidan. *Makara Sains*, 15(1), 48-52.

Yuniarto, A., Purwani, H., Juanda, D., Setiawan, F., & Kurnia, I. (2015). Kumis kucing (*Orthosiphon stamineus* [benth.]) leaves ethanol extract as anti-obesity agent in high-fat diet-induced obese mice. *Asian Journal of Pharmaceutical and Clinical Research*, 8(6), 234-236.

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Genetic Variation in Cytochrome b-*Hinf*1 and -*Alu*1 Gene Correlated to Body Size in Soang Gourami (*Osphronemus goramy*) from Single Spawning

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History Article

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Abstract

Soang gourami fingerling shows variable body sizes eventhough resulted from single spawning. Differences in body sizes among individuals is assumed to be correlated to their genetic component which can be studied using cytochrome b gene PCR-RFLP marker. This study aimed to determine specific PCR-RFLP marker among different sizes of soang gourami collected from single spawning. Genomic DNA was isolated using Chelex method. Cytochrome b gene were amplified and digested using four restriction enzymes. Specific markers were analyzed descriptivelly based on DNA band pattern appear in agarose gel. The result showed that PCR-RFLP markers of Cytochrome b-*Hinf*I of 315 bp, and 210 bp, and also Cytochrome b-*Alu*I of 334 bp and 189 bp are specific markers for large individuals, whereas small individuals are characterized by having Cytochrome b- *Hinf*I 366 bp, and 159 bp and Cytochrome b-*Alu*I 525 bp fragments. It is observed that genetic variation of Cytochrome b-*Hinf*I and -*Alu*I markers are possitively correlated to body size in soang gourami fingerling. Therefore, both cytochrome b-*Hinf*I and -*Alu*I gene can be reffered as specific markers to differentiate among different sizes of soang gourami strain fingerling from single spawning. This result proved that genetic divergences among individuals can be related with certain quantitative characters, such size related. Therefore our study can contribute on fisheries development, especially by providing new technique for fingerling selection to obtain high quality fingerling and also provide new insight the application of molecular technique in fisheries.

How to Cite

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INTRODUCTION

Giant gourami (*Osphronemus goramy* Lacepede, 1801) has been widely cultivated in Indonesia for many years. This species has high economic value due to its taste meat (Azrita, 2015). The high prices of gourami have encouraged the establishment of such fish as one focus of fisheries revitalization in Indonesia for the period of 2009-2025 (Nurdjana, 2008). In terms of cultivation, production of giant gourami is relatively low cost and it species has the ability to adapt to environmental condition with low oxygen levels (Setijaningsih et al., 2007). However, there are two major obstacles that hinder the success of giant gourami cultivation, namely slow growth rate and high mortality due to disease (Achmad et al., 2009).

Several giant gourami strains have been cultivated by the fish farmers, i.e. Soang, Jepang, Paris, Bastar, and Porselen strains (Setijaningsih et al., 2007). It was reported that growth performance of each strain is different (Nugroho et al., 1993) and fish farmers prefer to cultivate soang strain because it is believed to have a better growth rate than other strains. However, if we examined carefully in the fingerling of soang strain from single spawning, their body sizes are varies. These body size variations are suggested because of each individual have different growth rates. Differences in the ability to grow is assumed due to that the fish have different metabolic capabilities, primarily in harvesting energy from the feed (Yurisma et al., 2013), through celular respiration. The performance of cellular respiration assumed directly related to the variations in genetic component of each individual fingerling. The differences in genetic componen profile among individuals within population can be used for genetic diversity analysis in certain species (Millah et al., 2012). According to Kartikaningsih et al. (2001), genetic factors are among various factors that cause giant gourami growth rate is relatively slower than other fish species. Previous study proved that variation on growth hormone gene was possitivelly corelated with individual body size in certain population (Hua et al., 2009).

So far, detection of growth hormone gene 1 (GH 1) from soang gourami resulted incosistence DNA band pattern. Therefore, it could not be used as genetic marker to characterize individuals with different body sizes of that strain (Nuryanto et al., 2014). Meanwhile, cytochrome c oxidase 1 (CO1) which is belived to as the most variable mitochondrial DNA (mtDNA) and commonly use in animal population genetic studies, it was reported that the CO1 gene could not also be used to distinguish large and small individulas soang gourami from single spawning (Azizah et al., 2015). Therefore, it is necessary to seeks other molecular markers to be used as a candidate of specific marker on large and small individuals differentiation. Cytochrome b gene is among mtDNA genes which is familiar in population analysis. Previous studies, either using PCR-RFLP or sequences of cytochorme b gene have proven that this gene is variable enough to discriminate among populations on various fish species (Mesquita et al., 2001; Takehana et al., 2004; Cheng & Lu, 2005; Ma et al., 2010) and from pork in meatballs product (Fibriana et al., 2012). However, those studies were done to evaluate genetic differentiation among populations. So far, there was no study about intra-populational genetic diversity, especially on soang gourami fingerling from single spawning but having variable body sizes. Here we characterized soang giant gourami fingerling population which is consisted of two groups size individuals but originated from single spawning using PCR-RFLP marker of cytochrome b gene.

This study aims to develop PCR-RFLP marker to be used as specific marker for large and small individulas differentiation within population of soang gourami fingerling from single spawning.

The result is expected to have contribution in the development of giant gourami cultivation by providing new methods for fingerling selection using molecular character, especially RFLP characters. It is also expected that our study to provide new insight about the possibility of the application of molecular technique in applied sciences such as in aquaculture.

METHODS

This study was conducted from April to November 2016 and used purposive sampling. Fish samples were collected from Purbalingga, Central Java, but the fingerling were bought from Ciamis West Java. The samples of soang giant gourami fingerling were originated from single spawning. Fish samples were divided into two different groups based on their body size; i.e. small individulas with their total length less than 10 centimeter (<10 cm) and large individuals with their total length more than ten centimeter (> 10 cm). For DNA analysis, more or less of 0.5 centimeter of tissue samples were cut off from caudal fins while the individuals were kept alive. Tissues samples were preserved in 96% ethanol.

The DNA was extracted from all samples

using Chelex 5% method (Walsh et al., 1991) with small modification, especially on incubation times. Fragment of cytochrome b gene was amplified using a pair of primers as follow: *forward* 28FOR 5'-CGAACG TTGATA TGAAAA ACCATC GTTG-3' (Meyer et al., 1990) an *reverse* 34REV 5'-AAACTG CAGCCC CTCAGA ATGATA TTTGTC CTCA-3' (Cantatore et al., 1994). The PCR reaction was done in final volume of 25 µl. The final concentration of each reagen was 1X PCR buffer; 0.8 mM of each dNTP; 0.4 pM of each primer; 2 U Taq DNA polymerase, dan 0.8-3.52 ng/µl DNA template. Final volume of 25 µl was reached by adding ultrapure water (ddH_2O) as much as 18.4 µl. Amplification of the cytochrome gene fragments were conducted in thermal condition as follow. Predenaturation at 94 °C for 5 minutes and cotinued by 35 cycles with denaturation at 94 °C for 30 seconds, annealing at 55 °C for 45 seconds, and extension at 72 °C for 1 minute. Final extension was performed at 72 °C for 5 minutes. The amplicons were migrated in 1% of agarose gel and divisualized over UV-light transiluminator.

Consistent and strong amplicons were then digested using *Hinf*I, *Alu*I, *Hind*III, and *Hae*III restriction enzymes to obtain PCR-RFLP marker of the cytochrome b gene. Digestion procedures following the protocol provided by the company (Thermoscientific). Digestive products were migrated in agarose gel 1.2% and visualized over UV-light transluminator. Fragment length of the PCR-RFLP markers were estimated by comparing them to 100 base pair (bp) DNA ladder. Specific PCR-RFLP markers were defined descriptively based on the PCR-RFLP band pattern on agarose gel.

RESULTS AND DISCUSSION

Amplification processes using specific primer resulted amplicons with the size approximately of 525 base pair (bp) length. Similar amplicons sizes were always obtained when amplifications process were repeated. With this consistent result, we are sure that the amplicons are our target products. The obtained amplicons are presented in Figure 1.

The amplicons were then digested using four restriction enzymes, that were *Hinf*I, *Alu*I, *Hae*III and *Hind*III. Restriction fragments are wellknown as PCR-RFLP markers. The size of restriction fragments were estimated using regression analysis after compared to 100 bp DNA ladder. A complete PCR-RFLP markers measurements are presented in Table 1.

It can be seen from Table 1 that digestion of PCR amplicons using *Hinf*I enzymes resulting two different PCR-RFLP pattern from two group sizes. The PCR-RFLP marker from small individuals cosisted of two cytochrome b-*Hinf*I fragments with the sizes of 366 bp and 159 bp length, while from large individuals consisted of 315 bp and 210 bp length fragments. The cytochrome b-*Hinf*I fragments are presented in Figure 2.

The ability of *Hinf*I enzyme in cutting off the PCR amplicons proved that on cytochrome b gene of soang giant gourami fingerling has specific site which can be recognized by that enzyme. This is because restriction enzyme only able to recognize a specific site so that if there is no restriction site which can be recognized, the sequences will not be cut by the enzyme. The success of *Hinf*I enzyme cutting cytochrome b gene in this study similar to the studies from Hold et al. (2001) on 25 fish species on food products, (Chen & Lu, 2005) in fish *Coilia ectenes*, and Nebola et al. (2010) in several marine fish species, but different to Hold et al. (2001) for 11 other fish species. Similarities and differences among our study and those previous studies indicate that cytochrome b gene varies greatly between species so that some

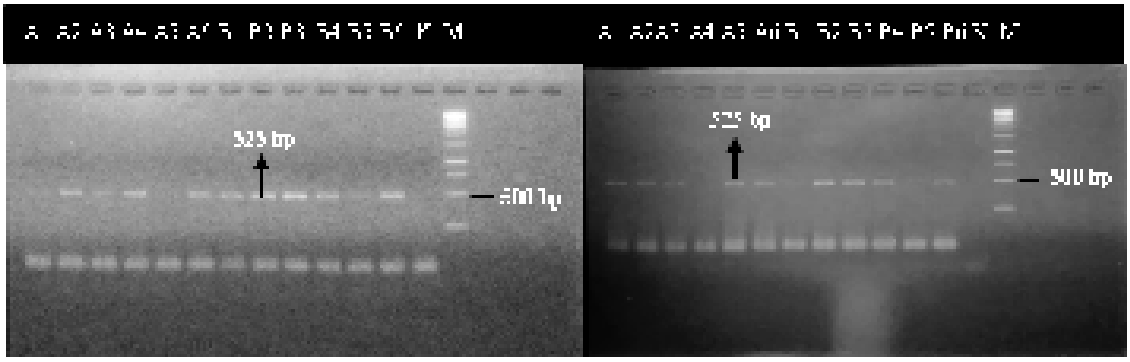

Figure 1. The amplicon of cytochrome b gene from soang giant gourami fingerling
Remarks: A1-A6. Small size fingerling; B1-B6. Large size fingerling; K. Negative control; M. DNA ladder 1 kb (kilo bases)

Table 1. The size of restriction fragment based on regression analysis measurement

Enzymes	Fingerling Size	Migration distance (cm)	y-value	Fragment Length (base pair)	Total Length (base pair)
HinfI	Small	2.17	2.569337	366	525
		2.9	2.20149	159	
	Large	2.31	2.498791	315	525
		2.66	2.322426	210	
AluI	Small	1.87	2.720507	525	525
	Large	2.26	2.523986	334	523
		2.75	2.277075	189	
HindIII	Small and Large	1.87	2.720507	525	525
HaeIII	Small and Large	2.31	2.498791	315	493
		2.8	2.25188	178	

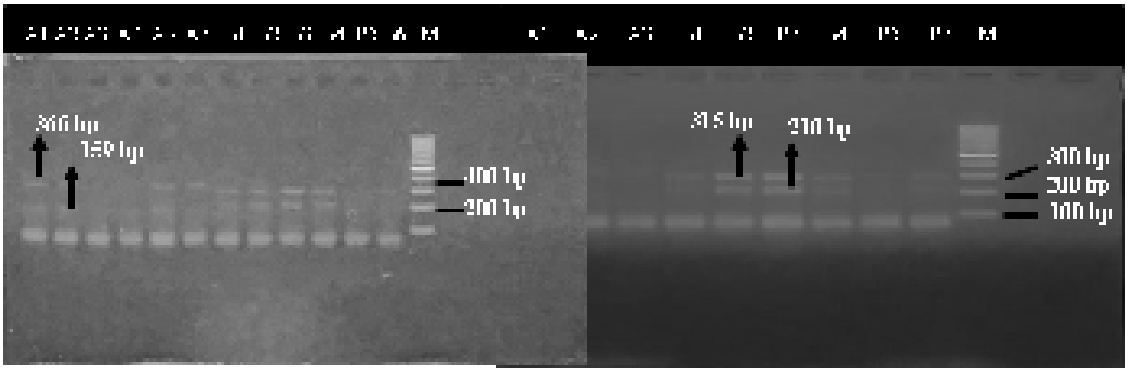

Figure 2. Restriction fragment resulted from *Hinf*I digestion
Remarks: A1-A6 = small individuals; B1-B6 = large individuals; M = 100 bp DNA ladder

species has restriction sites that could be recognize by *Hinf*I enzymes, while the other species has no such sites. The results of this study provide additional information that the cytochrome b gene not only varied between species but also within species, even within population such in this study which used soang giant gourami fingerling from single spawning but has variation in body size among its members.

The different between individual groups with different quantitative characters was also reported by Kusbiyanto et al. (2016) on Soang giant gourami fingerling from single spawning. However, comparison to the study from Kusbiyanto et al. (2016) was not congruence since we used different quantitative characters and different genetic markers. Here we used growth rate as quantitative character which was indicates by body sizes and RFLP of cytochrome b gene as genetic marker, while Kusbiyanto et al. (2016) used individual resistance as quantitative character and major histocompatibility complex (MHC) class II gene as genetic marker. Although not congruence comparison, however, at least we can learn from present study and the study from Kusbiyanto et al. (2016) that genetic divergence might occur among individuals from single spawning but with different quantitative characters, i.e. body size and resistances to diseases.

The results of *Hinf*I restriction generate different cytochrome b-*Hinf*I markers profiles betwen large size and small size of soang gourami fingerling. All large individuals have a uniform cytochrome b-*Hinf*I markers, as well as small fingerling have another uniform profile. This provides evidence that cytochrome b-*Hinf*I markers could be uses as a candidate of specific marker to distinguish fast-growing and slow-growing soang gourami fingerling. These differences are also providing information that there are two haplotypes of cytochrome b gene in Soang gourami fingerling with different body sizes. Each haplotype was only observed in certain group size. This means that genetic variation in cytochrome b-*Hinf*I of soang giant gourami fingerling is possitively related to their body size, especially to total length.

Amplicons digestion using *Alu*I enzyme

produce different pattern of PCR-RFLP markers for small and large individuals. On small individuals, the amplicons were not digested which was indicated by similar size of the DNA fragments between diggested and undiggested amplicons that is 523 bp length fragments. This means that in small individuals no restriction site was recognized by the enzyme, while in large individuals there was. In large individuals, the PCR amplicons were cut into two different Cytochrome b-*Alu*I fragments, i.e. 334 bp and 189 bp length fragments. Digestion poducts of *Alu*I enzyme are presented in Figure 3.

As presented in Figure 3, the *Alu*I enzyme able to cut the amplicons of the cytochrome b gene of large individuals only. This mean that restriction sites were only found in large individuals but not in small individuals, although both groups sizes were collected from single spawning. Our result similar to what were reported by Nebola et al. (2010) in several marine fish species, Cocolin et al. (2005) on rainbouw trout and dentex fish species, and Apostolidis et al. (1996) on *Salmo truta* L. Meanwhile, Ali et al. (2011) also reported that cytochrome b gene of mammal was successfully diggested by *Alu*1 enzyme. Similarities between our study and those previous studies were due to that the cytochrome b gene have specific site that can be recognized by *Alu*1 enzyme although that gene was isolated from different species. Our study and those previous studies proved that *Alu*1 restriction sites of cytochrome b gene are widely distributed acrross animal phyla, starting from fish up to mamalia.

Another information that can be summarized from Figure 3 is that *Alu*1 enzyme only able to digest cytochrome b gene from large individuals. The result shows that cytochrome b gene of soang giant gourami fingerling from single spawning are varies. This variation is possitively correlated with body size. Therefore, Cytochrome b-*Alu*I markers can be selected as a candidate marker for large and small individuals differentiation of soang giant gourami finggerling collected from single spawning.

The *Hae*III enzyme was able to digest PCR products and generated two PCR-RFLP fragments, that are Cytochrome b-*Hae*III of 315 bp and 178 bp length fragments. These PCR-RFLP fragments were obtained from all individuals whatever their total body length. The PCR-RFLP fragments resulted from *Hae*III digestion are presented in Figure 4.

All individuals have the same PCR-RFLP patterns (Figure 4). This fact proved that cytochrome b gene of soang giant gourami has restriction site for *Hae*III enzyme. This result is similar to the result from Nebola et al. (2010) on various marine fish species, Cheng & Lu (2005) on *Coilia ectenes*, Takehana et al. (2004) on *Oryzias latipes*, Lin et al. (2002) on freshwater eels, Hold et al. (2001) on 36 fish species, and Cocolin et al. (2000) on four marine fish species filet. This similarities could be due to that cytichrome b gene on our research object and those previous studies (Cheng & Lu, 2005; Takehana et al., 2004; Lin et al., 2002; Hold et al., 2001; Cocolin et al., 2000) have restriction sites which can be recognized by *Hae*III. This phenomenon indicated that *Hae*III restriction sites on cytrochrome b gene are widely distributed on many fish species, either on freshwater or marine water species.

If we look into detail on Figure 4, it can be observed that cytochrome b gene was digested. However, the Cytochrome b-*Hae*III markers shows similar pattern between small and large individuals. This means that only one allele of Cytochrome b-*Hae*III was resulted. Therefore, the Cytochrome b-*Hae*III marker could not be used as candidate marker to differetiante between fast-

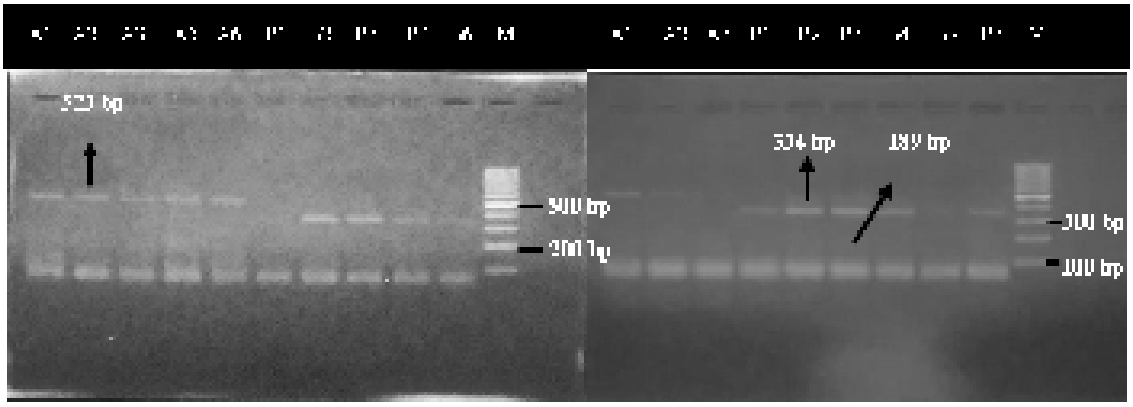

Figure 3. Restriction fragment resulted from *Alu*I digestion
Remarks: A1-A6 = small individuals; B1-B6 = large individuals; M= 100 bp DNA ladder

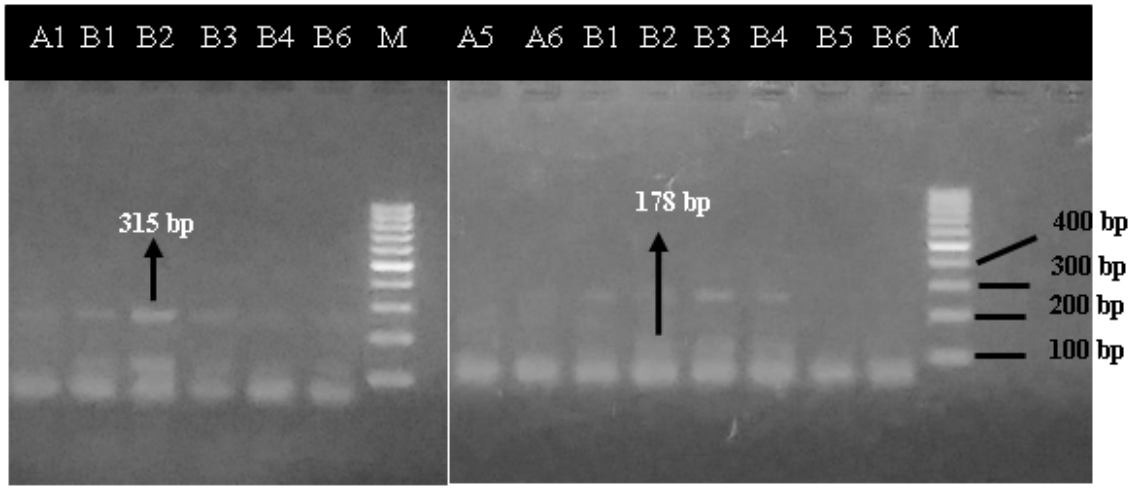

Figure 4. Restriction fragment resulted from *Hae*III digestion
Remarks: A1-A6 = small individuals; B1-B6 =large individuals; M. 100 bp DNA ladder

growing and slow-growing individuals of soang gourami fingerling collected from single spawning.

The *Hind*III enzyme was unable to digest cytochrome b gene. This was proven by a fact that digested products have similar sizes to undigested PCR products, i.e. 525 bp length fragments. The cytochrome b-*Hind*III fragments are presented in Figure 5.

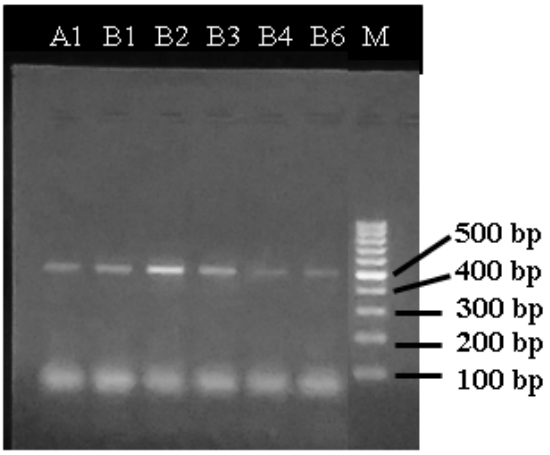

Figure 5. Restriction fragment resulted from *Hind*III digestion
Remarks: A1-A6 = small individuals; B1-B6 = large individuals; M = 100 bp DNA ladder

Inability of *Hind*III enzyme to cut the PCR product was due that on cytochrome b gene no restriction site available which can be recognized by the enzyme. We could not compare our study to previous studies because so far there were no studies in fish species that utilized *Hind*III enzyme to digest cytochrome b gene (Nebola et al., 2010; Cheng & Lu, 2005; Takehana et al., 2004; Lin et al. 2002; Hold et al., 2001; Cocolin et al., 2000). The reason could be due that those researchers realized that there were no *Hind*III restriction sites on cytochrome b gene on various fish species. Therefore, it was not surprising that we could not observed any *Hind*III restriction sites on cytochrome b gene of soang gourami fingerling so the gene was not digested by HindIII enzyme. Therefore, the cytochrome b-*Hind*III can not be used as specific marker for size selection on soang giant gourami fingerling collected from single spawning.

It was common that peoples studied growth variation among individuals from a single spawning using growth hormone gene or growth factor gene as genetic markers. However, we were unsuccessful to amplify those gene from soang giant gourami fingerling (Nuryanto et al., 2014). Different to previous studies, in this study, we used indirect estimation on observing genetic variation among individuals with different body sizes but resulted from single spawning using an alternative gene that is cytochrome b. This gene was selected due to its function as co-enzyme in cellular metabolisms. Therefore, it was expected that the variation in that gene will result in variation of metabolism rates among individuals which lead to different growth rate. The final result would be differences in body sizes among individuals. Therefore, the utilization of cytochrome b gene in such study is a novelty.

Our result proved that RFLP markers of cytochrome b-*Hinf*I and –*Alu*I were variable among individuals and their variations were related to body sizes of each individual. According to this result, those two RFLP markers can be used as a fundament for developing a new technique

for fingerling selection to obtain high quality fingerling. This is a new contribution of our study on the development of giant gourami cultivation. From scientific view, our study provide new insight about the application of molecular technique in applied sciences, e.g. in aquaculture.

CONCLUSIONS

During the study, it was observed that Cytochrome b-*Hinf*I and –*Alu*I variation in soang giant gourami fingerling population were correlated to their body size. Therefore, both PCR-RFLP markers can be used as specific markers to differentiate large and small individuals of sowang giant gourami fingerling from single spawning.

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REFFERENCES

Achmad, M., Alimuddin, Carman, O., Arfah, H. & Zairin, M. (2009). Penggunaan gen GH sebagai marka molekuler DNA gurami, *Osphronemus goramy* dalam pengembangan teknologi surrogate broodstock. *Jurnal Perikanan,* 11(2), 157-160.

Ali, M. E., Hashim, U., Mustafa, S. & Che Man, Y. (2011). Swine-Specific PCR-RFLP Assay Targeting Mitochondrial Cytochrome B Gene for Semiquantitative Detection of Pork in Commercial Meat Products. *Food Anallytical Methods*, 5(3), 613-623.

Apostolidis, A.P., Karakousis, Y. & Triantaphyllidis, C. (1996). Genetic differentiation and phylogenetic relationships among Greek *Salmo trutta* L. (brown trout) populations as revealed by RFLP analysis of PCR amplified mitochondrial DNA segments. *Heredity*, 77, 608-618.

Azizah, S. N., Nuryanto, A. & Pramono, H. (2015). Karakterisasi Molekuler Ikan Gurami Soang (*Osphronemus goramy* Lacepede, 1801) berbeda ukuran menggunakan PCR-RFLP Gen Sitokrom C Oksidase 1. *Biosfera*, 32(3), 185-193.

Azrita, S. (2015). Morphological character among five strains of giant gourami, *Oshpronemus goramy* Laceped, 1801 (Actinopterygii: Perciformes: Osphronemidae) using a truss morphometric system. *International Journal of Fisheries and Aquatic Studies*, 2(6), 344-350.

Cantatore, P., Roberti, M., Pesole, G., Ludovico, A., Milella, F., Gadaleta, M.N., & Saccone, G. (1994). Evolutionary analysis of cytochrome b sequence in some Perciformes: Evidence for a slower rate of evolution than in Mammals. *Journal of Molecular Evolution*, 39(6), 589-597..

Cheng, Q. Q. & Lu, D. R. (2005). PCR-RFLP analysis of cytochrome b gene does not support *Coilia ectenes taihuensis* being a subspecies of *Coilia ectenes*. *Journal of Genetics*, 84(3), 307-310.

Cocolin, L., D'agaro, E., Manzano, M., Lanari, D., & Comi, G. (2000). Rapid PCR-RFLP method for the identification of marine fish fillets (Seabass, Seabream, Umbrine, and Dentex. *Journal of Food Science*, 65(8), 1315-1317.

Fibriana, F., Widianti, T., & Retnoningsih, A. (2012). Deteksi Daging Babi Pada Produk Bakso di Pusat Kota Salatiga Menggunakan Teknik Polymerase Chain Reaction. *Biosaintifika: Journal of Biology & Biology Education*, 4(2).

Hold, G. L., Russell, V. J., Pryde, S. E., Rehbein, H., Quinteiro, J., Vidal, R., Rey-Mendez, M., Sotelo, C. G., Pe´rez-Martin, R. I., Santos, A. T. & Rosa, C. (2001). Development of a DNA-based method aimed at identifying the fish species present in food products. *Journal of Agricultural and Food Chemistry,* 49(3), 1175-1179.

Hua, G.H., Chen, S.L., Yu, J.N., Cai, K.L., Wu, C.J., Li, Q.L., Zhang, C.Y., Liang, A.X., Han, L., Geng, L.Y., Shen, Z., Xu, D.Q. & Yang, L.G. (2009). Polymorphism of the growth hormone gene and its association with growth traits in Boer goat bucks. *Meat science,* 81(2), 391-395.

Kartikaningsih, H., Suryanni, Y., Subagyo., Hariati, A. M. & Wiadnya, D. G. R. (2001). Peranan Chitin dari Limbah Pengolahan Udang sebagai Pemacu Pertumbuhan Ikan Gurami (*Ospheronemous goramy*, Lac.). *Jurnal Ilmu-ilmu Hayati (Life Science)*, 13(1).

Kusbiyanto, Nuryanto, A. & Soedibja, P. H. T. (2016). Deteksi gene *major histocompatibility complex class II* pada yuwana gurami soang, *Osphronemus goramy* Lacepede, 1801 asal satu pemijahan. *Jurnal Iktiologi Indonesia*, 16(3), 279-288.

Lin, Y. S., Poh, Y. P., Lin, S. M. & Tzeng, C. S. (2002). Molecular techniques to identify freshwater eels: RFLP analyses of PCR-amplified DNA fragments and allele-specific PCR from mitochondrial DNA. *Zoological Studies*, 41(4), 421-430.

Ma, C., Cheng, Q., Zhang, Q., Zhuang, P. & Zhao, Y. (2010). Genetic variation of *Coilia ectenes* (Clupeiformes: Engraulidae) revealed by the complete cytochrome b sequences of mitochondrial DNA. *Journal of Experimantal Marine Biology and Ecology*, 385(1), 14-19

Mesquita, M., Carvalho, G., Shaw, P., Crespo, E. &

Coelho, M. M. (2001). River basin-related genetic structuring in an endangered fish species, *Chondrostoma lusitanicum*, based on mtDNA sequencing and RFLP analysis. *Heredity*, 86(3), 253-264.

Meyer, A., Kocher, T., Basasibwaki, P. & Wilson, A. (1990). Monophyletic origin of Lake Victoria cichlid fishes suggested by mitchondrial DNA sequence. *Nature*, 347(6293), 550-553.

Millah, M. I., Habibah, N. A., Suwarni, E. & Retnoningsih, A. (2012). Analysis of genetic diversity and differentiation of java and madura teak based on microsatellite markers to support teak fingerprinting. *Biosaintifika: Journal of Biology & Biology Education*, 4(2). 113-120.

Nebola, M., Borilova, G. & Kasalova, J. (2010). PCR-RFLP analysis of DNA for the differentiation of fish species in seafood samples. *Bulletin of the Veterinary Institute in Pulawy*, 54(1), 49-53.

Nugroho, E., Satyani, D. & Rusmaedi. (1993). Evalusi potensi genetik dari beberapa ras gurame. *Buletin Penelitian Perikanan Darat*, 12(1), 30-36.

Nurdjana, M. L. (2008). *Roadmap mencapai industri perikanan budidaya yang tangguh di tahun 2025*. Safari Garden Hotel, Bogor, 2-5 Desember : Forum pemantapan konsep rencana pembangunan jangka panjang perikanan budidaya 2010-2025.

Nuryanto, A., Setyowati, E. A. & Kusbiyanto. (2014). Molecular characterization of soang giant gourami. *Research Report*. Research and Public Services of Unsoed, Purwokerto.

Setijaningsih, L., Arifin, O. Z. & Gustiano, R. (2007). Karakterisasi tiga strain ikan gurame (*Osphronemus goramy* Lac.) berdasarkan metode truss morfometriks. *Jurnal lktiologi Indonesia*, 7(1), 23-30.

Takehana, Y., Uchiyama, S., Matsuda, M., Jeon, S. R. & Sakaizumi, M. (2004). Geographic variation and diversity of the cytochrome b gene in wild populations of medaka (*Oryzias latipes*) from Korea and China. *Zoological Science*, 21(4), 483-491.

Walsh, P. S., Metzger, D. A. & Higuchi, R. (1991). Chelex-100 as a medium for simple extraction of DNA for PCR Based Typing From Forensic Material. *Biotechniques*, 10(4), 506-13.

Yurisma, E. H., Abdulgani, N. & Mahasri, G. (2013). Pengaruh salinitas yang berbeda terhadap laju konsumsi. *Jurnal Sains dan Seni*, 1(1), 1-4.

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Identification of Soybean Genotypes for Pod Shattering Resistance Associated with Agronomical and Morphological Characters

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Glycine max; pod shattering; resistance

Abstract

A yield loss caused by pod shattering is one of the obstacles to the improvement of soybean productivity in tropical areas. The aim of this study was to identify the resistance of soybean genotypes to pod shattering as affected by agronomical and morphological characters. The field study was conducted in Malang, Indonesia, using 150 soybean genotypes. Data were collected on agronomical traits, the percentage of pod shattering, and pod morphological traits. Identification for shattering resistance was done as per oven dry method. Percentage of pod shattering was ranged from 0 % up to 100 % shattering with a mean of 58.11 %. Pod shattering was found to be negatively correlated with a number of pod per plant, the thickness of the pod, and Y/Z (seed weight and pod weight ratio). The Identification obtained 66 very highly susceptible genotypes, 19 susceptible genotypes, 19 moderate genotypes, 38 resistant genotypes, and 8 very resistant genotypes. Two of eight very resistant genotypes (G511H/Anj//Anj///Anj////Anj-6-11 and G511H/Anj//Anj///Anj////Anj-5-4) have high yield, medium maturity day and large seed size. Those lines could be used as gene donor for soybean varietal improvement for shattering resistance, and recommended to propose as new improved soybean varieties resistant to pod shattering in Indonesia.

How to Cite

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INTRODUCTION

Soybean is the third most important crops after rice and maize in Indonesia. Pod shattering is one of major constraint associated with soybean production in the tropical ecology of Indonesia. It is due to soybean cultivation mostly planted in the second dry season (June / July until September / October), and characterized by hot and dry conditions, thereby pod shattering increases could lead to serious seed yield losses. In the USA, it was reported that seed shattering is considered as one of the major problems for soybean growers under the ESPS (early soybean production system) conditions (Zhang & Bellalouli, 2012).

Pod shattering refers to the opening of mature pods along the dorsal or ventral sutures of the soybean pod and dispersal of seed as the crop reaches maturity, as well as during harvesting (Bhor et al., 2014) resulting in seed loss. The yield loss due to pod shattering in soybean may range from 34 % to 100 % (Tefera et al., 2009; Khan et al., 2013) depend on the harvesting after maturity, environmental condition (Zhang & Boahen, 2010), chemical composition of the pod wall (Fitriana et al., 2009), plant growth regulator (Gulluoglu et al., 2006), anatomical structure of the pod, and genetic factor of the variety (Suzuki et al., 2009). Pod shattering is induced by low humidity, high temperature, and rapid temperature changes (Mohammed, 2010). Furthermore, it was also enhanced when dry-weather followed rains at harvesting (Liu et al., 2016; Kuai et al., 2016).

Pod shattering behavior of soybean variety was found to be associated with other agronomic, morphological, and physiological characteristics (Kang et al., 2009; Adeyeye et al., 2014). A report by Tiwari & Bhatia (1995) stated that that the thickness and length of the bundle cap on the dorsal side of the soybean pod and thickness of the pod were negatively and significantly correlated with the degree of pod shattering. Another study revealed that genotype with the small pod, less width and low volume/weight of seed was tolerant to pod shattering (Bara et al., 2013).

Shattering in soybean was the most important trait among the important characteristics of the soybean plant. Hence, the development of highly shattering-resistant cultivars in soybean is important to prevent significant seed loss. A study elucidated that resistance to pod shattering is one of great economic benefit to farmers in the hot tropics and areas where machines are used for harvesting regarding reduced yield losses and planning for time and labour (Zuo et al., 2014). A survey conducted in Benue state, Nigeria, revealed that resistance to pod shattering was a prerequisite for the adoption of any variety by the farming communities (Sanginga et al., 1999). A report by Funatsuki et al. (2008) stated that highly shattering-resistant cultivars had been preferably developed and cultivated in some regions where soybean cultivation has been carried out on a large scale with the use of combine harvesters.

A genetic variability is an important tool for the selection in the soybean varietal improvement program. Therefore, the breeding program for shattering resistance in soybean should be considered the affecting factors, the availability of gene source and suitable selection method. Further investigations are therefore needed in those aspects. Nowadays, the major emphasis of soybean variety improvement in Indonesia is focused on producing high-yielding cultivar, as well as for early maturity variety. Moreover, pod shattering becomes one of the problems in the improvement of potential soybean production. Hence, it is important to develop a new improved variety with pod shattering resistance to minimize yield losses. The objective of the research was to identify the resistance of soybean genotypes to pod shattering as affected by agronomical and morphological characters.

METHODS

The type of soil was Entisol Association and Inceptisol, the elevation was 335 m above sea level, and Oldeman climate type was C3. The research materials consist of 150 soybean genotypes, and the research was arranged in a randomized complete block design with two replications. Grobogan, Anjasmoro, and Argomulyo were used as check varieties. Each genotype was planted in 1.2 m × 4.5 m plot size with 40 cm × 15 cm planting distance, two plants per hill. Pests and diseases were controlled optimally. Drainage was applied to maintain optimum soil moisture. Fertilization with 250 kg ha^{-1} Phonska, 100 kg ha^{-1} SP 36, and 1 t ha^{-1} organic fertilizer at planting time. The data was collected started from days after planting to harvesting period (calculated if 95 % of the leaves have turned yellow), number of pods (taken from average of five randomly sample plants), 100 seed weight (g), and seed yield (randomly taken from the seed yield per plot and converted to t ha^{-1}).

Pod shattering identification was done as per oven dry method. The evaluation of pod shattering resistance sample was taken randomly,

that is 25 fully matured pods of each plot. The samples were drying to the oven at 30 °C for three days, and the temperature was elevated 10 °C for the next four days, respectively. On the 7th day, the numbers of shattered pods were counted, and every genotype was classified into a different category based on the percentage of shattered pods.

Observations on the pod morphological traits consists of: length of pod (A), width of pod (B), length-width ratio (A/B), width-length ratio (B/A), thickness of pod (C), width at mid part of the pod (D), thickness of pod and width ratio (C/B). Observation sample was from 25 fully matured pods of each plot, consists of: seed weight from 25 pods (X), pod wall weight of 25 pods (Y), pod weight (weight of pod wall and seed) of 25 pods (Z), seed weight and pod weight ratio (Y/Z), and pod wall weight and pod weight ratio (X/Z).

Data were subjected to analysis of variance (ANOVA) and continued with DMRT at 5% significance level. Data on pod shattering was subjected to arcsine-square root transformation before statistical analysis. The data were also subjected to Pearson correlation analysis to determine the relationship between the pod shattering and agronomical as well as pod morphological traits.

RESULTS AND DISCUSSION

Analysis variance elucidated a significant variation in all agronomic and morphological pod characteristics, except for a number of pods per plant and pod width. Pod shattering also showed significant variability among genotypes (Table 1). The significant value revealed the existence of genotypic differences among the genotypes tested. The coefficient of variation (CV) ranged from 1.90% to 27.16%.

Mean, range, and standard deviation for observed traits are presented in Table 2. The performances of different soybean genotypes under field conditions was indicated by days to maturity, a number of pod per plant, 100 seed weight, seed yield. Days to maturity ranged from 76 d to 84 d with a mean of 79 d. Days to maturity is classified into late maturity (> 90 d), medium maturity (80 d to 90 d), and short maturity (< 80 d), thus all the observed genotypes including to early and medium maturity. Early maturing soybean provides many benefits, i.e. minimizing the yield loss due to drought stress, and increase the cropping intensity within a year (Krisnawati & Adie, 2008).

A number of pod per plant ranged from 27 to 66 with a mean of 42. The seed size, which reflected by 100 seed weight, consists of medium

Table 1. The scoring rate was as follows (Krisnawati & Adie, 2017)

Score	Description	Category
1	No pod shattering	Very Resistant
2	< 25% pod shattering	Resistant
3	25 – 50% pod shattering	Moderately Resistant
4	51 – 75% pod shattering	Highly Susceptible
5	> 75% pod shattering	Very Highly Susceptible

Table 2. Analysis of variance of 150 soybean genotypes.

Parameter	Mean Square		CV (%)
	Replication	Genotype	
Days to maturity (d)	41.813**	8.691**	1.90
Number of pod/plant	55.987ns	100.747ns	23.28
100 seed weight (g)	1.594ns	4.092**	5.85
Seed yield (t ha^{-1})	3.257**	0.439**	19.05
Length of pod (cm)	0.102ns	0.244**	7.19
Width of pod (cm)	0.108*	0.022ns	12.86
Width at mid part of pod (cm)	0.033**	0.005**	5.88
Thickness of pod (cm)	0.068ns	0.312**	7.29
Pod shattering (%)	0.604ns	22.833**	27.16
Weight of 25 seeds (g)	0.002ns	3.361**	10.73

CV = coefficient of variation, **= significant at 1 % probability level (p < 0.01), ns = not significant

and large seeded. Seed yield varied from low yield (0.69 t ha^{-1}) to relatively high yield (2.96 t ha^{-1}) with a mean of 2.06 t ha^{-1}. In Indonesia, soybean seed size is divided into three categories: small (< 10 g per 100 seeds), medium (10 g to14 g per 100 seeds), and large size (> 14 g per 100 seeds) (Adie & Krisnawati, 2007). In this study, the average seed size was large seeded. Large seeded-size soybean is desirable trait in tempeh industry because it will produce tempeh with large volume (Krisdiana, 2005).

Observations on the physical traits consist of parts of the pod, i.e. the length of the pod, width of the pod, the width at mid part of the pod, the thickness of pod, and ratio of pod parts. A research by Bara et al. (2013) showed that those traits showed high genetic advance, which means that its phenotype reflects the genotype or assuming the absence of environmental effects. Hence, the selection for a specific genotype will be accurate within the limits imposed by the environmental effects. A further explanation by Rohman & Hussain (2003), a high genetic advance was associated with high value of heritability indicating additive gene effect in controlling the characters.

Percentage of pod shattering had a broad range; it was from no shattering up to 100% shattering. The mean shattering was 58.11%. The weight of seed from 25 samples of pods varied from 8.16 g to 15.92 g, with an average of 11.38 g. The weight of the pod wall also measured, and it ranged from 2.94 g to 7.24 g. Hence, the pod weight total (the weight of pod wall and seed) ranged from 11.09 g to 22.57 g (with a mean of 15.89 g). According to Bara et al. (2013), the thickness of pod as one of pod traits was more reliable for selection for improvement by simple selection procedure. This due to the trait was less influenced by the environment.

Identification for shattering resistance

Pod shattering as one of the major constraints in soybean could reduce the yield potential considerably. As a consequence, the management of pod shattering is great importance for achieving higher productivity. Furthermore, identification of resistant genotypes to pod shattering is one of the most important aspects of the management of pod shattering. However, the other countries (USA, Uganda, and Nigeria) have released soybean variety with pod shattering resistant, for example, 'Maksoy 1N' and 'Maksoy 2N' (Anonim, 2014a), Glenn (Anonim, 2014b), and TGX 1448-2E (Mohammed, 2010). In the present study, 150 soybean genotypes were evaluated for pod shattering resistance under laboratory condition. The pod shattering resistance

Table 3. Mean, range, and standard deviation for observed traits of 150 soybean genotypes.

Observation	Mean	Min	Max	SD
Days to maturity (d)	79	76	84	2.08
Number of pod per plant	42	27	66	7.10
100 seed weight (g)	15.80	13.18	22.13	1.43
Seed yield (t ha^{-1})	2.06	0.69	2.96	0.47
Length of pod (A) (cm)	4.36	3.55	5.32	0.34
Width of pod (B) (cm)	1.04	0.87	2.02	0.10
Width at mid part of pod (D) (cm)	0.91	0.75	1.05	0.05
Thickness of pod (C) (cm)	0.57	0.45	0.67	0.04
A/B	4.21	2.39	5.24	0.41
D/A	0.21	0.18	0.25	0.02
B/A	0.24	0.19	0.42	0.03
C/B	0.55	0.29	0.66	0.04
Pod shattering (%)	58.11	0.00	100.00	39.25
Weight of 25 seeds (X)	11.38	8.16	15.92	1.28
Weight of pod wall of 25 pods (Y)	4.51	2.94	7.24	0.77
Pod weight of 25 pods (Z)	15.89	11.09	22.57	1.92
X/Z	0.72	0.62	0.79	0.02
Y/Z	0.28	0.21	0.38	0.02

Min = minimal value, max = maximal value, SD = standard deviation

was classified into five categories (Fig.1) with pod shattering percentage ranged from 0% to 100%. The genotypes resistance consists of very highly susceptible (66 genotypes or 44%), highly susceptible (19 genotypes or 12.67%), moderate (19 genotypes or 12.67%), resistant (38 genotypes or 25.33%), and very resistant (8 genotypes or 5.33%).

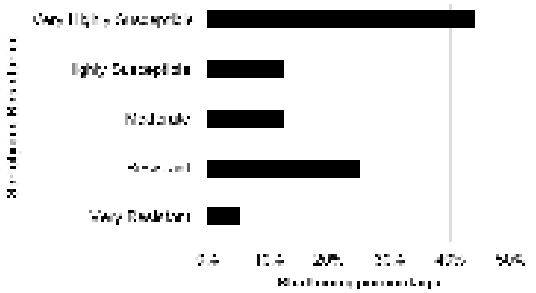

Figure 1. Pod shattering resistance category of 150 soybean genotypes.

The very resistant genotypes in this research showed no shattered pods. This finding is in line with research by Tukamuhabwa et al. (2002) which found three soybean genotypes (TGx 1448-2E, Duiker and Nam 2) demonstrated a high level of shattering resistance by showing no loss over the harvesting period. Thus, the use of resistant varieties was recommended as good sources of resistance in breeding for shattering resistance, and the use of susceptible varieties should be avoided since they start shattering on commencement of maturity resulting high yield loss. Another study by Khan et al. (2013) found that pod shattering percentage ranged from 8.7% (Himsoy-1560) to 93.3% (Punjab-1), and there is no variety resistant to pod shattering. Screening for shattering resistance by Bara et al. (2013) showed that shattering percentage ranged from 0.6730% (JSM 170) to 67.05% (JSM 131) with a mean of 19.11%. He also found that the rate of seed shattering accelerated after 7 days, and enhanced with the age of the matured pod. With the age of the plant, pod shattering is also influenced by the high temperature at the time of maturity. Screening for pod shattering resistance using oven method by Krisnawati et al. (2015), obtained the various degree of shattering (0% to 80%). Another study by Krisnawati & Adie (2016) found a number of shattered pods in the laboratory (oven method) ranged from 7 to 26 pods (22.2% to 87.2%). Enhancement in shattering resistance may promote productivity, harvesting of uniformly ripe seeds, efficiency of seed recovery and improved oil extraction. Moreover, it also promote the adjustment in harvesting and threshing time; reduction in cost of production, problem of volunteer plants (Morgan et al., 1998), and longevity of seed (Bara et al., 2013).

(A) (B)

Figure 2. Identification for pod shattering resistance using oven method; (A) Susceptible genotype; (B) Resistant genotype

Table 3 showed the effect of different agronomic parameters and morphological characteristics of soybean pod on pod shattering. Pod shattering behavior of soybean variety was reported to be associated with other agronomic characteristics. Adeyeye et al. (2014) observed many variabilities existed regarding vegetative growth, seed yield and shattering ability among the varieties tested. It revealed the existence of genotypic differences among varieties. In this research, the 100 seed weight, width of the pod, and width at mid part of the pod had no significant effect on a number of shattered pods per plant, which indicated that these parameters would not be useful as an index for pod shattering selection. Research by Tsuciya (1987) also found that 100 seed weight had no significant effect on pod shattering, whereas Bara et al. (2013) reported a significant and positive association of shattering percentage with pod width and width at mid part of the pod.

Furthermore, pod shattering was found to be negatively correlated with number of pod per plant, thickness of the pod, and Y/Z ratio. It implied that the increasing in the number of pod per plant, the thicker of the thickness of the pod, and the higher of Y/Z ratio will have a lower pod shattering, respectively. Meanwhile, the length of the pod and X/Z ratio were found to be significantly correlated to pod shattering. It means that the longer length of the pod, the pod shattering is also enhanced; as well as an increase of pod wall weight will result in a higher pod shattering percentage. This finding is in agreement with Adeyeye et al. (2014) and Bhatia & Tiwari (1994), which recommended the large seed (bigger diameter) and pod thickness as re-

liable index and indicator in selecting for shattering in soybean breeding program. Similarly, the newest study by Krisnawati & Adie (2017) revealed that pod length is one of the essential factors associated with pod shattering resistance, as well as pod wall thickness. However, this is not in agreement with the earlier report by Morgan et al. (1998) which stated that genotype with the small pod (with less width and weight of periphery region) and low volume/weight of seed has a low shattering percentage. The knowledge of correlation existing between characters is of great use in breeding programmes to easily identify those characters that may use as selection indices (Adeyeye et al., 2014).

In this study, the thicker pod and the higher of Y/Z ratio (larger seed size) will result to a lower pod shattering. Various studies of pod anatomy in detail have been conducted, and certain anatomical structures of the soybean pod have been recognized as important for resistance to shattering. Examination of the dehiscence zone of soybean pod and the expression analysis of the soybean endo polygalacturonase transcript revealed that the endo polygalacturonase was primarily found in dehiscence-related tissue and was presumably involved in the breakdown of the middle lamella before dehiscence (Christiansen et al., 2002). A study by Dong et al. (2014) revealed that the excessively lignified fiber cap cells (FCC) with the abscission layer unchanged in the soybean pod ventral suture as the key cellular feature of the shattering-resistant trait. Meanwhile, Funatsuki et al., (2014) revealed important aspects of pod shattering, namely, the dehiscing force and the associated regulatory gene.

The agronomic characters of genotypes with very resistant category were presented in Table 4. The eight selected genotypes have maturity from 78 days to 83 days, 100 seed weight ranged from 14.06 g per 100 seeds to 15.84 g per 100 seeds, and the seed yield ranged from 1.31 t ha^{-1} to 2.60 t ha^{-1}.

All the check varieties have short maturing day and large seed size. The popular variety of Anjasmoro was categorized as resistant to pod shattering. Meanwhile the varieties of Grobogan and Argomulyo categorized as very high susceptible, respectively.

The incorporation of high yielding and pod shattering resistant is one of pursued goal in Indonesian soybean breeding programmes. In this study, the genotype with the shortest maturing day was G511H/Anjasmoro//Anjasmoro-5-6, but it produced a low yield (1.70 t ha^{-1}). There were two high yielding genotypes (G511H/Anj//Anj///Anj////Anj-6-11 and G511H/Anj// Anj///Anj////Anj-5-4), higher than the check cultivars used. Both of lines have characteristics of medium maturity day, large seed size, and produced a yield of 2.52 t ha^{-1} and 2.60 t ha^{-1}, respectively. Since pod shattering is a qualitative heritable trait (Yamada et al., 2009; Mohammed, 2010; Sujata et al., 2012), thus these lines could be used as donor for shattering resistance, or could be proceed to the next selection step of breeding to be released as new soybean varieties with high yielding and pod shattering resistance, considering that those desirable characteristics are important for tropical area of Indonesia.

Table 4. Correlation analysis among agronomical, morphological characters and shattering percentage of 150 soybean genotypes.

Characteristic	1	2	3	4	5	6	7	8	9
(1) Pod shattering	1								
(2) Number of pod per plant	-0.171*	1							
(3) 100 seed weight	0.092ns	-0.210**	1						
(4) Length of pod (A)	0.440**	0.354**	-0.246**	1					
(5) Width of pod (B)	-0.139ns	0.124ns	-0.093ns	0.193*	1				
(6) Width at mid part of pod (D)	0.027ns	0.408**	-0.156ns	0.411**	0.277**	1			
(7) Thickness of pod (C)	-0.441*	0.291**	-0.069ns	-0.110ns	0.326**	0.274**	1		
(8) Y/Z ratio	-0.3375**	-0.1566ns	0.0792ns	-0.3942**	0.057ns	-0.1580**	0.1585ns	1	
(9) X/Z ratio	0.3374**	-0.156ns	0.079ns	-0.394**	-0.057ns	-0.158**	0.158ns	-1.000**	1

Y/Z = seed weight and pod weight ratio, X/Z = pod wall weight and pod weight ratio, **= significant at 1 % probability level ($p < 0.01$), * = significant at 5 % probability level ($p < 0.05$), ns = not significant.

Table 5. Agronomic characters of pod shattering resistance genotypes.

Genotypes	Days to maturity (d)	100 seeds weight (g)	Yield (t ha^{-1})	Category
G 511 H/Anjasmoro-1-7	81	14.60	1.98	VR
G 511 H/Anjasmoro//Anjasmoro-5-2	81	14.96	1.61	VR
G 511 H/Argom//Argom-2-1	81	14.73	2.15	VR
G 511 H/Anjasmoro//Anjasmoro-5-6	78	14.20	1.70	VR
G 511 H/Anj//Anj///Anj////Anj-6-11	82	15.84	2.52	VR
G 511 H/Anj//Anj///Anj////Anj-6-12	84	14.06	1.32	VR
G 511 H/Anjasmoro//Anjasmoro-5	81	15.69	2.03	VR
G 511 H/Anj//Anj///Anj////Anj-5-4	83	14.80	2.60	VR
Check varieties:				
Anjasmoro	79	14.65	2.37	R
Grobogan	77	18.71	1.46	VHS
Argomulyo	77	15.49	2.41	VHS

VR = very resistant, R = resistant, VHS = very highly susceptible

CONCLUSION

Pod shattering elucidated significant variability among genotypes. The genotypes resistance consists of very highly susceptible (66 genotypes), highly susceptible (19 genotypes), moderate (19 genotypes), resistant (38 genotypes), and very resistant (8 genotypes). The thicker of the thickness of the pod and the higher of Y/Z ratio (larger seed size) will result to a lower pod shattering. Two very resistant genotypes (G511H/Anj//Anj///Anj////Anj-6-11 and G511H/Anj// Anj///Anj////Anj-5-4) have high yield, medium maturity day and large seed size. Those lines could be proposed as new improved soybean varieties in Indonesia.

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REFERENCES

Adie, M. M., & Krisnawati, A. (2007). The Biology of Soybean Plant. In: *Production Technique and Development of Soybean*. Indonesian Agency for Agricultural Research and Development. Indonesian Center for Food Crops Research and Development. Bogor. pp. 45-73.

Adeyeye, A. S., Togun, A. O., Akanbi, W. B., Adepoju, I. O., & Ibirinde, D. O. (2014). Pod shattering of different soybean varieties, *Glycine max* (L) Merrill, as affected by some growth and yield parameters. *International Journal of Agricultural Policy and Research*, 2 (1), 010-015.

Anonim. (2014a). *Glenn Soybean Variety Description*. VCE (Virginia Cooperative Extension). Avalaible at http://www.virginiacrop.org/vcia.soyvar.htm. Accessed on 31 March, 2015.

Anonim. (2014b). *Grow New Soybean Varieties for Better Yields*. AGRA (Growing Africa's Agriculture). Available at http://www.soybeanafrica.com/Commercial-soybean-varieties-Uganda.pdf. Accessed on 31 March, 2015.

Bara, N., Khare, D., & Srivastava, A. N. (2013). Studies on the factors affecting pod shattering in soybean. *Indian Journal of Genetics*, 73 (3), 270-277.

Bhatia, V. S., & Tiwari, S. P. (1994). Pod-wall thickness as a selection criterion for shattering resistance in soybean. *Soybean Gene Newslett,* 21, 98-100.

Bhor, T. J., Chimote, V. P., & Deshmukh, M. P. (2014). Inheritance of pod shattering in soybean [*Glycine max* (L.) Merrill]. *Electronic Journal of Plant Breeding*, 5(4), 671-676.

Christiansen, L. C., Degan, F. D., Ulvskov, P., & Borkhardt, B. (2002). Examination of the dehiscence zone in soybean pods and isolation of a dehiscence-related endopolygaacturonase gene. *Plant, Cell & Environment,* 25(4), 479-490.

Dong, Y., Yang, X., Liu, J., Wang, B. H., Liu, B. L., & Wang, Y. Z. (2014). Pod dehiscence resistance associated with domestication is mediated by a NAC gene in soybean. *Nature communications, 5(3352)*, 1-11.

Fitriana, J., Pukan, K. D., & Herlina, L. (2009). The activity of nitrate reductase enzymes of soybean var. Burangrang in a variety of water levels at the commencement of the pod fill-up. *Biosaintifika: Journal of Biology & Biology Education*, 1(1), 1-8.

Funatsuki, H., Hajika, M., Hagihara, S., Yamada, T., Tanaka T., Tsuji H., Ishimoto, M., & Fujino, K. (2008). Confirmation of the location and the effects of a major QTL controlling pod de-

hiscence, qPDHI, in soybean. *Breeding Science,* 58(1), 63-69

Funatsuki, H., Suzuki, M., Hirose, A., Inaba, H., Yamada, T., Hajika, M., ... & Fujino, K. (2014). Molecular basis of a shattering resistance boosting global dissemination of soybean. *Proceedings of the National Academy of Sciences*, 111(50), 17797-17802.

Gulluoglu, L., Halis. A., Mehmet, A. (2006). Effects of some plant growth regulators and nutrient complexes on pod shattering and yield losses of soybean under hot and dry conditions. *Asian Journal of Plant Sciences*, 5, 368-372.

Kang, S. T., Kwak, M., Kim, H. K., Choung, M. G., Han, W. Y., Baek, I. Y., ... & Lee, S. H. (2009). Population-specific QTLs and their different epistatic interactions for pod dehiscence in soybean [Glycine max (L.) Merr.]. *Euphytica*, 166(1), 15.

Khan, M. H., Tyagi, D. S., & Dar, Z. A. (2013). Screening of soybean (*Glycine max* (L.) Merrill) genotypes for resistance to rust, yellow mosaic and pod shattering. *Intech*, Chapter 7, www.intechopen.com/download/pdf/42600.

Krisdiana, R. (2005). Preference of tofu and tempeh industry within using soybean raw material in East Java. *In*: Makarim AK, Marwoto, Adie MM, Rahmianna AA, Heriyanto, Tastra IK, editors. Research Performance Supporting the Legumes and Tuber Agribusiness, Bogor: Indonesian Center for Food Crops Research and Development, pp. 540-548.

Krisnawati, A., & Adie, M. M. (2008). Variability of days to maturity and its contribution to the soybean yield. *Agritek*, 17(11), 1-7.

Krisnawati, A., & Adie, M. M. (2016). Pod shattering resistance in different soybean genotypes. *In* Proceedings of ILETRI National Seminar. ILETRI, IAARD, Malang.

Krisnawati, A., & Adie, M. M. (2017). Variability on morphological characters associated with pod shattering resistance in soybean. *Biodiversitas*, 18(1), 73-77.

Krisnawati, A., Adie, M. M., & Harnowo, D. (2015). Variability of physical characters of pod related to the pod shattering resistance. *In* Proceedings of ILETRI National Seminar. ILETRI, IAARD, Malang.

Kuai, J., Sun, Y., Liu, T., Zhang, P., Zhou, M., Wu, J., & Zhou, W. (2016). Physiological mechanisms behind differences in pod shattering resistance in rapeseed (*Brassica napus* L.) varieties. *PLoS ONE*, 11(6), 1-12.

Liu, J., Wang, J., Wang, H., Wang, W., Zhou, R., Mei, D., ... & Hu, Q. (2016). Multigenic Control of Pod Shattering Resistance in Chinese Rapeseed Germplasm Revealed by Genome-Wide Association and Linkage Analyses. *Frontiers in plant science*, 7, 1058.

Mohammed, H. (2010). Genetic analysis of resistance to pod shattering in soybean (*Glycine max*. (L) Merrill). *Thesis*. The Department of Crop and Soil Sciences, Faculty of Agriculture of the College of Agriculture and Natural Resources, Kwame Nkrumah University of Science and Technology, Kumasi.

Morgan, C.L., Bruce, D.M., Child, R., Ladbrooke, Z.L., & Arthur, A.E. (1998). Genetic variation for pod shatters resistance among lines of oilseed rape developed from synthetic *Brassica napus*. *Field Crops Research*, 58(2), 153-165

Rohman, M. M. & Hussain, A. S. M. (2003). Genetic variability, correlation and path analyses in mugbean. *Asian Journal of Plant Sciences*, 2(17-24), 1209-1211.

Sanginga, P. C., Adesina, A. A., Manyong, U. M., Otite, O., & Dashiell, K. E. (1999). Social impact of soybean in Nigeria's southern Guinea savanna. IITA, Ibadan Nigeria.

Sujata, B., Basavaraja, G. T., & Salimath, P. M. (2012). Analysis of variability in segregation of soybean (*Glycine max* L Merill). *Karnataka Journal of Agricultural Sciences*, 25(2), 176-178.

Suzuki, M., Fujino, K., & Funatsuki, H. (2009). A major soybean QTL, *qPDH1*, controls pod dehiscence without marked morphological change. *Plant production science,* 12(2), 217-223.

Tefera, H., Bandyopadhyay, R., Adeleke, R. A., Boukar, O., & Ishaq, M. O. H. A. M. M. E. D. (2009). Grain yields of rust resistant promiscuous soybean lines in the Guinea savanna of Nigeria. In *9th African Crop Science, Conference Proceedings, Cape Town, South Africa, 28 September-2 October 2009* (pp. 129-134). African Crop Science Society.

Tiwari, S., & Bhatia, V. S. (1995). Characterization of pod anatomy associated with resistance to pod-shattering in soybeans. *Annals of botany,* 76(5), 483-485.

Tsuciya, T. (1987). Physiological and genetic analysis of pod shattering in soybeans. *Japan Agricultural Research Quarterly*, 21(3), 166-175.

Tukamuhabwa, P., Dashiell, K. E., Rubaihayo, P. R., & Nabasirye, M. (2002). Determination of field yield loss and effect of environment on pod shattering in soybean. *African Crop Science Journal*, 10(3), 203-209.

Yamada, T., Funatsuki, H., Hagihara, S., Fujita, S., Tanaka, Y., Tsuji, H., Ishimoto, M., Fujino, K., & Hajika, M. (2009). A major QTL, qPDH1, is commonly involved in shattering resistance of soybean cultivars. *Breeding science,* 59(4), 435-440

Zhang, L., & Bellalloui. (2012). Effects of planting dates on shattering patterns under early soybean production system. *American Journal of Plant Sciences*, 3(1), 119-124.

Zhang, L., & Boahen, L. (2010). Evaluation of critical shattering time of early maturity soybeans under early soybean production system. *Agriculture and Biology Journal of North America*, 1(4), 440-447.

Zuo, Q. S., Huang, H. D., Cao, S., Yang, S. F., Liao, Q. X., Leng, S. H., ... & Zhou, G. S. (2014). Effects of harvesting date on yield loss percentage of mechanical harvest and seed quality in rapeseed. *Acta Agronomica Sinica*, 40, 650-656.

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Capability of Vitamin E as a Radioprotector in Suppressing DNA Damage Determined with Comet Assay

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Abstract

Radiation has a potent to damage cells. Radiation may act directly or indirectly on deoxyribonucleic acid (DNA) that results in the degeneration of tissues and necrotic, and thereby it needs a potent radioprotector to prevent these damages. Vitamin E is natural product known as an antioxidant which has potential as radioprotector. This research aimed to determine the capability of vitamin E with emphasized on the searching for its optimal concentration as radioprotector of DNA damage. This study used blood samples of healthy person irradiated with gamma rays at a dose of 6 Gy as the lethal dose to lymphocytes. The cocentrations of vitamin E from 0 to 0.8 mM was added into blood 15 minutes before irradiation. Isolation of lymphocytes was done using gradient centrifugation method. Evaluation on the capability of this compound in suppressing DNA damage was done by using alkaline Comet assay and data analysis was done using CaspLab program. The results show that addition of vitamin E could suppres these DNA damages and 0.8 mM of vitamin could reduce DNA damage up to 94.2%. We conclude that vitamin E effectively suppresed DNA damages induced by radiation. This information may benefit to the patient from negative impacts of radiotherapy.

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INTRODUCTION

When a track of ionising radiation passes through a cell it will deposit energy which can disrupt the organic molecules mainly that most sensitive molecule within the cell i.e. deoxyribonucleic acid (DNA) which is the most target and termed as the blueprint of life. It is well known that free radicals formed by the radiolysis of cellular aqueous milieu, and their interaction with one another and with oxygen are primary mediators of radiation injury (Hall & Giaccia, 2006; Tetriana et al. 2015). Ionizing radiation induce the production of Reactive Oxygen Species (ROS) through hydrolysis of water. Including superoxide, hydrogen peroxide, and hydroxyl radicals as the most reactive radicals. Such ROS can initiate oxidative cellular injury as well as activated intracellular signaling pathways and stimulated cytochrome c release from mitochondria leading to apoptosis (programmed cell death) (Azzam et al., 2012).

Radioprotectors are compounds that are designed to reduce damage occured in normal tissues caused by ionizing radiation. These compounds are mostly antioxidants and must be exist before or at the time of radiation for effectiveness as protector (Citrin et al., 2010). This radiation modifier or protectors is expected to alter the response of normal tissues to irradiation via free radical scavenging and/or H atom donation (Rahman et al., 2015). Radioprotectants are also important in suppressing the accumulation of genetic mutations, cell death or tissue disorganisation in patients undergoing radiotherapy or individuals exposed to non-lethal, but higher than normal, levels of radiation in accidental event (Liu, 2010).

Although endogenous antioxidant systems such as glutathione, thioredoxin, superoxide dismutase, and catalase normally inhibit the deleterious effects of ROS, these systems may be overwhelmed in irradiated cells. Exogenously supplemented antioxidants, or agents that stimulate endogenous antioxidant systems within cells, have shown promise in terms of suppressing the harmful effects of irradiation. A variety of reducing agents, such as vitamin E analogs, polyphenols, thiols and superoxide dismutase mimetics have been described as potential radiation countermeasures in the recent past (Singh et al., 2012; Weiss et al., 2009; Dumont et al., 2010).

Natural products which benefit to human health have been attractive targets for research (Singh et al., 2012). For both prevention and therapy of human diseases, these compounds are common in our diets and are often perceived as being more 'natural' and better suited for medicinal purposes due to being well tolerated and minimally toxic even at the upper ranges of dietary intake. Vitamins are prominent among natural compounds considered beneficial for human health (Satyamitra et al., 20111). Vitamin E is well known as antioxidant, neuroprotector, and also anti-inflammatory properties (Singh et al., 2013). It is essential because body cannot produce vitamin E so it should be obtanied from food supplements. Vitamin E represents a generic term for all tocopherols and their derivatives with naturally occurring and biologically active stereoisomeric compounds of α-tocopherol (AT) (Palozza et al., 2008).

Comet assay, also known as single-cell gel electrophoresis, is a simple method for measuring DNA strand breaks in eukaryotic cells. The technique includes cells embedding in agarose on a microscope slide, lysis with detergent and high salt to form nucleoids containing supercoiled loops of DNA linked to the nuclear matrix, electrophoresis at high pH results in structures resembling comets, and observation with fluorescence microscopy. Finally the analysis is that the intensity of the comet tail relative to the head reflects the number of DNA breaks (Olive et al., 2006; Speit & Hartmann, 2005; Singh et al., 1988). The purpose of this study was to examine the capability of vitamin E as radioprotector in suppressing the radiation induced DNA damage The benefits of this research are to protect normal tissue from radiation damage due to radiotherapy in cancer patients..

METHODS

Vitamin E was provided as dl-α-tocopherol soft capsule at a concentration of 250 IU (Catalent, Australia). A 1 mM stock solution of vitamin E was prepared in polyethilene glicol (PEG) mixed wtih Tween 20 solution and serial dilutions with PBS were made in order to achieve a working concentration of 0.2 mM; 0.4 mM; 0.6 mM dan 0.8 mM of vitamin E .

The study was performed on peripheral blood samples obtained from two healthy males, non-smoking, non-alcoholic donors with ages of 30 and 50 years. The donors are never exposed to ionizing radiation. Three mL venous blood were collected under sterile conditions in vacutainer tubes (Becton Dickinson, NJ, USA) containing lithium heparin as anticoagulant. After collection, the blood was divided into 6 tubes of samples.

The present study was carried out in 6

groups, group 1: control (cells without radiation), group 2 : radiation control (RC: cells were exposed to 6 Gy of gamma radiation), group 3: cells treated with 0.2 mM vitamin E before radiation exposure, group 4: cells treated with 0.4 mM vitamin E before radiation exposure, group 5; cells treated with 0.6 mM vitamin E before radiation exposure, and group 6: cells treated with 0.8 mM vitamin E before radiation exposure.

Before radiation, blood samples were incubated for 15 minutes with the serial concentration of vitamin E. The blood samples were then irradiated with gamma radiation in the ice. The source of gamma radiation used was Cobalt-60 (IRPASENA, PATIR, BATAN). All of the blood samples were irridiated with dose of 6 Gy, at dose rate of 1 Gy/minutes as a lethal dose of gamma radiation for lymphocytes cells. Lymphocyte cells were isolated 5–15 minutes after radiation and examined for induced DNA damages using comet assay. One blood sample without antioxidant served as control in both series, and these samples were also irradiated with 6 Gy.

After radiation, lymphocyte were isolated from the blood samples using Histopaque (Sigma) according to standard method (Panda et al., 2012). Three mL of fresh heparinized blood was mixed with the equal volume of phosphate buffered saline (PBS, Merck), which was overlaid on 3 mL of Histopaque. Lymphocytes were separated using density gradient centrifugation. Then the separated lymphocytes were washed twice with PBS by centrifugation for 15 min at 1000 rpm and cells were suspended in minimum volume of RPMI -1640 (Gibco).

Cell viability was measured using trypan blue dye exclusion method (Chung et al., 2015). The lymphocytes were mixed with equal volume of 0.4% trypan blue dye for 3 minutes and counted using haemocytometer. Viable and dead cells were scored under the microscope.

About 10^4 cells per 100 µL of medium was taken from each dose treatment for Comet assay by following the standard procedure with slightly modification (Singh et al., 1988). Fully-frosted microscopic slides were prepared. Each slide was covered with 1% Normal Melting Point (NMP) agarose (Sigma). After solidification, the slides were then coated with 0.6% NMP agarose. A Low Melting Point (LMP) agarose was melted and stabilized in a waterbath (RTE10) at 37°C. For each sample and control, 5 µL of cell homogenate was mixed with 100 µl of 1% LMP agarose and placed on the slides. After 10 minutes of solidification on ice, the slides were covered with 0.5% LMP agarose. The slides were then immersed in a pre-chilled lysis solution ((2.5 M NaCl, 100 mM Na2EDTA, 10 mM Tris–HCl, adjust until pH 10 with NaOH (Sigma) and added 1% Triton X-100 (Sigma) and 10% dimethyl sulfoxide (Sigma) and kept in at 4°C for 60 minutes. The slides were placed horizontally in a humidity chamber at 37 °C for 30 minutes. All slides were then immersed in an alkali solution (0.3 M NaOH, 1 mM Na2EDTA; pH 12.1) for 40 minutes. Electrophoresis in a pre-chilled alkali solution (0.3 M NaOH, 1 mM Na2EDTA; pH 13) at 1 V/cm was done for 20 minutes in refrigerator (4°C). After electrophoresis, the slides were rinsed gently three times with neutralization buffer (0.4 M Tris–HCl, pH 7.5) to remove excess alkali and detergents. Each slide was fixed with methanol, stained with ethidium bromide (20 µg/ml) and covered with a coverslip. Slides were stored at 4°C in sealed boxes until analysis.

The stained samples with ethidium bromide were observed using a Nikon flourescence microscope. A total of 50 randomly captured comets from each slide were examined at 250x magnification using an epifluorescence microscope that connected through computerized to an image analysis. Cells were piled not counted. The image of comet was digitally analyzed using *CASPLab comet assay software.*

Each experimental set consists of duplicated slides. The various parameters measured in the exposed and control groups were evaluated using Excell program (StaSoft, Tulsa, USA). Each sample was characterized for the extent of DNA damage by considering the mean, SE (standard error of the mean), median and range of the comet parameters.

RESULTS AND DISCUSSION

The viability cells in this research was observed under microscope. The results showed that the number of cells in all groups was in enough number (around 10^7 cells/mL) and suitable for Comet assay. In this research, the DNA damage of irradiated lymphocytes was assessed by comet assay by staining the cells with ethidium bromide and the comet that mainly consist of single stranded DNA can be seen with a fluorescence microscope as presented in Figure 1.

Results of visualization showed that lymphocyte cells irradiated with gamma ray formed tail of comet due to DNA damage in the form of breaking one DNA strand (single strand break/SSB) and the rupture of both strands of DNA at the opposite position (double strand breaks/DSB). The DNA damage can be estimated

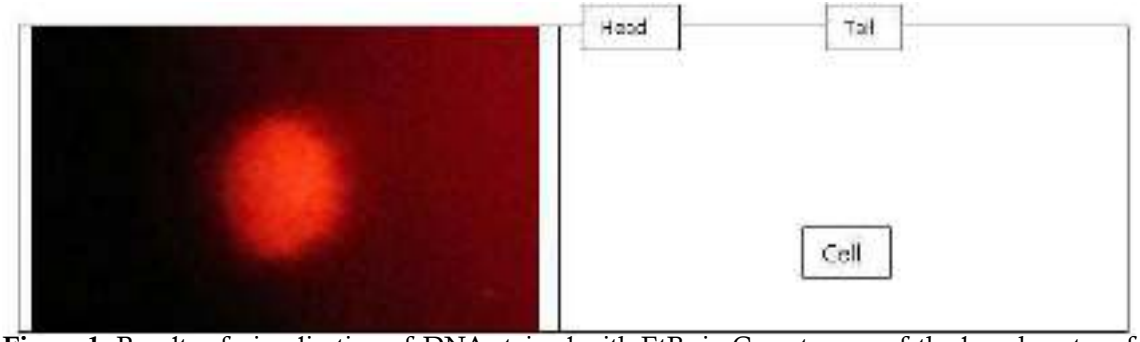

Figure 1. Results of visualisation of DNA stained with EtBr in Comet assay of the lymphocytes of control blood sample (left) and that exposed to 6 Gy dose of gamma radiation with tail of comet (right).

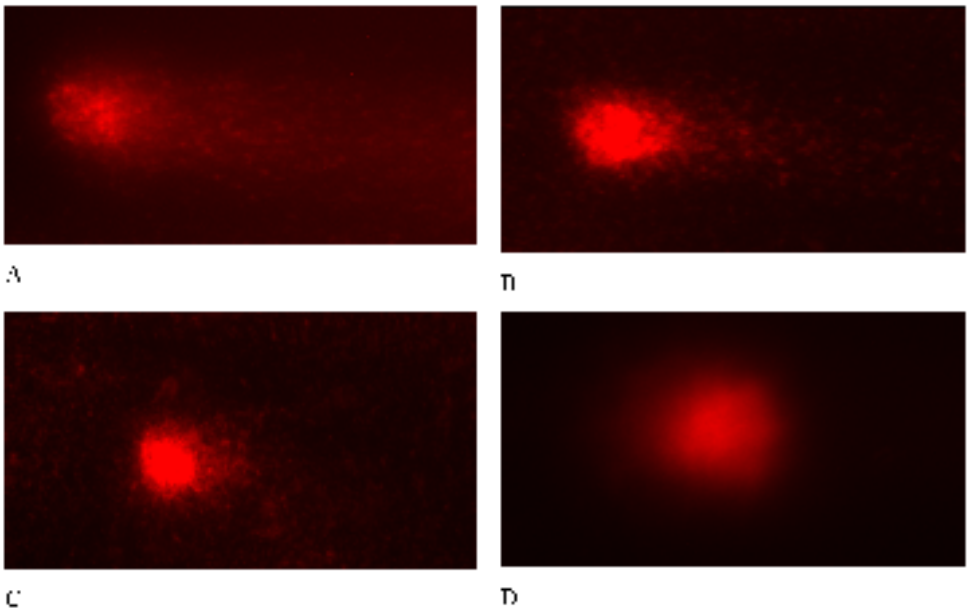

Figure 2. Visualization of comet of lymphocyte irradiated with 6 Gy and treated with vitamin E at consentrations of 0.2 mM (A), 0.4 mM (B), 0,6 mM (C), and 0.8 mM (D).

by measuring the length of the comet tail using an ocular scale fitted in the eyepiece of the microscope or by visual scoring of degree of damage from 0 to 4 according to comet appearance [Figure 1]. Alternatively, there are numerous image analysis software to quantitate additional DNA damage parameters such as percentage of DNA in head, percentage of DNA in tail, tail moment (product of tail length and percentage of DNA in tail), and tail area.

In order to avoid or inhibit these DNA damages we treated the blood with an antioxidant vitamin E before irradiation. In this study four concentrations of vitamin E (0.2; 0.4; 0.6; and 0.8 mM) were added to the blood 15 minutes before irradiation. All of the results are presented in Figure 2.

From the visualization presented in Figure 2, it can be seen that there is a shortening the tail of comet of lymphocytes treated with vitamin E. Higher concentration of vitamin E resulted in shorter tail of comet which means that there is a reduction of DNA damages in the presence of vitamin E and this treatment effectively reduce the deleterous effects of gamma radiation.

The comet itself can be analysed by visual scoring or computerised image analysis. In this research the comet were analyzed using CASPLab Comet Assay software which reported using a range of different endpoints. This comet assay sofware can measure 8 parameters of the digital image of comet value : Long-tailed comet (TL), DNA Tail, Tail Moment (TM), Olive tail moment (OTM), DNA head, DNA Percentage in head, and the length of comet (L.comet). Head DNA that indicating the number of DNA in head of comet is an additional parameter in computerized program of CASP Lab.

The graph in Figure 3 demonstrate the relationship between vitamin E concentration

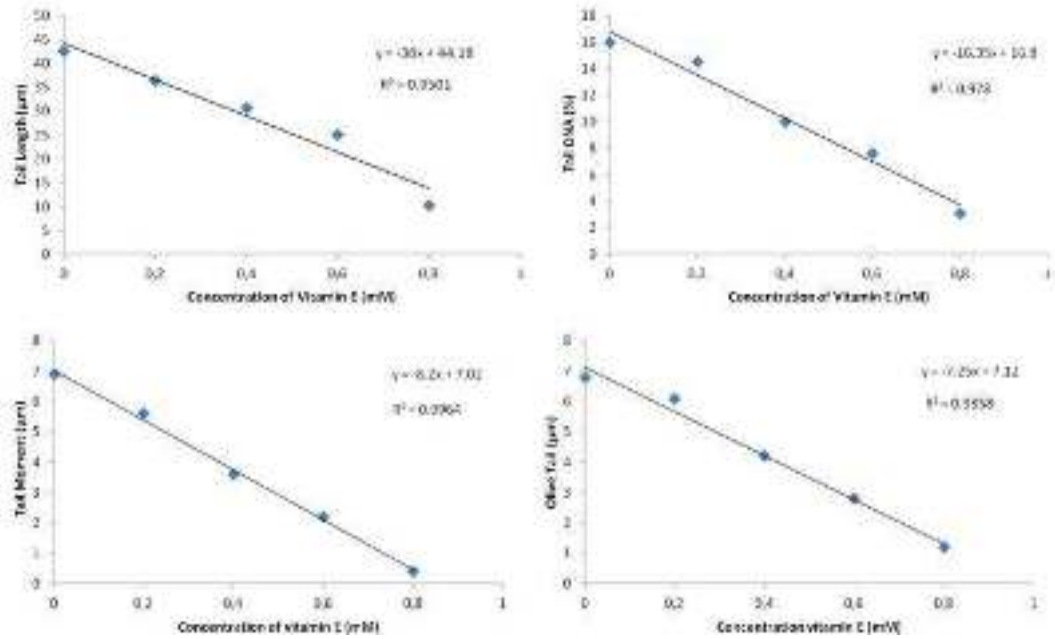

Figure 3. The effect of vitamin E addition on the four parameters of Comet assay (DNA fragment) where DNA damage was reduced by vitamin E with each correlation coefficient (R^2).

and DNA damages induced by 6 Gy gamma radiation. Here we found that each parameter has tendention to decrease by increasing the concentration of vitamin E added (Figure 3). The correlation coefficient (R2) for every parameter : (a) tail moment (TM), (b) olive tail moment (OTM), (c) tail DNA, and (d) tail length (TL) more than 0,9 indicating a very good relationship.

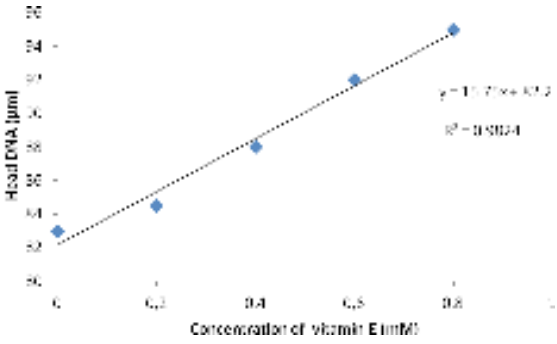

Figure 4. Relationship between *head DNA* with the increasing of vitamin E concentration

Figure 4 shows that head DNA and the concentration of vitamin E have a positive correlation meaning that higher concentration of vitamin E higher number of DNA in head. This finding indicating that vitamin E effectively reduce DNA damage due to ionizing irradiation. Among comet tail parameters, TM gave the highest percentage of reducing damage given with 0.8 mM of vitamin E. Based on 5 parameters in comet test it is known that vitamin E is a good radioprotector in suppressing DNA damage post irradiation with R^2 more than 0.9.

The percentage reduction in DNA damage to increase the concentration of vitamin E in the fourth comet tail parameters are shown in Table 1. Seen the higher the concentration of vitamin E is added before irradiation can reduce radiation-induced DNA damage. The value of TM provides the highest percentage decrease DNA damage the four parameters of comet tails. The decreasing of DNA damage percentage shown in the highest value of TM at a concentration of 0.8 mM vitamin.

The cell-type-of-choice in biomonitoring research activities is mostly the lymphocyte because blood is easily collected and lymphocytes have proved to be good surrogate cells. In Comet assay the process of electrophoresis is done under the alkaline conditions where the strand breaks through their ability to relax DNA supercoiling, allow the negatively charged DNA loops to extend towards the positively charged anode (Singh et al., 1988).

Similar research was performed by Singh et al. (2013) who has done an *in vivo* study to determine the effect of tocopherol succinate (TS) treatment against DNA damage caused by ionizing radiation in peripheral blood mononuclear cells, splenocytes and thymocytes of mouse. The mice were treated with vehicle or TS (400 mg/kg) and exposed to high dose (9.2 Gy that is the $LD_{90/30}$ dose that causes hematopoietic injury) of ^{60}Co γ-radiation 24 h after drug injection. Peripheral blood, spleen and thymus were collected 30

Table 1. Percentage of decreasing in DNA damage due to the administration of vitamin E± SD.

Concentration of Vitamin E (mM)	Decreasing in DNA damage (%)			
	Tail length	Tail DNA	Tail moment	Olive tail moment
0	0	0	0	0
0.2	14.6±8.3	9.3±6.5	18.8±4.0	10.3±2.0
0.4	28.0±6.6	37.5±4.4	47.8±2.4	39.1±8.0
0.6	41.4±13.0	52.5±5.7	68.1±2.5	58.8±2.3
0.8	75.8±5.0	80.6±2.0	94.2±0.4	82.3±0.9

minutes and 4 h after irradiation, and used for alkaline comet assay. The administration of TS significantly inhibited DNA damage in peripheral blood cells and thymocytes compared with vehicle-treated mice, evidenced by the shorter tail length and smaller percentage of DNA in the comet tail. Another study conducted by Yassa et al. (2011) aimed to investigate potential protective vitamin E against pesticide diazinon (DZN) in murine. The results showed that mice treated with vitamin E reduced DZN- induced DNA damage where the length TL shortened by up to 50%. These results suggest that vitamin E has a protective effect on DZN-induced DNA and showing that vitamin E prevent genotoxicity induced by DZN. It indicates that vitamin E is also effectively suppressing the harmfull effects induced by other chemical.

Since many types of radiation are now being frequently used in clinical treatment of patients with cancer and in exprimental research, it is essential that more detailed information on the chemical capabilities in minimizing the effect of irradiation be obtained. In this research, like other studies, we are searching for the efficacy of natural chemical in suppressing the negative effects of ionizing radiation. And many studies had shown that vitamin E can scavenge molecular oxygen, peroxide and hydroxyl radicals and atomic oxygen radicals induced by ionizing radiation.

The understanding in radiation effects has placed emphasis on the search for antioxidant agents that are suitable as radiation countermeasures (Sing et al., 2012; Weiss & Landauer, 2009; Dumont et al., 2010). Although endogenous antioxidant systems (glutathione, thioredoxin, superoxide dismutase, and catalase) normally inhibit the deleterious effects of ROS, these systems may be overwhelmed in irradiated cells. Exogenously supplemented antioxidants, or agents that stimulate endogenous antioxidant systems within cells, have shown promise in terms of suppressing the harmful effects of irradiation. If present in the cells at the time of radiation exposure, such antioxidants may protect cells from radiation damage by scavenging ROS before they act on cellular components. A variety of reducing agents, such as vitamin E analogs, polyphenols, thiols and superoxide dismutase mimetics have been described as potential radiation countermeasures in the recent time.

Here we tested the potentail of vitamin E as a Radioprotector to lethal dose of gamma ray. It was approved that administration of vitamin E modulated the expression of antioxidant enzymes and inhibited expression of oncogenes in irradiated cells (Singh et al., 2013). Vitamin E is a group of eight structurally related fat-soluble vitamins, four tocopherols (α, β, γ, and δ), and in more detail, acting as antioxidants by preventing the propagation of free radical reactions by donating hydrogen from their phenolic group to stabilise the radicals, and thereby break the chain of events leading to oxidative damage. The tocopherols protect the structure and function of human cell membranes (Vasilyeval & Bespalov, 2015; Niki, 2014; Satyamitra et al., 2011). And here we treat the blood with vitamin E 15 minutes before irradiation. This is due to the fact from some other published results (Singh et al., 2013; Maurya et al., 2006; Ghosh et al., 2009; Citrin et al., 2010) that vitamin E did not protect mice when administered as a mitigator after irradiation and it can be used only as a radio-protector of DNA which is a target for mutagens and carcinogens, and induce changes in DNA structure giving rise to mutations and/ or cell death.

Nair et al. (2003) had been done a very comprehensive study on the efficacy of tocopherol monoglucoside (TMG) as radioprotector and they found that, this chemical, which is a water soluble derivative of vitamin E, offers protection against the deleterious effects of ionizing radiation, either in vivo or in vitro studies, to biological systems. It has a potent antioxidant and an effective free radical scavenger, so that it protects DNA from radiation-induced strand breaks formation. It also protected thymine glycol formation induced by gamma-radiation. It prevents gamma-radiation-induced loss of viability of EL-

tumor cells and peroxidation of lipids in microsomal and mitochondrial membranes. It reduce upto 75% embryonic mortality resulting from exposure of pregnant mice to ionizing radiation (2 Gy) by ip administration (0.6 g/kg, body wt) prior to irradiation. This chemical TMG also offered protection to mice against whole body gamma-radiation-induced lethality and weight loss. Study also showed that the LD50(30) of mice increased from 6 to 6.72 Gy upon post irradiation administration of a single dose of TMG (0.6 g/kg, body weight) by intraperotoneal route.

Vitamin E can alleviate radiation induced decrement in delayed-type hypersensitivity and as adjuvant to other radioprotectant. A very old and simple research conducted by Mahdy (1991) from Middle Eastern Regional Radioisotope Centre for the Arab Countries, Cairo, in Egypt found that intraperitoneally injection of vitamin E before whole body gamma irradiation at the dose of 7 Gy to female albino rats remarkably recovered in the serum protein content at all post-irradiation days, while it slightly recovered in the level of serum urea.

Basically there are some mechanisms of this chemical in its action. One investigator revealed that vitamin E act by preventing lipid peroxidation which does not generally play a major role in cell killing by ionizing radiation. Other suggested the chemical by scavenging of secondary radicals but it needs a very high concentration to effectively prevent DNA damage which is responsible for classical reproductive cell death, or by suppressing the protracted oxidative stress which is difficult to be distingusihed from its role as scavenging radicals, decreasing oxygen concentration which is due to the fact that hypoxic cells are radioresistant so that treatments that decerase microenvironmental oxygen can be radioprotective, enhancing the DNA repair which is a major factor in determining the radiosensitivity (Bump, 1998).

Borek (2008) proposed that vitamin E is an antioxidant and its radiprotective action is depend on the oxygen partial pressure in tissue and it was shown that this chemical effectively protect tissue from deletirius effects of ionizing radiation in high oxyen pressure such as lung. Recent studies have suggested several proposed alternative mechanisms: most notably, an indirect effect of tocopherols in eliciting specific species of radioprotective growth factors or cytokines such as granulocyte colony-stimulating factor (G-CSF). The vitamin E treatment in irradiated group of rat presented more acinar cells than the irradiated group, but no statistically significant difference was observed ($p>0.05$). They conclude that vitamin E seems to have failed as a radioprotective agent on acinar cells in rat parotid glands (Gomes et al., 2013).

Our results demonstrate that vitamin E has the potential to protect DNA damage lynphocyte cell from radiation injury . As is known radiotherapy given to patients often results in immunologic cell damage resulting in decreased immunity. Lymphocyte cells have immune cells an important role in immunity. This study are useful to enrich radioprotectant information that can protect radiotherapy patients from the effects of radiation

CONCLUSIONS

Ionizing radiation at a dose of 6 Gy, which is equivalent to highly lethal doses for humans, effectively cause the DNA damage. This study shows that the addition of vitamin E is significantly suppressed the DNA damages at all concentration tested, concentration of 0.8 mM of vitamin E could reduce DNA damage up to 94.2%. It was given just before the irradiating gamma rays

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REFERENCES

Azzam, E. I., Jay-Gerin, J. P. & Pain, D. (2012). Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury. *Cancer Letter*, 327(0), 48–60.

Borek, C. (2008). Dietary antioxidants and phytochemicals in radioprotection and therapy, in : Herbal Radiomodulators: Applications in Medicine, Homeland Defence and Space (Rajesh Arora ed.). pp. 141-143.

Bump, E. A. (1998). Introduction, In: Radioprotectors: chemical, biological, and clinical perspectives (Edward A. Bump and Kamal Malaker). CRC Press, Boca Raton, pp. 3-10.

Chung, D. M., Kim, J. H. & Kim, J. K. (2015). Evaluation of MTT and Trypan Blue assays for radiation-induced cell viability test. *International Journal of Radiation Research,* 13(1), 331-335.

Citrin, D., Cotrim, A. P., Hyodo, F., Baum, B. J., Krishna, M. C., & Mitchell, J. B. (2010). Radioprotectors and mitigators of radiation-induced normal tissue injury. *Oncologist,* 15(4), 360-371.

Dumont, F., Le Roux, A., & Bischoff, P. (2010). Radia-

tion countermeasure agents: an update. *Expert opinion on therapeutic patents,* 20(1), 73-101.

Ghosh, S. P., Kulkarni, S., Hieber, K., Toles, R., Kao, T. C., Hauer-Jensen, M. (2009). Gamma-tocotrienol, a tocol antioxidant as a potent radioprotector. *International journal of radiation biology,* 85(7), 598-606.

Gomes , C. C., de Moraes Ramos-Perez F. M., da Cruz Perez, D. E., Novaes P. D., Bóscolo F. N., & de Almeida, S. M. (2013). Radioprotective effect of vitamin E in parotid glands: a morphometric analysis in rats. *Brazilian Dental Journal,* 24(3), 183-187.

Hall, E. J. & Giaccia, A. J. (2006). Radiobiology for the Radiologist. Sixth Edition. Philadelphia: Lippincott Williams & Wilkins; pp. 5-15.

Liu, S. Z. (2010). Biological effects of low level exposures to ionizing radiation: theory and practice. *Human & experimental toxicology,* 29(4), 275-281.

Mahdy, A. M. (1991). Vitamin E as A chemical radioprotector for controlling the radiation induced changes in the levels of protein and urea in the serum of irradiated rats. *Isotope and Radiation Research,* 23(2), 117-123.

Maurya, D. K., Devasagayam, T. P. A., & Nair, C. K. K. (2006). Some novel approaches for radioprotection and the beneficial effect of natural products. *Indian Journal of Experimental Biology*, 44(2), 93-114.

Nair, C. K., Devi, P. U., Shimanskaya, R., Kunugita, N., Murase, H., Gu, Y. H. & Kagita, T. V. (2003). Water soluble vitamin E (TMG) as a Radioprotector. *Indian Journal of Experimental Biology*, 41(12), 1365-1371.

Niki, E. (2014). Role of vitamin E as a lipid-soluble peroxyl radical scavenger: in vitro and in vivo evidence. *Free Radical Biology and Medicine,* 66, 3-12.

Olive P. L. & Banáth, J. P. (2006). The comet assay: a method to measure DNA damage in individual cells, *Nature protocols,* 1(1), 23-29.

Palozza, P., Simone, R., Picci, N., Buzzoni, L., Ciliberti, N., Natangelo, A. (2008). Design, synthesis, and antioxidant potency of novel alpha-tocopherol analogues in isolated membranes and intact cells. *Free Radical Biology and Medicine,* 44(7), 1452-1464.

Panda, S. K., Kumar, S., Tupperwar, N. C., Vaidya, T., George, A., Rath, S., Bal, V. & Ravindran, B. (2012). Chitohexaose activates macrophages by alternate pathway through TLR4 and blocks endotoxemia. *PLoS Pathog,* 8(5), e1002717.

Rahman, M. M., Islam, M. B., Biswas, M. & Alam, A. H. M. K. (2015). In vitro antioxidant and free radical scavenging activity of different parts of *Tabebuia pallida* growing in Bangladesh. *BMC research notes*, 8(1), 621.

Satyamitra, M., Kulkarni, S., Ghosh, S. P., Mullaney, C. P., Condliffe, D., & Srinivasan, V. (2011). Hematopoietic recovey and amelioration of radiation-induced lethality by the vitamin E isoform, delta-tocotrienol. *Radiation research,* 175(6), 736-745

Singh, N. P., McCoy, M. T., Tice, R. R. & Schneider, E. L. (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental cell research,* 175(1), 184-191.

Singh, P. K., Wise, S. Y., Ducey, E. J., Fatanmi, O. O., Elliott, T. B., & Singh, V. K. (2012). Alpha-tocopherol succinate protects mice against radiation-induced gastrointestinal injury. *Radiation research,* 177(2), 133-145.

Singh, V. K., Ducey, E. J., Brown, D. S., & Whitnall, M. H. (2012). A review of radiation countermeasure work ongoing at the Armed Forces Radiobiology Research Institute. *International journal of radiation biology,* 88(4), 296-310.

Singh, V. K., Singh, P. K., Wise, S. Y., Posarac, A. & Fatanmi, O. O. (2013). Radioprotective properties of tocopherol succinate against ionizing radiation in mice. *Journal of Radiation Research* 54(2), 210–220.

Speit, G. & Hartmann, A. (2005). The comet assay: a sensitive genotoxicity test for the detection of DNA damage. *Molecular toxicology protocols*. 291, 85–95.

Tetriana, D., Mailana, W., Kurnia, I., & Syaifudin, M. (2015). Preliminary Study on the Single Nucleotide Polymorphism (SNP) of XRCC1 Gene Identificationto Improve the Outcomes of Radiotherapy for Cervical Cancer. *Biosaintifika: Journal of Biology & Biology Education*, 7(2), 79-86.

Vasilyeval, I. N. & Bespalov, V. G. (2015). Release of Extracellular DNA after Administration of Radioprotective Combination of α-Tocopherol and Ascorbic Acid. *Radiatsionnaia biologiia, radioecologiia,* 55(5), 495-500.

Weiss, J. F. & Landauer, M. R. (2009). History and development of radiation-protective agents. *International journal of radiation biology,* 85(7), 539-573.

Yassa V. F., Girgis, S. M., & Abumourad I. M. K. (2011). Potential protective effects of vitamin E on diazinon-induced DNA damage and some haematological and biochemical alterations in rats. *Journal of Mediterranean Ecology* 11, 31-39.

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Application of *rps16* Intron and *trnL-trnF* Intergenic Spacer Sequences to Identify Rengas Clone Riau

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Gluta renghas; PCR; Riau; rps16 intron; trnL-trnF intergenic spacer

Abstract

Rengas clone Riau has been identified using morphological characters and molecular technique with a *psbA-trnH* intergenic spacer, however, this method can only determine its taxonomic status at genus level, namely *Gluta* sp. This study reports application two DNA barcodes, i.e. *rps16* intron and *trnL-trnF* intergenic spacer, to identify Rengas clone Riau. The methods included collection of the leaves from Kajuik Lake, total DNA isolation, electrophoresis, PCR (polymerase chain reaction), gel purification and sequencing. The *rps16* intron size was 659 bp and the *trnL-trnF* intergenic spacer was 527 bp. The BLASTn analysis showed that sequences of the *rps16* intron and the *trnL-trnF* intergenic spacer of *Gluta* sp clone Riau had 100% similarity to those of *G. renghas* deposited in GenBank. These results were supported by high max score, high total score, query cover = 100%, and E-value = 0. The dendrograms also showed the closest relationship of *Gluta* sp clone Riau with *G. renghas* deposited in GenBank compared to other species of *Gluta*. In conclusion, this study succeeded in identifying Rengas clone Riau as *Gluta renghas* by using sequences of the *rps16* intron and the *trnL-trnF* intergenic spacer. A combination of DNA barcodes could be applied to identify various plants as long as the database for the DNA barcodes is available in public database such as GenBank.

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INTRODUCTION

Riau Province in Indonesia has some flood plains and one of them is Kajuik Lake which is located in Pelalawan Regency. This lake is the floodplain of Kampar river and plays an important role for the life of the floodplain ecosystem, especially for the Riau endemic fish namely selais. In the rainy season, fish migrate to the lake and use the sideline of the roots of the trees that grow in and around the lake for spawing, laying eggs, caring fries, and providing shelters to protect fries from predators. In addition, the trees also contribute by maintaining erosion, to maintain water quality, and provide nutrients to the lake. Rengas is one of the trees that are growing there (Elvyra & Yus, 2012; Roslim, 2017).

Rengas from Kajuik Lake has been identified by Elvyra & Yus (2012) using morphological characters of leaf and stem without flower and fruit and then they determined it as *Gluta* sp clone Riau. Roslim (2016) then continued the identification using a DNA barcode such as *psbA-trnH* intergenic spacer region and expected that the species name could soon be determined. Unfortunately, this effort did not succeed because the sequence database of the *psbA-trnH* intergenic spacer of genus *Gluta* had not reached that point yet.

Further analysis of the morphology characters shows that there is similarity between *Gluta* sp clone Riau with *G. renghas* (Fern, 2014). At that time, the DNA barcode sequences for *Gluta renghas* was available in GenBank is *rps16* intron and *trnL-trnF* intergenic spacer (Roslim, 2017). Therefore, the next research to determine the identity of *Gluta* sp clone Riau would be better off if it uses those DNA barcode sequences.

The DNA barcode is a piece of short DNA (approximately 700 bp) which position is known to be in the genome of organism, in the nuclear or organelle (mitochondria and chloroplast), and is used to identify an organism. The technique is called DNA barcoding (Hebert et al., 2003). Basically, this technique is developed to assist and to put organism identification at ease. People who are not experts in the field of taxonomy – such as employees of quarantens of animals, plants, geneticists, ecologists, etc. - can identify or determine the taxonomic status of the observed organism. In addition, molecular identification can still execute broken and incomplete specimen or in the condition of which even taxonomists are not able to identify it (Hebert et al., 2003).

Since 2003, scientists has developed some DNA barcodes and they have deposited these barcodes in public database such as GenBank (National Center for Biotechnology Information's GenBank - NCBI, GenBank), EMBL (the European Molecular Biology Laboratory), and BOLD (the Barcode of Life Data System) (Stoeckle, 2003; Hebert & Gregory, 2005). Two DNA barcodes, i. e. *matK* and *rbcL*, have been agreed as a standard 2-locus barcode for plant identification due to the quality of the sequence, the ability to recover, and the ability to discriminate plant species (CBOL Plant Working Group, 2009). In addition, the other DNA barcode such as a *rps16* intron and a *trnL-trnF* intergenic spacer has been developed. Unlike the *matK* and *the rbcL* which encode the functional protein, the *rps16* intron and the *trnL-trnF* intergenic spacer are parts of plant chloroplast genome that do not encode the functional protein (Sugita & Sugiura, 1996; Shaw et al., 2007; Borsch & Quandt, 2009).

The *rps16* intron is an intron of a ribosomal protein S16 gene. Intron is a region in a gene that is transcribed but not translated in other words intron is a type of a non coding region in a genome. Generally, the non coding region is more vary and easier to mutate than the coding region (Borsch & Quandt, 2009). Coding region is also called exon that is a region in a gene which is transcribed and translated into protein, not easier to mutate, and relative conserved. There are 12 genes encoding ribosomal protein small subunit in soybean (*Glycine max*) (Daniell et al., 2016). The *rps16* intron has been used for phylogenetic study of Angiosperms (Shaw et al., 2007) and Urophyleae (Smedmark et al., 2008).

Quite similar to the *rps16* intron, the *trnL-trnF* intergenic spacer region is also a non coding region but located between two genes, i.e. *trnL* (UAA) gene and *trnF* (GAA) gene. This region has higher rate mutation and variation than the coding region such as *matK* and *rbcL* (Borsch & Quandt, 2009). The *trnL*(UAA) is located between the *trnF*(GAA) and *trnT*(UGU) in the plant chloroplast genome. The lengths of the *trnL*(UAA) exon on tobacco, rice, and *Marchantia*, respectively, are 577 bp, 614 bp, and 389 bp (Taberlet et al., 1991). The lengths of the *trnL-trnF* intergenic spacer on tobacco, rice, and *Marchantia*, respectively, are 438 bp, 324 bp, and 158 bp (Taberlet et al., 1991). The the *trnL-trnF* intergenic spacer region has been used to discriminate the species in genus *Lophophora* (Adrienne et al., 2015), to determine a new species of *Atraphaxis* (Yurtseva et al., 2016), to analyze the phylogenetic relationships within Pandanaceae (Buerki et al., 2012) and ferns (de Groot et al., 2011), to identify the tea plants which are used to produce commercial

tea and to provide information about the varieties used to make tea (Lee at al., 2016), and to analyze diversity and phylogeny of Myrtaceae (Vasconcelos et al., 2017) and *Cycas chenii* (Yang et al., 2016), to explain the genetic diversity of allopolyploid wheatgrass *Elymus fibrosus* (Schrenk) Tzvelev which is a member of Poaceae: Triticeae that is caused by its origin (Wu et al., 2016).

Scientists agree that it would be better to use multilocus DNA barcodes for plant molecular identification (Fazekas et al., 2008; CBOL Plant Working Group, 2009). The hypervariable non coding region, like the *rps16* intron and the *trnL-trnF* intergenic spacer, is easier and preferred to be used for identification and discovery of a new species (Kress et al., 2009; Adrienne et al., 2015; Yurtseva et al., 2016). Therefore, this study reports the application of 2 DNA barcodes, i.e. the *rps16* intron and the *trnL-trnF* intergenic spacer, to identify Rengas clone Riau. This research will provide information that identification of plants can be performed using a combination of DNA barcodes.

METHODS

Plant material used in this study was fresh leaves of *Gluta* sp clone Riau that grows in and around Kajuik Lake located in Langgam, Pelalawan Regency, Riau Province, Indonesia. The primer pairs for amplification of the *rps16* intron and the *trnL-trnF* intergenic spacer were designed based on both sequences available in GenBank (Tabel 1).

Total DNA was extracted from fresh leaves of *Gluta* sp clone Riau using DNeasy plant mini kit (Qiagen). 0.5 gram of leaves was weighed and cut with scissors. After that, the pieces were crushed using mortar and pestel in liquid Nitrogen into powder. The powder was then poured into 1.5 ml tube for the next step according to the manufacture instruction (Qiagen). The pellet was then diluted with 50 µl of TE (Tris EDTA pH 8.0) and stored at 4°C. The quality and the quantity of the total DNA were predicted using electrophoresis technique.

Amplification of the DNA regions was performed using PCR technique with the following components: 1X PCR buffer (plus Mg^{2+}), 0.1 mM dNTPs, 2.4 µM primer forward, 2.4 µM primer reverse, 2 U enzim Dream *Taq* DNA polymerase (Thermo Scientific), 1 ng DNA total, and water until 50 µl. The PCR conditions are as follows: 5 minutes at 94 °C for 1 cycle followed by 45 seconds at 94 °C, 45 seconds at 47 °C, and 1 minute at 72 °C for 35 cycles, and ended with 1 cycle of post-PCR for 10 minutes at 72 °C.

Electrophoresis is a technique used to separate the DNA fragments on a porous matrix under the influence of an electrical field. In this study, the electrophoresis was conducted to predict the quality and the quantity of the total DNA and also to check the success rate of PCR. It was done on 1.2% agarose gel in 1X TBE buffer (Tris Borate EDTA pH 8.0) at 65 volts for 30 minutes. Afterwards, the gel was stained by immersion in 5 µg/ml of ethidium bromide solution for 5 minutes then soaked in water for 5 minutes. Visualization of the DNA bands on the gel was performed using a UV lamp transilluminator (WiseUv WUV-M20, Daihan Scientific) and then photographed using a digital camera (Olympus SP-500 UZ).

Gel purification and sequencing of the PCR products were conducted by 1st Base in Malaysia via PT Gentika Science Jakarta, Indonesia. The PCR primers were used for the bidirectional sequencing.

The nucleotide sequence was then aligned using BLASTn program (Basic Local Alignment Search Tool) at http://www.ncbi. nlm.nih.gov/BLAST (Altschul et al., 1997) to find the similarity to the sequences in the GenBank database. Software of MEGA version 6.06 (Build#: 6140226) (Molecular Evolutionary Genetics Analysis) (Tamura et al., 2013) was used to create a dendrogram by Kimura 2-parameter model and UPGMA (Unweighted Pair Group Method with Arithmetic mean) with 1000 bootstrap.

RESULTS AND DISCUSSION

The PCR products for the *rps16* intron and the *trnL-trnF* intergenic spacer were approxima-

Table 1. Primer pairs for amplification of the *rps16* intron and the *trnL-trnF* intergenic spacer.

Primer	5'----------3'	Annealing Temperature (°C)	Region
G-rps-F	CATCCTTTGTTCGGTTCTAC	45.0	ribosomal protein S16 gene, intron
G-rps-R	GTCTCGAGAAAAATGATTCG		
G-trn-F	AGGATAGGTGCAGAGACTCA	49.9	*trnL-trnF* intergenic spacer
G-trn-R	TCATTCACATGGAGATTCCT		

tely 650 bp and 500 bp, respectively (Figure 1). The bands on the gel were clear and feasible for sequencing purposes.

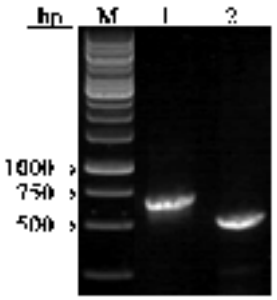

Figure 1. The DNA band profiles of (1) the *rps16* intron and (2) the *trnL-trnF* intergenic spacer. M = 1 kb DNA ladder (Thermo Scientific).

Analysis of The *rps16* Intron Sequence

The length of the *rps16* intron sequence was 659 bp. The sequence had been registered in GenBank with the accession number KX365741 (Figure 2). The BLASTn analysis to the sequence showed that the *rps16* intron sequence of *Gluta* sp clone Riau had 100% similarity to the *G. renghas* sequence deposited in GenBank. This result was supported by the high value of max score, total score, and query cover, and the low value of E-value (Table 2).

Figure 2. The *rps16* intron sequence of *Gluta* sp clone Riau.

Analysis to the *rps16* intron sequence demonstrated that there were 19 nucleotide variations on some species of *Gluta* compared to *Gluta* sp clone Riau. Those variations were caused by mutations, such as insertion, deletion, and subtitution, wether transition subtitution and transversion subtitution. The mutations occured on *Gluta* sp clone Riau were the same as *G. renghas* deposited in GenBank (Figure 3).

Dendrogram generated from the *rps16* intron sequences also showed that *Gluta* sp clone Riau had the closest relationship (distance = 0) with *G. renghas* as compared to others (Figure 4). This result confirmed that the species name of *Gluta* sp clone Riau was *G. renghas*.

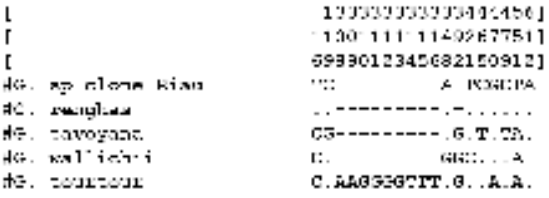

Figure 3. Differences among species of *Gluta* based on the *rps16* intron sequences. The numbers ordered low showed the the position of the base according to *Gluta* sp clone Riau.

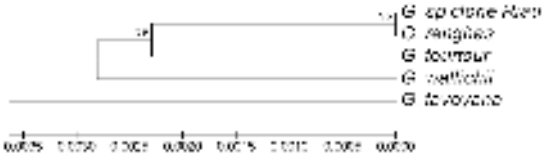

Figure 4. Dendrogram based on the *rps16* intron sequences calculated using UPGMA method with 1000 bootstrap. G = *Gluta*.

Analysis of The *trnL-trnF* Intergenic Spacer Sequence

The *trnL-trnF* intergenic spacer sequence was 527 bp in size. The sequence had been regis-

Table 2. BLASTn result of the *rps16* intron of *Gluta* sp clone Riau.

Description	Max score	Total score	Query cover	E value	Ident	Accession
Gluta renghas voucher Pell 806 (BKL) ribosomal protein S16 (*rps16*) gene, intron; chloroplast	1189	1259	100%	0.0	100%	KP055393.1
Gluta tavoyana voucher Pell 1075 (NY) ribosomal protein S16 (*rps16*) gene, intron; chloroplast	1160	1230	100%	0.0	99%	KP055394.1
Gluta wallichii isolate 51 *rps16* gene, intron; chloroplast	1157	1221	100%	0.0	99%	AY594600.1
Gluta tourtour voucher Randrianasolo 770 (MO) ribosomal protein S16 (*rps16*) gene, intron; chloroplast	1148	1218	100%	0.0	98%	KP055395.1

tered in GenBank with the accession number was KX365742 (Figure 5). The BLASTn analysis showed that the *trnL-trnF* intergenic spacer sequence of *Gluta* sp clone Riau also had 100% similarity to *G. renghas*. This result was supported by the values of BLASTn parameters (Table 3).

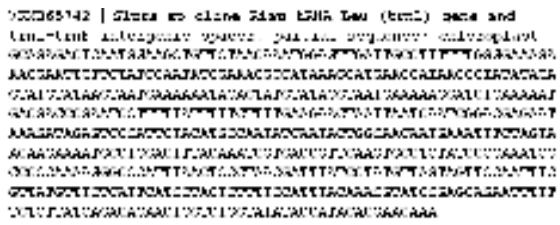

Figure 5. The *trnL-trnF* intergenic spacer sequence of *Gluta* sp clone Riau.

Analysis to the *trnL-trnF* intergenic spacer sequence demonstrated that there are 15 variations between species of *Gluta* observed and those variations were due to mutation, for example, insertion, deletion, and subtitution. Mutations occured on the sequence of *Gluta* sp clone Riau were the same as *G. renghas* (Figure 6).

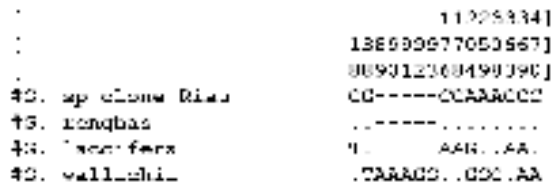

Figure 6. Differences among species of *Gluta* based on the *trnL-trnF* intergenic spacer sequences. The numbers ordered low showed the the position of the base according to *Gluta* sp clone Riau.

Dendrogram created using the *trnL-trnF* intergenic spacer sequences also showed the close relationship of *Gluta* sp clone Riau with *G. renghas* than *G. laccifera* and *G. wallichii* (Figure 7). Like the analysis results based on *rps16* intron sequence, the analysis using the *trnL-trnF* intergenic spacer also succeeded confirming the taxonomic status of *Gluta* sp clone Riau as *G. renghas.*

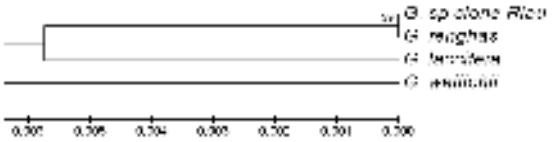

Figure 7. Dendrogram based on the *trnL-trnF* intergenic spacer sequences using UPGMA method with 1000 bootstrap. G = *Gluta*.

Types of study in biology require a clear identity or a clear taxonomic status of the organisme observed. The identification to determine the taxonomic status can be performed using morphological characters and DNA sequences. The determination of the organism identity using DNA sequences or DNA barcodes is called DNA barcoding (Hebert et al., 2003). One program, such as BLASTn, is frequently used to analyze the DNA barcodes (de Groot et al., 2011; Pang et al., 2012; Schoch et al., 2012; Selvaraj et al., 2012; Sivalingam et al., 2016; Nithaniya & Parani, 2016).

In this study, the BLASTn analysis is used to find the database of interest and to detect the similarity of the observed sequence (as query) to the sequences available in GenBank database. Some parameters in BLASTn program need to be considered to make a decission, for instances, the values of max score and total score should be high, the values of query cover dan the ident should be 100%, and E-value should be zero (Madden, 2013; Fassler & Cooper, 2008; Roslim, 2017; Roslim et al., 2016a).

The taxonomic status confirmation using the DNA barcode was also performed on tuntun angin (*Elaeocarpus floribundus*). Tuntun angin is a plant that was previously identified based on morphological characters as *Elaeocarpus floribundus* and then succeeded in being verified using 2

Table 3. BLASTn result of the *trnL-trnF* intergenic spacer sequence of *Gluta* sp clone Riau.

Description	Max score	Total score	Query cover	E value	Ident	Accession
Gluta renghas voucher Pell 806 (BKL) *tRNA-Leu* (*trnL*) gene and *trnL-trnF* intergenic spacer, partial sequence; chloroplast	951	951	100%	0.0	100%	KP055514.1
Gluta laccifera isolate Quyen 08 *tRNA-Leu* (*trnL*) gene and *trnL-trnF* intergenic spacer, partial sequence; chloroplast	924	924	100%	0.0	99%	KY067412.1
Gluta wallichii tRNA-Leu (*trnL*) gene and *trnL-trnF* intergenic spacer, partial sequence; chloroplast	910	910	100%	0.0	98%	AY594516.1

DNA barcodes, namely *matK* and ITS sequences, and also BLASTn analysis. The parameters values obtained were max score = 937, E-value = 0.0, identity value = 100%, and query cover = 100% (Roslim et al., 2016a). This success was supported by the availability of the *matK* and the ITS sequences for *Elaeocarpus floribundus* in GenBank.

The identification using DNA barcoding does not work if the DNA barcode sequences for the organism of interest do not exist in a public database like GenBank (Will & Rubinoff, 2004). Moreover, if the DNA barcode sequences of the organism of interest do not match the sequences existed in the public database, there will be 2 possibilities. First, the organism is a known species but the DNA barcode database is not available in the public database. Second, the organism is a new species. If the choice falls on the second possibility, the morphological justification must be conducted by following the taxonomic rules to make a conclusion that the organism is a new species (Will & Rubinoff, 2004; DeSalle, 2006; Roslim et al., 2016b).

In this study, few mutations have occured in both of the *rps16* intron and the *trnL-trnF* intergenic spacer on some species of *Gluta*. Mutation is a change on the DNA such as insertion, deletion, subtitution, translocation, and inversion. Mutations detected in both of the sequences are insertion, deletion, and subtitution, wether transition and transversion. Mutation can cause variation between species and this is favorable for phylogenetic analysis and determination of organism identity (Kelchner, 2002; Smedmark et al., 2008). Ryzhkova et al. (2013) has analyzed the indels and subtitution mutations on the *rps16* intron sequence to identify 6 haplotypes on *Solanum*. In addition, due to variation caused by mutation on the *trnL-trnF* intergenic spacer, Adrienne et al. (2015) can identify a species in genus *Lophophora*. A new species of *Atraphaxis* is also determined based on the *trnL-trnF* intergenic spacer (Yurtseva et al., 2016).

The DNA barcoding technique is basically developed to assist and to facilitate in identifying an organism using the DNA barcode sequences. However, there are some considerations in using this technique for plant identification, namely: (1) prediction of the genus of the observed plant based on morphological characters; (2) determination of tpyes of the DNA barcodes which will be used by examining and selecting 1 to 4 of the DNA barcodes related to the observed genus which amount is abundant in public database; (3) amplification of the DNA barcodes using universal primer or own designed primer based on the conserved region; (4) performance of the BLASTn analysis and conclusion based on the BLASTn parameters. If the sequence of the observed plant has similarity (with ident value = 100%) to sequences in GenBank database, the plant is the same; (5) the conclusion should be better verified with the morphological or other corroborating data.

CONCLUSIONS

This study has succeeded in applying sequences of the *rps16* intron and the *trnL-trnF* intergenic spacer to identify Rengas clone Riau as *Gluta renghas*. This success is supported by the availability of those sequences in GenBank. Thus, the DNA sequences database availability is critical for plant molecular identification.

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REFERENCES

Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic acids research,* 25(17), 3389-3402.

Adrienne, E. Ng., Sandoval, E., Murphy, T. M. (2015). Identification and Individualization of Lophophora using DNA Analysis of the trnL/trnF Region and rbcL Gene. *Journal of forensic sciences,* 61(S1)

Borsch, T. & Quandt, D. (2009). Mutational dynamics and phylogenetic utility of noncoding chloroplast DNA. *Plant Systematics and Evolution,* 282(3), 169-199.

Buerki, S., Callmander, M. W., Devey, D. S., Chappell, L., Gallaher, T., Munzinger, J., Haevermans, T., Forest, F. (2012). Straightening out the screw-pines: A first step in understanding phylogenetic relationships within Pandanaceae. *TAXON,* 61(5), 1010-1020.

CBOL Plant Working Group. (2009). A DNA barcode for land plants. *Proceedings of the National Academy of Sciences,* 106, 12794–12797 (2009).

Daniell, H., Lin, C., Yu, M., Chang, W. (2016). Chloroplast genomes: diversity, evolution, and applications in genetic engineering. *Genome Biol-*

ogy, 17, 134.

de Groot, G. A., During, H. J., Maas, J. W., Schneider, H., Vogel, J. C., Erkens, R. H. J. (2011). Use of rbcL and trnL-F as a Two-Locus DNA Barcode for Identification of NWEuropean Ferns: An Ecological Perspective. *PLoS ONE,* 6(1), e16371

DeSalle, R. (2006). Species discovery versus species identification in DNA barcoding efforts: response to Rubinoff. *Conservation Biology*, 20(5), 1545–1547.

Elvyra, R. & Yus, Y. (2012). *Ikan Lais dan Sungai Paparan Banjir di Provinsi Riau*. Pekanbaru: UR Press Pekanbaru.

Fassler, J. & Cooper, P. (2008). *BLAST Help*. Bethesda (MD): National Center for Biotechnology Information (US).

Fazekas, A. J., Burgess, K. S., Kesanakurti, P. R., Graham, S.W., Newmaster, S. G., Husband, B. C., Percy, D. M., Hajibabaei, M., Barrett, S. C. H. (2008). Multiple multilocus DNA barcodes from the plastid genome discriminate plant species equally well. *PLoS ONE*, *3(7)*, e2802.

Fern, K. (2014). *Useful Tropical Plants Database*. Creative Commons Attribution - Non Commercial – Share Alike 3.0 Unported License. http://tropical.theferns.info/image.php?id=Gluta+renghas). Accessed 1 January 2016.

Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B*, 270, 313–321.

Hebert, P. D. N. & Gregory, T. R. (2005). The promise of DNA barcoding for taxonomy. *Systematic Biology,* 54(5), 852–859.

Kelchner, S. A. (2002). Group II introns as phylogenetic tools: structure, function, and evolutionary constraints. *American Journal of Botany,* 89(10), 1651–1669.

Kress, W. J., Erickson, D. L., Jones, F. A., Swenson, N. G., Perez, R., Sanjur, O., & Bermingham, E. (2009). Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. *Proceedings of the National Academy of Sciences,* 106(44), 18621-18626.

Lee, S-C., Wang, C-H., Yen, C-E., & Chang, C. (2016). DNA barcode and identification of the varieties and provenances of Taiwan's domestic and imported made teas using ribosomal internal transcribed spacer 2 sequences. *Journal of Food and Drug Analysis*, 25(2), 260-274.

Madden, T. (2013). *The NCBI Handbook, 2nd edition*. Bethesda (MD): National Center for Biotechnology Information (US).

Nithaniya, S. & Parani, M. (2016). Evaluation of chloroplast and nuclear DNA barcodes for species identification in Terminalia L. *Biochemical Systematics and Ecology,* 68, 223-229.

Pang, X., Liu, C., Shi, L., Liu, R., Liang, D., Li, H., Cherny, S. S., Chen, S. (2012). Utility of the trnH–psbA intergenic spacer region and its combinations as plant DNA barcodes: A meta-analysis. *PLoS ONE*, 7, e48833.

Roslim, D. I. (2017). Pandan (*Pandanus* sp), Rotan (*Calamus* sp), and Rengas (*Gluta* sp) from Kajuik Lake, Riau Province, Indonesia. *Brazilian Archives of Biology and Technology,* 60, e17160419.

Roslim, D. I., Khumairoh, S., Herman. (2016a). Confirmation of tuntun angin (*Elaeocarpus floribundus*) taxonomic status using *matK* and *ITS* sequences. *Biosaintifika: Journal of Biology & Biology Education*, 8(3), 392-399.

Roslim, D. I., Nurkhairani, P., Herman, Elvyra, R. (2016b). Identification of durik-durik plant (*Syzygium* sp) using the psbA-trnH intergenic spacer and ITS regions. *Transaction of Persatuan Genetik Malaysia,* 3, 11-16.

Ryzhkova, N. N., Slugina, M. A., Kochieva, E. Z., Skryabin, K. G. (2013). Polymorphism and structural variation of rps16 group-II intron in the *Solanum* species. *Genetika*, 49(7), 824-9.

Schoch, C. L., Seifert, K. A., Huhndorf, S., Rober,. V., Spouge, J. L., Levesque, C. A., Chen, W., Fungal Barcoding Consortium. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences,* 109(16), 6241–6246.

Selvaraj, D., Shanmughanandhan, D., Sarma, R. K., Joseph, J. C., Srinivasan, R. V., Ramalingam, S. (2012). DNA barcode ITS effectively distinguishes the medicinal plant *Boerhavia diffusa* from Its adulterants. *Genomics Proteomics Bioinformatics,* 10(6), 364–367.

Shaw, J., Lickey, E. B., Schilling, E. E., Small, R. L. (2007). Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in Angiosperms: the Tortoise and the Hare III. *American Journal Of Botany*, 94(3), 275–288.

Sivalingam, D., Rajendran, R., Anbarasan, K. (2016). Studies on DNA barcoding of sacred plant - *Ficus religiosa* L. *Journal of Applied and Advanced Research,* 1(1), 29-39.

Smedmark, J. E. E., Rydin, C., Razafimandimbison, S. G., Khan, S. A., Liede-Schumann, S., Bremer, B. (2008). A phylogeny of Urophylleae (Rubiaceae) based on *rps16* intron data. *TAXON*, 57(1), 24–32.

Stoeckle, M. (2003). Taxonomy, DNA, and the bar code of life. *BioScience*, 53(9), 796-797

Sugita, M. Sugiura, M. (1996). Regulation of gene expression in chloroplasts of higher plants. *Plant Molecular Biology,* 32(1), 315-326.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution,* 30(12), 2725-2729.

Taberlet ,P., Gielly, L., Pautou, G., Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology,* 17(5), 1105-1109.

Vasconcelos, T. N. C., Proença, C. E. B., Ahmad, B., Aguilar, D. S., Aguilar, R., Amorim, B. S., Campbell, K., Costa, I. R., De-Carvalho, P. S.,

Faria, J. E. Q., Giaretta, A., Kooij, P. W., Lima, D. F., Mazine, F. F., Peguero, B., Prenner, G., Santos, M. F., Soewarto, J., Wingler, A., Lucas, E.J. (2017). Myrteae phylogeny, calibration, biogeography and diversification patterns: Increased understanding in the most species rich tribe of Myrtaceae. *Molecular Phylogenetics and Evolution,* 109, 113-137.

Will, K. W. & Rubinoff, D. (2004). Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. *Cladistics,* 20, 47–55.

Wu, D-C., He, D-M., Gu, H-L., Wu, P-P., Yi, X., Wang, W-J., Shi, H-F., Wu, D-X., Sun, G. (2016) Origin and evolution of allopolyploid wheatgrass *Elymus fibrosus* (Schrenk) Tzvelev (Poaceae: Triticeae) reveals the effect of its origination on genetic diversity. *PLoS ONE,* 11(12), e0167795.

Yang, R., Feng, X., & Gong, X. (2016). Genetic structure and demographic history of *Cycas chenii* (Cycadaceae), an endangered species with extremely small populations. *Plant Diversity,* 39(1), 44-51.

Yurtseva, O. V., Kuznetsova, O. I., Mavrodiev, E. V. (2016). A broadly sampled 3-loci plastid phylogeny of *Atraphaxis* (Polygoneae, Polygonoideae, Polygonaceae) reveals new taxa: I. *Atraphaxis kamelinii* spec. nov. from Mongolia. *Phytotaxa,* 268(1), 001–024.

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Seedling Production of Pak Choy (*Brassica rapa* L.) using Organic and Inorganic Nutrients

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Brassica rapa L. var. chinensis; hydroponic; organic nutrients; Pak Choy; seedling production

Abstract

Pak Choy or Bok Choy (*Brassica rapa* L. var. chinensis) is one of favorite Chinese leafy vegetable for various dishes in Indonesia. In this study, it was used as a plant model to identify the appropriate organic hydroponic nutrient solution for leafy vegetable seedling production. The seed was sown on rock wool slabs submerged with 200 ml of a nutrient solution containing biofertilizer of *Beyonic StarTmik@Lob* (0, 25, 50, 75, and 100%). commercial hydroponic solution (0, 25, 50, 75, and 100%) and its combination (25, 50, and 75%). The experiments were arranged in a CRD. Meanwhile, the obtained data was analyzed using ANOVA followed by DMRT. The relationship among growth parameters was observed using Pearson correlation analysis. The result of the study showed that the combination of organic and inorganic nutrient (25% *Beyonic StarTmik@Lob* and 75% commercial hydroponic solution) resulted in the highest seedling growth parameters and leaf indices as well as the perfectly positive correlations among growth parameters. This result indicated that the use of organic nutrient alone was not appropriate for hydroponic seedling production of Pak Choy. Therefore, further study needs to be done to identify the hydroponic solution without inorganic nutrients towards the organic vegetable production.

How to Cite

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INTRODUCTION

Pak Choy or Bok Choy (*Brassica rapa*) was introduced to South-East Asia in the 15th century. Nowadays it is widely cultivated in this region including Indonesia. Pak Choy is one of favorite Chinese leafy vegetable in Indonesia. All above ground part of this vegetable is edible mainly its succulent petiole. It is not commonly eaten raw but used in main ingredients for soup and stir-fried dishes. Each of 100 g edible part of Pak Choy contains protein 1.7 g, fat 0.2 g, Carbohydrate 3.1 g, vitamins and minerals such as β-carotene (2.3 mg), vitamin C (53 mg) and Calcium (102 mg) (Tay & Toxopeus, 1994). The national production of *Brassica* in 2015 is 600,200 tons (Central Agency on Statistics of Indonesia, 2015). This vegetable is commonly produced by conventional farming using inorganic fertilizer to enhance crop productivity. Nowadays, the demand of organic vegetable is increased due to the public concern about healthy food, free from chemical residues. Organic farming of cauliflower (*B. oleacera* var. botrytis) in lowland area showed a better vegetative growth but not for generative growth (Widiatningrum & Pukan, 2010). Organic farming of another *Brassica* species (cauliflower) was successfully done. Therefore, the organic vegetable seedling production plays an important role, because it is the initial step in the practice of biological farming. Moreover, Kubota et al. (2013) stated that the use of high-quality planting materials is critical for success in greenhouse plant production.

The viability of small seed including Pak Coy is usually tested using a paper substrate (Purbojati & Suwarno, 2006). However, seedling production is commonly using a locally organic substrate such as peat and vermicompost (Tuzel et al., 2014), spent mushroom compost (Priadi et al., 2016) on tomato seedling, and coconut coir dust on lettuce (*Lactuca sativa* L.) (Hossain et al., 2016). Moreover, production of organic transplants involves more than organic fertilizer and substrates and avoiding the use of non-approved pesticides. These organic substrates are also as an organic nutrient source with or without the addition of inorganic and organic fertilizer. A study on the feasibility of organic nutrient solution for the hydroponic culture of leafy green vegetable was conducted by Ferguson et al. (2014). Also, the seedling production of peppermint and spearmint using inorganic and organic fertilization was previously performed by Akoumianaki-Ioannidou et al. (2010). In this study, we use both inorganic and organic nutrients using a hydroponic culture technique to identify the appropriate organic nutrient solution for Pak Choy seedling production prior a transplanting to the soil or soilless medium.

METHODS

This study was conducted from October to December 2016 in the screen house of the Germplasm Garden of RC for Biotechnology-LIPI, Cibinong, West Java. The average temperature in the screen house was 33.4°C with the relative humidity (RH) of 60.7%.

The commercial seed of Pak Choy (*Brassica rapa* Var. Nauli), produced by East-West Seed Company were obtained from a local farm shop in Bogor; West Java was used in this study. The organic nutrient solution was obtained from bio-organic fertilizer of *Beyonic StarTmik@Lob* (25 ml/1 water) produced by Research Center for Biology-LIPI, whereas a commercial hydroponic solution (Raja Hidroponik) (5 ml/l) was used for the inorganic nutrient source. The seeds were sown manually on each 1 cm^3 rock wool slabs submerged in a 200 ml of nutrient solution in a plastic container (25 x 20 x 3 cm). Groundwater moistened the rock wool slabs before seed sowing. Each container consists of 25 rock wool slabs containing inorganic and organic nutrient (Table 1).

The degree of acidity (pH) and electrical conductivity (EC) of the nutrient solution were done using water/soil (2:1) extraction method at the beginning and the end of germination period using a digital portable pH and EC meter (Adwa AD1000). Seedling height was measured using a digital caliper (Nankai). Seedling leaf area was calculated using digital image analysis method (Bradshaw et al., 2007). This approach has been used by researchers in a variety of application (Priadi et al., 2016) due to the simple, inexpensive and accurate method. The water content of Pak Coy seedling was measured on a fresh weight basis method according to ISTA (2006) using a drying oven (Zenith Lab DHG9053A) at 130°C for 2 hours.

Germination parameter of the Pak Choy seedlings was germinability and germination rate. Seedling growth was observed daily and taken from 25 seedlings for each replication. Seedling height and diameter, whole leaves and roots, and leaf area were observed at the end of germination period (day-14)taken from five seedlings of each replication. Seedling emergences were recorded per day when a normal seedling was visible above the rock wool slabs. The leaf indices of SLA

Table 1. Composition of nutrient solution for hydroponic seedling production of Pak Choy

Code	Composition
K0-1	100% groundwater
K0-2	100% commercial hydroponic solution
K0-3	*Beyonic StarTmik@Lob* at the producer's recommended concentration*
K1	25% K0-3+75% groundwater
K2	50% K0-3+50% groundwater
K3	75% K0-3+25% groundwater
K4	25% K0-3+75% K02
K5	50% K0-3+50% K02
K6	75% K0-3+25% K02

Note*=25 ml in 1 liter of water

(Specific Leaf Area) and LAR (Leaf Area Ratio) were recorded to evaluate the seedling resistance at transplant (Herrera et al., 2008). The SLA is the ratio of leaf area to leaf dry weight. Meanwhile, LAR is the ratio of leaf area to dry seedling weight.

The experiments were arranged in a Completely Randomized Design (CRD) with 3 replications. Obtained data was analyzed using Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The relationship among growth parameters was observed using Pearson correlation analysis. Data was processed using statistical software SPSS 16.0.

RESULTS AND DISCUSSION

Data in Table 2 showed that pH and EC value of the nutrient solution at the end of germination period were higher than that the initial. The higher pH is caused by anion uptake by the plant during plant growth period. Meanwhile, the higher EC is due to the high level of nutrient due to the additional nutrient solution for adjustment (Hossain et al., 2016).

Seedling Emergence

Seedling emergence is one of the important parameter as well as emergence rate. The highest all seedling growth parameter was obtained by the Pak Choy seed sown on the rock wool slabs containing 100% of groundwater (K0-1). It was not significantly different with K0-1 when the seed was sown on the rock wool slabs containing the mixture of organic and inorganic solution (25% *Beyonic StarTmik@Lob* and 75% commercial hydroponic solution). Contrary, the mixture of 75% *Beyonic StarTmik@Lob* and 25% commercial hydroponic solution (K3) resulted in the lowest seedling growth. A biofilm was formed on the root surface of the seedling in the hydroponic solution containing 100% *Beyonic StarTmik@Lob* (K0-3). Seedling growth of Pak Choy on K-03 was lower due to the biofilm formation. A study conducted by Chinta et al. (2015) found that a biofilm was formed on the root surface of organic hydroponic lettuce; in contrast, chemical hydroponics resulted in a lack of biofilm. Biofilm is formed as an interaction between beneficial microorganism and lettuce roots. Another study conducted by Fujiwara et al. (2012) on tomato seedlings found that a rhizosphere biofilm in the organic hydroponic may be responsible for the suppression of the bacterial wilt. In this study, we found that the use of *Beyonic StarTmik@Lob* alone as a nutrient solution for Pak Choy hydroponic seedling production seemed to be not appropriate to obtain optimal growth (Table 3).

Leaf Indices

The highest leaf number (5.067) was obtained from the hydroponic solution containing 100% commercial hydroponic solution (K0-2). It was not significantly different with those containing both K4 and K5. The mixture of inorganic and organic nutrient solution of K4 and K5 resulted in the best leaf area. The best SLA index was obtained from K4 nutrient solution. According to Herrera et al. (2008), the lower SLA value indicated, the higher transplant stress resistance. The LAR represents the relationship between photosynthetic material and respiratory material in the plant. It is also used for evaluation of seedling resistance at transplant. Higher LAR indicates more biomass production for seedling growth. Table 4 showed that there was not significantly different in both of SLA and LAR among the nutrient solution used except the K0-3.

Table 2. Characteristic of nutrient solution of Pak Choy hydroponic culture

Nutrient Solution	Initial		Final	
	pH	EC (dS m^{-1})	pH	EC (dS m^{-1})
K0-1	5.91	0.0	7.25	0.3
K0-2	4.53	2.2	6.44	9.7
K0-3	3.98	0.7	6.79	4.7
K-1	4.09	0.5	6.88	1.0
K-2	4.12	0.3	6.91	1.5
K-3	4.62	0.1	6.86	2.4
K-4	5.06	1.5	7.20	3.1
K-5	4.86	1.2	7.43	4.1
K-6	4.15	1.5	7.48	4.3

Table 3. Growth parameter of Pak Choy seedlings after 14-days sowing on rock wool slabs containing inorganic and organic nutrient solution in the screen house

Nutrient Solution	Emergence (%)		Rate of emergence		Height (cm)		Diameter (cm)		Total roots	
K0-1	98.667	a	7.047	a	1.580	bcd	0.9933	abc	11.800	a
K0-2	85.333	ab	6.093	ab	1.800	bc	1.0733	a	12.267	a
K0-3	60.000	bcd	4.288	bcd	2.033	b	0.7500	d	6.933	d
K-1	77.333	abc	5.524	abc	1.587	bcd	0.9267	abc	10.467	b
K-2	49.333	cd	3.520	cd	1.373	cd	0.9067	bc	10.400	b
K-3	36.000	d	2.570	d	1.200	d	0.8633	cd	8.733	c
K-4	78.667	abc	5.620	abc	2.847	a	1.0633	ab	12.400	a
K-5	66.667	bcd	4.763	bcd	2.573	a	1.0667	ab	12.067	a
K-6	56.000	bcd	3.997	bcd	1.873	b	0.9600	abc	10.333	b

Note: Means in the same column followed by the different letter are significantly different ($p<0.05$) according to DMRT

Seedling dry weight

The highest Pak Choy seedling dry weight was obtained in the hydroponic solution containing biofertilizer of Beyonic StarTmik@Lob (K-03) (Table 5 and Figure 1). This biofertilizer was supported by PGPR (Plant growth promoting rhizobacteria) (Dewi et al., 2015). It was suggested that the higher seedling dry weight was affected by the hydroponic solution containing PGPR as explained by Bashan & de-Bashan (2010), since PGPR at the very early stage leading to better absorption of water and minerals. The result of this study agrees with a study conducted by Das et al. (2014) on organically cultivated mungbean using the organic manure containing PGPR.

The lower seedling dry weight was obtained on a mixture of organic and inorganic nutrient solution (25% *Beyonic StarTmik@Lob* and 75% inorganic nutrient solution). The dry seedling weight of Pak Choy seems to be affected by the availability of *Beyonic StarTmik* in the nutrient solution. A study conducted by Ferguson et al. (2014) showed that hydroponic culture of Bok Choy using organic nutrient solution resulted in the lower yield compared with those low or high-level inorganic nutrients. Nevertheless, the price of the organic product is much higher than the conventional product.

Correlation among growth parameters

Correlation among Pak Choy seedling growth parameters in the nutrient solution used is varied. There was perfectly positive correlation between total leaves (TL) and both stem dry weight (SW) and whole seedling dry weight (TW) as well as the correlation between leaf area (LA) and TW and between SW and TW on K4 nutrient solution (Table 6). It was reasonable that the increased in the whole seedling dry weight was affected by the increased of total leaves, leaf area and stem dry weight. Table 6 showed that the correlation among those seedling growth pa-

Table 4. Leaf indices of Pak Choy seedlings after 14-days sowing on rock wool slabs containing inorganic and organic nutrient solution in the screen house

Nutrient Solution	Leaf number		Leaf area (mm^2)		SLA (mm^2/mg)		LAR (mm^2/mg)	
K0-1	4.067	c	88.000	c	84.137	a	53.887	a
K0-2	5.067	a	162.365	b	84.767	a	57.170	a
K0-3	2.933	d	52.540	e	9.250	b	5.187	b
K-1	4.000	c	71.111	d	112.763	a	68.903	a
K-2	3.800	c	62.753	de	132.373	a	67.907	a
K-3	3.667	c	53.663	e	132.440	a	65.480	a
K-4	5.000	a	231.022	a	95.697	a	61.287	a
K-5	5.000	a	238.703	a	107.717	a	75.223	a
K-6	4.533	b	154.699	b	119.843	a	75.380	a

Note: Means in the same column followed by the different letter are significantly different ($p<0.05$) according to DMRT

Table 5. Dry weight of Pak Choy seedlings after 14-days sowing on rock wool slabs containing inorganic and organic nutrient solution in the screen house

Nutrient Solution	Stem (mg)		Leaves (mg)		Roots (mg)		Whole (mg)	
K0-1	0.157	cd	1.047	de	0.437	b	1.637	cd
K0-2	0.523	cd	2.017	bcd	0.457	b	2.993	bc
K0-3	1.900	a	5.687	a	2.640	a	10.227	a
K-1	0.090	d	0.633	e	0.313	b	1.033	d
K-2	0.137	cd	0.483	e	0.317	b	0.937	d
K-3	0.140	cd	0.380	e	0.250	b	0.767	d
K-4	1.017	b	2.993	b	0.550	b	4.560	b
K-5	0.607	bc	2.277	bc	0.380	b	3.267	bc
K-6	0.467	cd	1.433	cde	0.327	b	2.227	cd

Note: Means in the same column followed by the different letter are significantly different ($p<0.05$) according to DMRT

Figure 1. Seedling performance of Pak Choy after 14-day sowing in various hydroponic nutrient solution (A= K0-1, B= K0-2, C= K0-3, D=K1, E=K2, F=K3, G=K4, H=K5, I=K6)

rameters seemed to be affected by the biofertilizer and inorganic nutrient proportion in the hydroponic solution of K-4 (25% K0-3+75% K02). In contrast, there was not any significant difference correlation among growth parameters in K-6 (75% K0-3+25% K02). A different result showed by tomato seedling grown on various compost types; there was a very significant correlation between leaf area and both dry leaf weight and whole seedling dry weight, as well as seedling height and diameter (Priadi et al., 2016). The correlation among seedling growth parameters seemed to

Tabel 6. Correlation of growth parameters of Pak Choy seedling in various nutrient solution

Parameter K01		Nutrient Solution								
		K02	K03	K1	K2	K3	K4	K5	K6	
HT	DM	-1.000*	-0.993	-.905	-0.954	-0.422	0.723	0.347	0.854	0.589
	TL	0.596	-0.596	0.270	n/a	0.973	0.827	0.667	n/a	-0.858
	LA	0.885	0.924	0.919	-0.034	0.614	-0.127	0.951	0.123	-0.536
	TR	0.986	0.993	0.969	0.019	1.000**	0.667	-0.950	-0.979	0.225
	SW	0.984	0.979	-0.248	1.000*	-0.229	0.205	0.659	-0.881	-0.988
	LW	0.255	0.931	0.460	-0.987	0.989	-0.006	0.707	-0.498	-0.967
	RW	0.966	0.998*	0.237	-0.985	0.993	-0.418	0.228	-0.962	-0.944
	TW	0.985	0.958	0.200	0.345	0.999*	-0.175	0.659	-0.655	-0.969
DM	TL	-0.610	0.500	-0.655	n/a	-0.619	0.986	0.930	n/a	-0.091
	LA	-0.893	-0.962	-0.999*	-0.266	0.456	0.594	0.039	-0.412	0.366
	TR	-0.989	-1.000**	-0.982	-0.317	-0.412	0.997*	-0.622	-0.942	-0.655
	SW	-0.987	-0.996	-0.189	-0.948	-0.786	0.825	0.934	-0.999*	-0.456
	LW	-0.272	-0.966	-0.795	0.893	-0.287	0.686	0.909	-0.877	-0.364
	RW	-0.961	-0.985	-0.629	0.991	-0.524	0.325	0.992	-0.963	-0.289
	TW	-0.988	-0.984	-0.599	-0.610	-0.376	0.554	0.934	-0.953	-0.371
TL	LA	0.901	-0.243	0.628	n/a	0.417	0.453	0.403	n/a	0.894
	TR	0.721	-0.500	0.500	n/a	0.971	0.971	-0.866	n/a	-0.693
	SW	0.731	-0.419	0.866	n/a	0.000	0.721	1.000**	n/a	0.928
	LW	0.929	-0.261	0.979	n/a	0.930	0.558	0.998*	n/a	0.961
	RW	0.367	-0.640	0.999*	n/a	0.993	0.165	0.877	n/a	0.980
	TW	0.724	-0.339	0.997*	n/a	0.961	0.410	1.000**	n/a	0.959
LA	TR	0.950	0.962	0.988	0.999*	0.623	0.654	-0.807	0.082	-0.943
	SW	0.955	0.983	0.155	-0.053	-.909	0.945	0.394	0.362	0.662
	LW	0.676	1.000*	0.773	0.195	0.722	0.993	0.453	0.799	0.734
	RW	0.734	0.901	0.601	-0.137	0.519	0.954	-0.085	0.152	0.785
	TW	0.951	0.995	0.570	0.926	0.653	0.999*	1.000**	0.669	0.729
TR	SW	1.000**	0.996	0.000	0.000	-0.240	0.866	-0.861	0.959	-0.375
	LW	0.412	0.966	0.666	0.143	0.991	0.741	-0.892	0.665	-0.466
	RW	0.910	0.985	0.470	-0.189	0.992	0.397	-0.520	0.997*	-0.534
	TW	1.000**	0.984	0.437	0.945	0.999*	0.617	-0.860	0.796	-0.459
SW	LW	0.426	0.986	0.746	-0.990	-0.367	0.977	0.998*	0.849	0.995
	RW	0.903	0.966	0.882	-0.982	-0.115	0.803	0.882	0.977	0.984
	TW	1.000**	0.996	0.900	0.327	-0.277	0.928	1.000**	0.935	0.996
LW	RW	-0.004	0.908	0.972	0.945	0.966	0.911	0.850	0.716	0.997
	TW	0.416	0.997	0.962	-0.189	0.995	0-.006	0.998*	0.981	1.000**
RW	TW	0.908	0.940	0.999*	-0.500	0.986	0.967	0.883	0.837	0.996

Note: HT=Height; DM=Diameter; TL=Total leaves; LA=Leaf area; TR=Total roots; SW=Stem dry weight; LW=Leaf dry weight; RW=Root dry weight; TW=Whole seedling dry weight; Means in the same column followed by the same letter are not significantly different ($p<0.05$)*) or very significantly different ($p<0.01$) **), n/a =not available

be affected by the plant species and growing medium types. Furthermore, a study conducted by Shangjie et al. (2011) on a hydroponic of lettuce and Pak Choy showed that the vegetable types varied on optimal solution concentration, and different vegetable cultivars exhibited specific growth characteristics.

The result of the study as explained above, found that the inorganic nutrient in the hydroponic solution could be decreased by using biofertilizer. However, further study needs to be done to obtain organic substances from inexpensive, and easily available materials for hydroponic seedling production towards the organic product since the hydroponic culture of vegetable crops is commonly applied by the urban community which has no space for conventional farming. By using the organic nutrient in the hydroponic solution is expected to be produced more healthy vegetable.

CONCLUSIONS

From this study, we concluded that the nutrient solution containing 25% Beyonic *StarTmik@Lob* and 75% commercial hydroponic solution (K4) was the appropriate nutrient solution for hydroponic seedling production of Pak Choy. The organic seedling production plays an important role because it is the initial step in the practice of biological farming. Therefore, further study needs to be done to obtain organic substances for hydroponic seedling production towards the organic product.

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REFERENCES

Akoumianaki-Ioannidou, A., Rasouli, M., Podaropoulou, L., & Bilalis, D. (2010). Seedlings production of *Mentha× piperita* (peppermint) and *Mentha spicata* (spearmint) in float system with organic and inorganic fertilization. In *XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on 937* (pp. 1307-1311).

Bashan, Y., & De-Bashan, L. E. (2010). Chapter two-how the plant growth-promoting bacterium Azospirillum promotes plant growth—a critical assessment. *Advances in agronomy*, *108*, 77-136.

Bradshaw, J. D., Rice, M. E., & Hill, J. H. (2007). Digital analysis of leaf surface area: effects of shape, resolution, and size. *Journal of the Kansas Entomological Society*, 80(4), 339-347.

Chinta, Y. D., Eguchi, Y., Widiastuti, A., Shinohara, M., & Sato, T. (2015). Organic hydroponics induces systemic resistance against the air-borne pathogen, Botrytis cinerea (gray mould). *Journal of Plant Interactions*, 10(1), 243-251.

Central Agency on Statistics of Indonesia. (2015). Production of Vegetables 2015. Retrieved from https://www.bps.go.id/site/resultTab.

Das, I., & Singh, A. P. (2014). Effect of PGPR and organic manures on soil properties of organically cultivated mungbean. *Bioscan*, *9*(1), 27-29.

Dewi, T. K., Arum, E. S., Imamuddin, H. & Antonius, S. (2015). Characterization of plant growth promoting rhizobacteria (PGPR) supporting organic biofertilizer. In *Prosiding Seminar Nasional Masarakat Biodiversitas Indonesia* (Vol. 1, No. 2, pp. 289-295).

Ferguson, S. D., Saliga III, R. P., & Omaye, S. T. (2014). Investigating the effects of hydroponic media on quality of greenhouse grown leafy greens. *International Journal of Agricultural Extension*, *2*(3), 227-234.

Fujiwara, K., Aoyama, C., Takano, M., & Shinohara, M. (2012). Suppression of *Ralstonia solanacearum* bacterial wilt disease by an organic hydroponic system. *Journal of General Plant Pathology*, 78(3), 217-220.

Herrera, F., Castillo, J. E., Chica, A. F., & Bellido, L. L. (2008). Use of municipal solid waste compost (MSWC) as a growing medium in the nursery production of tomato plants. *Bioresource technology*, 99(2), 287-296.

Hossain, S. M. M., Imsabai, W., & Thongket, T. (2016). Growth and quality of hydroponically grown lettuce (*Lactuca sativa* L.) using used nutrient solution from coconut-coir dust and hydroton substrate. *Advances in Environmental Biology*, 10(4), 67-80.

ISTA. (2006). *International Rules For Seed Testing 2006*. Bassersdorf: International Seed Testing Association.

Kubota, C., Balliu, A., and Nicola, S. (2013). Quality of Planting Materials. *In Good Agricultural Practices for Greenhouse Vegetable Crops: Principles for Mediterranean climate Areas.* FAO Plant Production and Protection Paper 217, 355–378.

Priadi, D., Arfani, A., Saskiawan, I. & Mulyaningsih, E.S. (2016). Use of grass and spent mushroom compost as a growing medium of local tomato (*Lycopersicon esculentum* Miller) seedling in the nursery. *Agrivita,* 38(3), 242-250.

Purbojati, L., & Suwarno, F. C. (2006). Studi alternatif

substrat kertas untuk pengujian viabilitas benih dengan metode uji diatas kertas. *Jurnal Agronomi Indonesia*, 55-61.

Shangjie, R., Wei, F., Guomin, Z., & Yuyang, Z. (2011). Growth and quality of hydroponically cultured lettuce and pakchoi under two different solution concentration. *Crops,* 3, 011.

Tay, D. C. S., & Toxopeus, H. (1994). *Brassica rapa* L. cv. group pak choi, in PROSEA (Plant Resources South-East Asia) No. 8: Vegetables (2nd edition), ed. by Siemonsma JS and Piluek K. Pudoc, Prosea Foundation Bogor. P. 30-34 Wageningen. pp: 130-134.

Tuzel, Y., Oztekin, G. B., & Tan, E. (2014). Use of different growing media and nutrition in organic seedling production. In *XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014): 1107* (pp. 165-175).

Widiatningrum, T., & Pukan, K. K. (2010). Pertumbuhan dan produksi kubis bunga (*Brassica oleracea* var botrytis) dengan sistem pertanian organik di dataran rendah. *Biosaintifika: Journal of Biology & Biology Education*, *2*(2), 115-121.

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The Effect of Physical Activity agains the Telomere Length in the Leukocytes Cells of KONI Athletes

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Abstract

Telomeres are strands of non coding DNA at the ends of chromosomes that have the primary function to protect DNA from damage and maintain chromosomal stability. Physical exercise will increase the antioxidant activity can increase telomere proteins, lengthen telomeres and or protein networks associated with telomere so that the telomere remains long, or stopping telomere shortening. Telomere length was also associated with age. The purpose of the research was to determine telomere length of leukocyte cells in the KONI (Indonesian National Sports Committee) athletes in Jakarta. The research method is descriptive, by measuring telomere length using quantitative PCR on leukocyte cells. Samples are KONI athletes from several sports, including men and women athletes, with ages between 15-20 years. Used a control group (not athletes) is students of the Faculty of Medicine, University of YARSI. The results showed that there was no significant difference ($p > 0.05$) between telomere length group of athletes with the control group in both sexes. Similarly, telomere length between athlete male with female athletes also showed no significant difference ($p > 0.05$). It was concluded that physical exercise in athletes KONI at the age of 15- 20 years had no effect on telomere length in leukocytes. The results of this study provide information about the telomere length in Indonesian athletes at an early age.

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INTRODUCTION

Telomeres are the ends of chromosomes that are non-coding DNA replication in eukaryotic cells, which in humans is a replication of hexanucleotide TTAGGG. Hexanucleotide a core protein of the complex telomere shelterin and is associated with telomere function (Sabenroth et al., 2015) Cloning DNA strand can be done thoroughly with their typical structure of telomeres and the enzyme telomerase. If the cells do not have telomerase, the cells were not able to double-stranded telomere DNA. Thus causing DNA strand telomeres become shorter (Theimer & Feigon, 2006).

Telomere function is as a cover, which is essential for maintaining chromosome stability of recombination, fusion and degradation. Therefore, loss of telomere function might have had a great effect in the maintenance and integrity of chromosomes.

Telomere length can be used as an index of the biological age of a person or as biomarker of cell aging and can used to predict related incidence of morbidity and mortality (Laine et al., 2015). In addition to telomere length is often associated with the state of human health and at a certain age-related diseases in humans such as diseases related to the cardiovascular system (Hunt et al., 2008; Chen et al., 2011, Sabenroth et al., 2015)

Physical activity will increase the activity of antioxidant that can boost the protein telomere, extending telomeres and or tissue proteins associated with telomeres. Furthermore, physical activity can keep telomeres long stays or stop telomere shortening (Ludlow & Roth, 2011).

The research objective was to determine telomere length in KONI athletes leukocyte cells in some sports include men and women athletes. Benefits of the research is include the information about the length of telomeres athletes at a young age and become a reference for further research related to telomere length at the same age or different age.

METHODS

The study used a descriptive survey by measuring the telomere length on leukocyte cell of KONI athletes from several branches of sports. The research was conducted in the Laboratory Clinic Prodia and Integrity Laboratory YARSI University, Jakarta.

Subjects were students of SMP / SMA Ragunan, South Jakarta, about 40 people i.e. 20 men and 20 women. The controls are students of the Faculty of Medicine, University of YARSI totaled 38 people, including 17 men and 21 women). The age ranged from 15-20 years. The sampling technique is random sampling and differentiates the active individuals exercising with individuals who are not actively exercising. The sample criteria is aged 15 - 20 years,and healthy.Each subject of research conducted venous blood sampling as much as 5 cc, further isolation of lymphocytes and measurement of telomere length.

Samples were collected in tubes containing EDTA (Ethylene diamine tetra acid), then dilution with PBS solution (Phosphate Buffered Saline). Tubes labeled EDTA, then blood EDTA samples were rocked to prevent blood clotting. EDTA blood was transferred to a special tube and added a solution of PBS with comparing 1: 1. Mixture of blood EDTA (Ethylene diamine tetra Acid,) and PBS were transferred into tubes already containing Ficoll, then centrifuged at a speed of 400g, for 10 minutes. The middle layer (monocytes) were taken and transferred to a new tube, then added Ficoll and centrifuge back on the speed of 100g for 10 minutes. Centrifugation is done 2 times.

Measurement of telomere length were calculated using quantitative PCR (O'Collaghan et al., 2011), includes the step of DNA isolation, measuring the quality and quantity of DNA and genotyping. Measurement of relative elomere length is relatively proceed with the method Cawthon (2002).

DNA was extracted using the QIAamp DNA blood mini kit (Qiagen, Germany) according to the protocol indicated on the kit DNA is then stored in a freezer at -20°C.

The quality and quantity of DNA were analyzed using a spectrophotometer NanoDrop ND1000. This measurement is important to do in order to determine the concentration of DNA to be used in PCR and sequencing.

PCR (polymerase chain reaction) is a technique or method of reproduction (replication) enzymatic DNA without using organisms. With this technique, the DNA can be produced in large quantities at relatively short time so as to facilitate a variety of other techniques that use DNA. PCR was performed to determine the length of telomeres through repetition TTAGGG generated. Here is a primer sequence that was used to observe telomere length.

Telomere length data were analyzed by ANOVA followed by multiple comparison test using SPSS 20 version

Table 1. Oligomere used to measure the length of telomeres in human and rodent

	Oligomer Name	Species	Oligomer sequence (5'-3')	Amplicon size
Standards	Telomere standard	Human/ rodent	(TTAGGG)14	84 bp
	36B4 standard	Human	CAGCAAGTGGGAAGGTGTAATCCGTCTCCA-CAGACAAGGCCAGGACTCGTTTG TACCCGTTGAT-GATAGAATGGG	75 bp
PCR Primers	teloF	Human/ rodent	CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGG TTTGGGTT	>76 bp
	teloR	Human/ rodent	GGCTTGCCTTACCCTTACCCTTACCC TTACCCTTACCCT	
	36B4F	Human	CAGCAAGTGGGAAGGTGTAATCC	75 bp
	36B4R	Human	CCCATTCTATCATCAACGGGTACAA	
	b-globinF	Human	GCTTCTGACACAACTGTGTTCACTAGC	82 bp
	b-globinR	Human	CACCAACTTCATCCACGTTCACC	
	36B4F	Rodent	ACTGGTCTAGGACCCGAGAAG	78 bp
	36B4R	Rodent	TCAATGGTGCCTCTGGAGATT	

(O'Collaghan et al., 2011),

This research has been getting Description Escaped Airworthiness Conduct of Research Ethics Committee, Research YARSI with No. 004 / KEP-UY / BIA / V / 2014 dated May 9, 2014

RESULTS AND DISCUSSION

Measurement of telomere length is done by creating a standard curve of telomeres compared to the standard curve Beta-globin with Quantitative PCR methods (O'Collaghan et al., 2011) and was followed by measuring the relative telomere length by calculating the ratio between telomere to single copy gene (T / S) Quantitative PCR method refers to a method of Cawthon (2002). The results of measurements of the relative telomere length of 38 samples are presented in Table 1. As supporting data presented data from anthropometric examination (Table 2, 3, and 4)

Statistical analysis with Levence test showed no difference between groups of athletes with the control group with a significance value of $p > 0.05$. By sex obtained telomere length between male athletes with female athletes did not show a difference with a significance value of $p > 0.05$).

Telomeres are nucleoprotein structures located at the ends of chromosomes of eukaryotic cells. Telomere length can be shortened with age and is involved in cellular aging. Therefore, the telomere length is a biomarker for aging (Mather et al., 2011). Telomeres consist of nucleotides TTAGGG replication. In humans, there is a repeat of 2000

Table 2. Relative telomere length value in athlete and control group leukocyte (ratio T / S)

Repetition	Athlete Group		Control Group	
	Male	Female	Male	Female
1	0.76	0.77	0.82	0.82
2	0.75	0.90	0.85	0.80
3	0.75	0.78	0.82	0.81
4	0.79	0.76	0.83	0.79
5	0.78	0.79	0.82	0.82
6	0.78	0.84	0.83	0.80
7	0.78	0.79	0.82	0.79
8	0.78	0.80	0.82	0.78
9	0.88	0.79	0.80	0.80
10	0.79	0.79	0.81	0.82
11	0.78	0.81	0.74	0.82
12	0.79	0.84	0.82	0.86
13	0.76	0.80	0.78	0.83
14	0.80	0.81	0.79	0.84
15	0.83	0.83	0.80	0.81
16	0.80	0.83	0.83	0.81
17	0.82	0.85	0.86	0.84
18	0.81	0.85		0.81
19	0.78	0.82		0.87
20	0.79	0.86		1.04
21				0.93
Mean	0.7900	0.8156	0.8141	0.8329
SD	0.02974	0.03456	0.02740	0.05789

In this study, the sample used is young people aged between 17-20 years, male and female gender, include athletes with some sports include athletics, football, volleyball, hangars, and boxing. Measurement of telomere length is taken from the cell leukocyte. The study by some researchers previously reported that telomere length at the individual has a different telomere length in different tissues or organs, such as the kidneys, liver, lungs or blood cells, lymphocyte cells have telomere length varied. Similarly, different species, length of telomeres is also different in different species (Cherif et al., 2003)

Various studies show that the influence of exercise to delay aging and prolong life. People who regularly exercise generally held steady despite the young age of aging. Compared with those who did not exercise, athletes runners had cells that looked much younger when observed under a microscope. According to Ludlow and Roth (2011), physical exercise will increase the activity of antioxidant that can boost the protein telomere, extending telomeres and or tissue proteins associated with telomeres. Sport / physical activity keep telomeres long stays or stop telomere shortening. Studies on 69 men and women volunteer aged 50-70 years showed that regular physical activity can maintain to the telomere length (Ludlow et al., 2008; Collin et al., 2003)

The results of this research showed that telomere length in KONI athletes leukocytes did not differ with the control group. This research was supported by previous studies reported that the leukocyte telomere length in cells of young

Table 3. Data of weight / height in a control and athlete group

Repetition	Athlete group				Control Group			
	Male		Female		Male		Female	
	Body Weight (kg)	Body height (cm)	Body Weight (kg)	Body height (cm)	Body Weight (kg)	Body height (cm)	Body Weight (kg)	Body height (cm)
1	60	170	47	158	91	187	39	155
2	53	156	49	167	83	169	46	152
3	68	168	46	158	66	175	52	157
4	58	162	82	172	70	165	56	156
5	59	165	68	167	56	160	50	161
6	62	168	60	169	160	160	58	157
7	64	169	46	155	69	172	61	163
8	74	185	44	158	62	165	44	149
9	60	165	46	150	110	176	49	159
10	55	167	69	169	51,5	170	42	158
11	54	174	62	165	77,5	171	61	158
12	67	176	55	163	96	165	61	155
13	54.5	176	58	170	80	173	55	153
14	53	163	71	154	87	174	51	155
15	57	170	77	172	66	166	60	153
16	66	178	60	174	67	178	46	161
17	62	168	72	175	87	184	49	159
18	60	164	46	155			49	158
19	61	171	50	168			58	163
20	50	163	48	161			60	160
21	45	154					60	163
Mean	59.40	168.19	57.80	164.00	81.12	171.18	52.71	157.38
SD	6.73	7.18	11.91	7.42	25.33	7.54	6.92	3.81

athletes with an average age of 20.6 years is not different from the control group, are volunteers who are not actively doing physical activity. Instead of continuous exercise in athletes older the average age of 51.6 years, showed that the telomeres are longer than those without exercise (Werner et al., 2008).

Another study on adolescent group Caucasians and African Americans ages 14 to 18 years also show telomere length (ratio T / S) does not difference compared to the control group. But the telomere length in Afrika.-American race is greater than Caucasians. It shows that the race can affect telomere length, whereas adipose tissue is not related to telomere length at this age. Physical activity causes the anti-aging effects are quite strong at a young age, particularly in women (Zhu et al., 2011)

Moderate physical exercise or aerobics, especially in women can maintain telomere length or increasing telomere length than those who are inactive. It is especially in women over the age of 40 years. Regular physical activity nothing to do with a decrease in oxidative stress and inflammation as well help prevent the onset of chronic diseases. It has been reported also that telomere length is influenced by various factors such as age, sex, race, smoking, physical activity, socioeconomic status, obesity, intake of multivitamins, alcohol consumption and hormone replacement therapy despite inconsistent findings (Enokido et al., 2014)

Relative telomere length (ratio T / S) at a young age (22-27 years) and elderly (66-77 years) between the group of athletes with non-athletes men have also been reported. At a young age, te-

Table 4. Waist circumference ratio data (LPI) cm) and Pelvic circumference (LPA) cm) data of athletes and the control group

Repetition	Athlete Group				Control Group			
	Male		Female		Male		Female	
	Wc	Pr	Wc	Pr	Wc	Pr	Wc	Pr
1	77	85	76	90	107	103	63	83
2	70	75	82	88	93	104	87	90
3	90	93	62	83	89	93	77	83
4	77	85	96	112	87	97	46	89
5	73	83	70	97	72	95	87.5	98
6	85	70	76	94	77	93	82,5	98
7	82	90	68	87	84	96	89	102
8	83	92	67	85	79	98	71	84
9	72	87	69	88	98	114	69	94
10	79	86	77	102	26	87	76	81
11	76	86	80	89	88	103	98	96
12	85	95	75	83	108	112	74	95
13	80	88	78	83	101	102	75	97
14	70	89	88	95	103	109	69	86
15	76	93	86	95	90	95	78	95
16	76	95	81	88	87	89	66	89
17	78	96	82	89	89,5	107	69	89
18	76	93	68	82			70	87
19	80	93	73	83			76	97
20	69	88	71	83			77	99
21	65	85					76	96
Mean	77.10	87.95	76.2 5	89.80	86.97	99.82	75.05	91.81
SD	6.05	6.47	8.23	7.61	18.69	7.80	10.76	6.18

Notes: Wc =waist circumference; Pr = pelvic ring

lomere length athletes are no different from non-athletes, while in old age, telomere length was significantly different. Group of athletes had longer telomeres than non-athletes (Osthus et al., 2012)

Results of a meta-analysis on the effect of physical activity on telomere length have also been reported. From a meta-analysis of randomized conducted 35 research results convering 41 329 samples reported that 20 the results showed no difference between telomere length group of athletes with the control group, while 15 other research showed significant differences (Mundstock et al., 2015).

In this study, telomere length between male athletes with female athletes is the same. There are no reports of previous studies related to telomere length among women athletes to male athletes. It has been reported that in male athletes . telomere length at a young age did not different from the control group and physical activity in athletes is not related to the relative telomere length in later life (Laine et al., 2015)

In individual non-athletes, researchers previously reported that telomere length between men and women did not differ significance. It is reported that telomere length-related changes with increasing age and male gender. There were no significant differences in telomere length between male and female. It was further reported also that socio-economic status, poor, diet and smoking habits may be associated with one's biological aging process. (Hunt et al., 2008; Shiels et al., 2011).

Things contradictions reported by other studies that found that newborn female babies had longer telomere than male babies. (Aubert et al., 2012). This is supported by other studies that also reported the difference in telomere length

Table 5. Thick fat data in athletes group and the control group

Repetition	Athlete Group						Control Group					
	Male			Female			Male			Female		
	Biceps	Triceps	S.Iliaca	Biceps	Triceps	S.Iliaca	Biceps	Triceps	S.Iliaca	Biceps	Triceps	S.Iliaca
1	6	5	4	5	7	9	6	9	14	6	11	17
2	5	4	6	5	6	6	10	14	20	7	9	11
3	4	4	5	6	8	8	5	7	7	4	6	11
4	5	6	16	10	14	15	11	14	18	6	9	14
5	5	5	7	10	14	15	11	12	14	8	13	13
6	5	5	5	7	14	10	5	11	12	9	28	18
7	6	5	5	6	9	8	6	11	16	6	8	10
8	4	6	5	6	9	7	5	8	12	10	13	16
9	5	4	6	6	11	10	7	22	26	9	13	15
10	5	6	6	6	12	9	3	4	6	6	9	8
11	2	5	6	5	5	10	5	9	18	6	9	18
12	6	5	6	4	5	5	12	19	28	12	18	19
13	5	6	5	5	4	5	8	6	15	10	19	17
14	4	6	6	9	9	10	7	12	13	8	14	13
15	5	6	6	5	5	10	5	8	10	9	11	15
16	6	10	7	3	5	5	4	7	6	5	12	11
17	4	8	6	5	6	5	8	15	19	6	9	13
18	4	6	5	3	5	5				6	8	15
19	5	5	5	4	5	5				11	15	21
20	5	5	6	3	5	5				4	10	15
21	4	6	6							11	16	16
Mean	4.76	5.62	6.14	5.65	7.90	8.10	6.94	11.06	14.94	7.57	12.38	14.57
SD	0.94	1.36	2.37	2.06	3.43	3.14	2.68	4.70	6.25	2.36	4.94	3.25

between both the sexes. It was reported that the white blood cells, women have longer telomeres than men (Nawrot et al., 2004; Barrett & Richardson, 2011, Gardner et al*.,* 2014; Dalgard et al*.,* 2015)

Physical activity spare time can increase telomere length of about 200 nucleotides in both men and women compared to inactive. Not reported differences in telomere length in male athletes and female athletes. Physical activity can be potentially as anti-aging. In addition to, intensive physical activity in men aged 20-30 years at least 10 years can extend the telomeres (Cherkas et al*.,* 2008; Sabenroth et al*.,* 2015).

Regular physical exercise can maintain telomere length, induces anti-aging, and has the effect of protective. Besides physical activity in men and women athlete with an average age of 20 years have anti-apoptotic effects on endothelial cells. Physical exercise will increase the activity of antioxidant that can boost the protein telomere, extending telomeres and or proteins associated with telomeres. Sport/physical activity keep telomeres long stays or stop telomere shortening. Studies on 69 volunteer men and women aged 50-70 years showed that regular physical activity to maintain telomere length (Ludlow et al., 2008; Ludlow & Roth, 2011; Werner et al., 2008)

In this study, the sample used is young people aged between 17-20 years, including male and female. Measurements taken from the telomere length of leukocyte cells in healthy individuals. A previous study reported that telomere length in individuals to variations, i.e. different organs have different telomere length, such as the kidneys, liver, lungs or blood cells, lymphocyte cells have telomere length varied. Similarly, different species, length of telomeres is also different (Cherif et al*.,* 2003).

Telomere length associated with a person's nutritional status. Assessment of nutritional status is calculated values between body weight and height person who is described as a person's body mass index. Overview nutritional status among athletes and non athletes depicted in Table 3.

Total fat content of a person's body is important to know because it determines whether a person can be classified as obese or not obese. Percentage of body fat in athletes and control group illustrated in Table 4 and 5.

Levels of body fat based on waist circumference and sex in athletes eligible men and women classified as normal because the percentage is greater than non-athletes in the same age group. Physical activity in athletes can prevent the accumulation of body fat (Azwar, 2004). A previous study reported that obesity is associated with increased oxidative stress, inflammation and telomere shortening is associated with increased body mass index and increased waist circumference and hip circumference, especially in women (Kim et al*.,* 2010)

The results of this study provide information about the telomere length in Indonesian athletes at an early age and provide information to the public about the effect of exercise on telomere length that can become the biomarker of the aging process.

CONCLUSIONS

Telomere length in KONI athletes in the 15-17 age equal to the length of telomeres with non athletes at the same age level. The length of telomeres in women athletes to male athletes are the same.

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REFERENCES

Aubert, G., Baerlocher, G. M., Vulto, I., Poon, S. S., & Lansdorp, P. M. (2012). Collapse of telomere homeostasis in hematopoietic cells caused by heterozygous mutations in telomerase genes. *PLoS Genet*, 8(5), e1002696.

Azwar, A. (2004). Tubuh sehat ideal dari segi kesehatan. In *Makalah disampaikan pada Seminar Kesehatan Obesitas, Senat Mahasiswa Fakultas Kesehatan Masyarakat UI*, Sabtu (Vol. 15, pp. 1-7).

Barrett, E. L., & Richardson, D. S. (2011). Sex differences in telomeres and lifespan. *Aging cell*, 10(6), 913-921.

Cawthon, R. M. (2002). Telomere measurement by quantitative PCR. *Nucleic acids research*, 30(10), e47-e47.

O'Callaghan, N. J., & Fenech, M. (2011). A quantitative PCR method for measuring absolute telomere length. *Biological procedures online*, 13(1), 3.

Chen, W., Kimura, M., Kim, S., Cao, X., Srinivasan, S. R., Berenson, G. S., ... & Aviv, A. (2011). Longitudinal versus cross-sectional evaluations of leukocyte telomere length dynamics: age-dependent telomere shortening is the rule. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences*, 66(3), 312-319.

Cherif, H., Tarry, J. L., Ozanne, S. E., & Hales, C. N. (2003). Ageing and telomeres: a study into organ-and gender-specific telomere shortening. *Nucleic acids research*, 31(5), 1576-1583.

Cherkas, L. F., Hunkin, J. L., Kato, B. S., Richards, J. B., Gardner, J. P., Surdulescu, G. L., ... & Aviv, A. (2008). The association between physical activity in leisure time and leukocyte telomere length. *Archives of internal medicine*, 168(2), 154-158.

Collins, M., Renault, V., Grobler, L. A., Gibson, A. S. C., Lambert, M. I., Derman, E. W., ... & Mouly, V. (2003). Athletes with exercise-associated fatigue have abnormally short muscle DNA telomeres. *Medicine & Science in Sports & Exercise*, 35(9), 1524-1528.

Dalgård, C., Benetos, A., Verhulst, S., Labat, C., Kark, J. D., Christensen, K., ... & Aviv, A. (2015). Leukocyte telomere length dynamics in women and men: menopause vs age effects. *International journal of epidemiology*, 44(5), 1688-1695.

Enokido, M., Suzuki, A., Sadahiro, R., Matsumoto, Y., Kuwahata, F., Takahashi, N., ... & Otani, K. (2014). Parental care influences leukocyte telomere length with gender specificity in parents and offsprings. *BMC psychiatry*, 14(1), 277.

Gardner, M., Bann, D., Wiley, L., Cooper, R., Hardy, R., Nitsch, D., ... & Bekaert, S. (2014). Gender and telomere length: systematic review and meta-analysis. *Experimental gerontology*, 51, 15-27.

Hunt, S. C., Chen, W., Gardner, J. P., Kimura, M., Srinivasan, S. R., Eckfeldt, J. H., ... & Aviv, A. (2008). Leukocyte telomeres are longer in African Americans than in whites: the national heart, lung, and blood institute family heart study and the Bogalusa heart study. *Aging cell*, 7(4), 451-458.

Kim, S., Parks, C. G., DeRoo, L. A., Chen, H., Taylor, J. A., Cawthon, R. M., & Sandler, D. P. (2009). Obesity and weight gain in adulthood and telomere length. *Cancer Epidemiology and Prevention Biomarkers*, 18(3), 816-820.

Laine, M. K., Eriksson, J. G., Kujala, U. M., Raj, R., Kaprio, J., Bäckmand, H. M., ... & Sarna, S. (2015). Effect of intensive exercise in early adult life on telomere length in later life in men. *Journal of sports science & medicine*, 14(2), 239.

Ludlow, A. T., Zimmerman, J. B., Witkowski, S., Hearn, J. W., Hatfield, B. D., & Roth, S. M. (2008). Relationship between physical activity level, telomere length, and telomerase activity. *Medicine and science in sports and exercise*, 40(10), 1764.

Ludlow, A. T., & Roth, S. M. (2011). Physical activity and telomere biology: exploring the link with aging-related disease prevention. *Journal of aging research*, 2011.

Mather, K., Milburn, P., Parslow, R., Anstey, K., Jorm, A., Christensen, H., & Easteal, S. (2007). Is telomere length a biomarker of ageing?. *Australasian Journal on Ageing*, 26(2), A48-A49.

Mundstock, E., Zatti, H., Louzada, F. M., Oliveira, S. G., Guma, F. T., Paris, M. M., ... & Sarria, E. E. (2015). Effects of physical activity in telomere length: systematic review and meta-analysis. *Ageing research reviews*, 22, 72-80.

Nawrot, T. S., Staessen, J. A., Gardner, J. P., & Aviv, A. (2004). Telomere length and possible link to X chromosome. *The Lancet*, 363(9408), 507-510.

Østhus, I. B. Ø., Sgura, A., Berardinelli, F., Alsnes, I. V., Brønstad, E., Rehn, T., ... & Nauman, J. (2012). Telomere length and long-term endurance exercise: does exercise training affect biological age? A pilot study. *PloS one*, 7(12), e52769.

Saßenroth, D., Meyer, A., Salewsky, B., Kroh, M., Norman, K., Steinhagen-Thiessen, E., & Demuth, I. (2015). Sports and exercise at different ages and leukocyte telomere length in later life–Data from the Berlin Aging Study II (BASE-II). *PloS one*, 10(12), e0142131.

Shiels, P. G., McGlynn, L. M., MacIntyre, A., Johnson, P. C., Batty, G. D., Burns, H., ... & McGinty, A. (2011). Accelerated telomere attrition is associated with relative household income, diet and inflammation in the pSoBid cohort. *PloS one*, 6(7), e22521.

Theimer, C. A., & Feigon, J. (2006). Structure and function of telomerase RNA. *Current opinion in structural biology*, 16(3), 307-318.

Werner, C., Fürster, T., Widmann, T., Pöss, J., Roggia, C., Hanhoun, M., ... & Haendeler, J. (2009). Physical exercise prevents cellular senescence in circulating leukocytes and in the vessel wall. *Circulation*, 120(24), 2438-2447.

Zhu, H., Wang, X., Gutin, B., Davis, C. L., Keeton, D., Thomas, J., ... & Van Der Harst, P. (2011). Leukocyte telomere length in healthy Caucasian and African-American adolescents: relationships with race, sex, adiposity, adipokines, and physical activity. *The Journal of pediatrics*, 158(2), 215-220.

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Chromium Phytoremediation of Tannery Wastewater using *Ceratophyllum demersum*

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Abstract

Tanning industry produces liquid waste containing heavy metals, especially chromium – harmful for ecosystems and human health. Phytoremediation is a technique that utilizes the physiologicalpotential of plants to transform contaminants to be less or non-hazardous. The aim of the research is to determine the efficiency of *Ceratophyllum demersum* L. to remediate chrome in tannery wastewater. The research used 84 strands of *C. demersum* compound leaves of 30 g wet weight. The treatments consist of the use of 7.74 mg/L, 11.30 mg/L, 17.00 mg/L and 23.73 mg/L concentrations of chromium and a control. The research was conducted using a static method. The design of the experiment was the complete random design with 3 replication of treatment in 14 days. The parameters observed were the efficiency of chromium phytoremediation, water turbidity, BOD, and total chlorophyll level of the leaves. The results showed that the highest efficiency was at the concentration of 7.74 mg/L with 1.7% chromium, 17.3% water turbidity, and 46% BOD. Meanwhile, the highest efficiency of total chlorophyll level was 3.88 mg/L, reached at the concentration of 17.00 mg/L. In conclusion, *C. demersum* is good to use as a *phytoremediator* of tannery wastewater at the concentration of 7.74 mg/L, subsequently, these results can be used as a basis for the consideration of the application implementation in the process of liquid waste reduction.

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INTRODUCTION

Tanning industry is one of the industries using hazardous and toxic materials in the production; that is chromium (Cr). Among the well-known centers of tanning industry is the one located in Sukaregang Village of Garut Sub-district of Garut Regency. Based on the survey and sampling conducted by the Ministry of Environment on 4-6 July 2002, Ciwalen river is highly polluted, exceeding the water quality-based effluent limits (Kementrian Lingkungan Hidup, 2002).

Cr in waters is a potential pollutant for the ecosystem. The bioaccumulation of heavy metal (Cr) in the tissue of water plants could chronically affect their growth by slowing it down (Shanker et al., 2005), as well as cause kidney disease and lung cancer in humans (Wilbur et al., 2012), if consumed. In plants, the Cr can affected to reduction of seedling growth on *Sorghum bicolor* (L.) Moench species (Kasmiyati et al., 2016). Therefore, decreasing its level in waters is necessary.

One of the ways to decrease the level of Cr in waters is by phytoremediation, which is the utilization of plants with their biosorption ability. Besides, phytoremediation is easy to conduct, as well as offers lower budget and simpler technology than the so-called engineering-based methods (Hidayati, 2005).

Ceratophyllum demersum (coontail) is a water plant that could be used as a phytoremediator. It could absorb heavy metals with its root tissue. Among the previous studies is the one conducted by Maryam et al. (2011). The study remediates ferrous metals (Fe) for 18 days by 40% using treated municipal wastewater (TMW) method and 67.5% using raw municipal wastewater (RMW) method. It also remediates nickel (Ni) for 14 days by 50% (Chorom & Jaafarzadeh, 2012), as well as absorbs Cd, Pb, Zn, Co, Cu, and Ni metals as much as 2.35 mg/L, 208.71 mg/L, 1172.8 mg/L, 23.5 mg/L, 96.3 mg/L, and 48.09 mg/L respectively. Other serults show that *C. demersum* can used for remediation of heavy metals such as Cadmium (Cd) (Al-Ubaidy & Rasheed, 2015), Cu, Pb & Zn in river sediment (Fawzy et. al., 2012). In addition, the plant is easy to find in Indonesia and able to adapt well in a polluted environment.

The use of *C. demersum*as a phytoremediator is expected to decrease the level of Cr in the river polluted by the liquid waste of the tanning industry in Sukaregang, Garut to a non-hazardous level. For the society, the result of the research could be one of the solutions to the problems of tannery waste, both individually and in groups.

METHODS

The materials used were approximately 80 strands of mature *C. demersum* compound leaves of 30 g wet weight, taken from aquascape shop in Jakarta. The liquid waste was taken from the tanning home industry in Sukaregang, Garut Regency.

The research used static method. It means that there was no addition of materials during the observation/experiment. The research used complete random design, consisting of four treatments by using waste concentrations of 7.74 mg/L (K1), 11.30 mg/L (K2), 17.00 mg/L (K3), and 23.73 mg/L (K4), as well as the control (water) (K0), 1 liter each. The treatmentsare repeated three times.

*Ceratophyllum demersum*is acclimated for seven days by cutting the tip of the stems to broaden the absorption surface. The concentrations of tannery liquid waste of 25%, 50%, 75%, and 100% as well as the control are put into 1500 ml-sized water bottles. The experiment was conducted in 14 days in accordance with the doubling time. The measured parameters were the efficiency of chrome phytoremediation (*SNI* 06-6989.17-2004, *AAS* method), the water turbidity (*SNI* 06-6989.25-2005, using nephelometer), BOD (*SNI* 6989.2-2009, spectrophotometry method), and the total chlorophyll level of the leaves (Arnon-1949 method, 96% alcohol solvent, 649 nm and 665 nm λ spectrophotometer). The measurement of chrome, water turbidity and BOD was observed at the Health Laboratory of West Java in Bandung. Mean while, the measurement of total chlorophyll level was conducted at the Aquatic Ecology Laboratory of Biology Department of Science and Technology Faculty of Sunan Gunung Djati State Islamic University, also in Bandung. The research was conducted from June to November 2015.

The efficiency value of the phytoremediation of tannery wastewater was determined by using the equation: $E = \frac{Co-Ci}{Co} \times 100\%$, with E=Efficiency (%), C_0=initial level (mg/L), C_i=final level (mg/L). Data were analyzed using analyses of variance and Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

The initial levels of chrome in the tannery wastewater in this research were 7.74 mg/L, 11.30 mg/L, 17.00 mg/L, and 23.73 mg/L. *Ceratophyllum demersum* shows the potential to remediate chrome in the concentration of 7.74 mg/L and

11.30 mg/L, meaning that there was a positive value of efficiency. However, potential remediation was not found in the concentration of 17.00 mg/L and 23.73 mg/L, meaning that the efficiency value was negative (Figure 1). The highest efficiency level of chrome phytoremediation was shown in the 7.74 mg/L waste concentration, which is 1.7% in 14 days. This phytoremediation ability is in line with the research conducted by Teymouri et al. (2013) which uses *C. demersum* in a NaCl-modified chrome solution. The highest result of Teymouri's research was gained in the condition where the pH is 2, the biosorbent dosage is 8 g/L, the treatment is in 60 minute contact, and there was 10.20 mg/L maximum adsorption capacity of *C. demersum*.

According to Priyanto (2006), the liquid waste taken from the joint waste storage pond belonging to a number of tanning home industries in Sukaregang Village of Garut Regency contains 1,265.05 mg/L of BOD and 8,554.05 mg/L of chrome. Two metal ions found there are Cu (cooper) and Cr (chromium). The highest amount of metal ion found is chromium. Chromium (VI) is known to be a thousand times more toxic than Chromium (III); and Chromium (VI) is found more than Chromium (III) in the tanning industry (Villegas et al., 2008). Chromium could cause problems of respiratory system, skin, blood vessels, and kidney (Tchounwou et al., 2012). Contact with the skin could cause irritation, and it could result in stomachache and vomiting if swallowed. The negative effects of heavy metals on plants are weakened organism activities and degraded soil fertility. As a result, the production quantity also decreases. The most fatal effect of heavy metal pollution on plants is the contaminated food chain (McGrath et al., 2002). Cr (VI) could affect the growth of mung beans (Turner & Rust, 1971), lettuce, wheat and tomato (Moral et al., 1995), *Albizialebbek*, *Acasialebbek*, and green beans (Sharma & Sharma, 1993) as well as rice and beans (Prasad & Freitas de Oliviera, 2003). Those results show that Cr (VI) – on some specific concentrations – affects the growth of roots, leaves, and seeds.

Chrome accumulated in *C. demersum* decreases the level of chlorophyll. It is resulted by the hampered photosynthesis process marked by chlorosis of the leaves. Moreover, there is a loss of leaf buds, marked by the amount of leaf buds floating on the wastewater. *Ceratophyllum demersum* experiences oxidative stress caused by over-limit chrome toxicity in its tissues – and Reactive Oxygen Species (ROS) such as H_2O_2 occurs. According to Panda & Patra (1997), the accumulation of chrome in plants could hamper their growth, cause chlorosis in new leaves, reduce the pigment content, change the enzymatic functions, damage root cells, and cause ultrastructural modification of chloroplast and cell membrane. Heavy metals in a plant would trigger ROS as a result of the deactivation of antioxidant enzymes such as s*uperoxidedismutase* (SOD), catalase (CAT), and glutathione peroxidase (GPOD). ROS occurring in a plant is also caused by oxidative stress. ROS would easily damage peroxide fat in the lipid-membrane, cell membrane of the phospholipid and lipoprotein by spreading through chained reaction (Hazra et al., 2010). ROS itself could affect any kinds of biomolecule such as nucleic acid, protein, and amino acid; disturbing the cell metabolism.

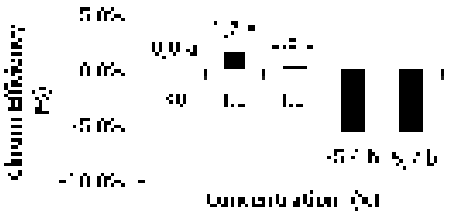

Figure 1. The efficiency of *Ceratophyllum demersum* in remediating chrome in tannery wastewater (K0=Control, K1=7.74 mg/L, K2=11.30 mg/L, K3= 17.00 mg/L, K4=23.73 mg/L)

A plant develops a complex mechanism where they could control the absorption and accumulation of heavy metals. This mechanism involves chelation and execution of some specific class of heavy metals. They would compose *Phytochelatin* Currency (PC) and *metallothienins* (MTs) (Cobbet, 2000).

The other indicator of potential phytoremediation of tannery wastewater by *C. demersum* is the remediated water turbidity. Its potentials in the waste concentrations of 7.74 mg/L, 11.30 mg/L, 17.00 mg/L and 23.73 mg/L were positive (Figure 2). The efficiency value of the water turbidity phytoremediation is decreased as the concentration was increased: 17.3% in the waste concentration of 7.74 mg/L, 2.7% in the concentration of 11.30 mg/L, 0.8% in the concentration of 17.00%, and 0% in the concentration of 7.74 mg/L. The highest efficiency was shown in the concentration of 23.73 mg/L of tannery wastewater.

The phytoremediation efficiency by *C. demersum* on the water turbidity was below 20%; it means the turbidity level of the wastewater was still high (Figure 3). In the concentration of 25%

liquid waste, the level of turbidity was between 180-370 NTU/L. Even with the highest value of efficiency, the water clarity was still below standard because the turbidity is more than 20 NTU/L. According to Yusuf (2008), water withover 20 NTU/L turbidity is dangerous for the biota living in it, as it could interfere with their activities and metabolisms.

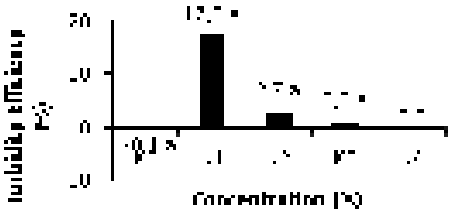

Figure 2. *C. demersum* efficiency in remediation of tannery wastewater turbidity (K0=Control, K1=7.74 mg/L, K2=11.30 mg/L, K3= 17.00 mg/L, K4=23.73 mg/L)

The higher the concentration of tannery wastewater is, the lower the turbidity efficiency value becomes. *C. demersum* could remediate the turbidity best in the treatments on the 7.73 mg/L concentration. Meanwhile, in the treatments onthe 11.30 mg/L, 17.00 mg/L and 23.73 mg/L concentrations the efficiency values were low. It happened because particles in the liquid waste clog the pores of the cut leaf stems that they could not optimally absorb the contaminant.

Remédios et al., (2012) points out that the deposition and colloidal in the water would prevent the sunlight to go through. It would not reach the bottom. That is why the photosynthesis of *C. demersum* is hampered. As a result, the lives of the microorganisms are bothered. Water turbidity restrains the incoming light. It occurs as there are floating materials and decomposition of certain substances, such as organic materials, microorganisms, muds and other minute floating things (Bilotta & Braizer, 2008).

The initial condition of the researched tannery waste water BOD in the treatments 7.70 mg/L, 11.30 mg/L, 17.00 mg/L, and 23.73 mg/L were 228.07 mg/L, 412.80 mg/L, 530.43 mg/L, and 647.40 mg/L respectively. The *C. demersum* showed that its phytoremediation efficiency on BOD in 14 days was fluctuating (Figure 4). The value decreased in the 7.70 mg/L and the 11.30 mg/L concentrations, and increased in the 17.00 mg/L and the 23.73 mg/L concentrations. The efficiency values of phytoremediation using *C. demersum* on the BOD from the highest to the lowest waste concentrations respectively were 46%, 14%, 25%, and 29%.

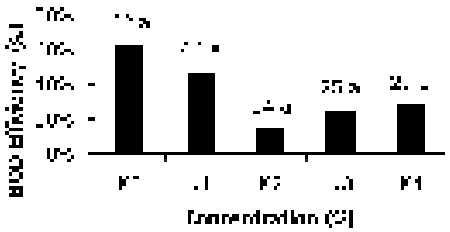

Figure 4. *C. demersum* efficiency in the remediation of tannery wastewater BOD (K0=Control, K1=7.74 mg/L, K2=11.30 mg/L, K3= 17.00 mg/L, K4=23.73 mg/L)

During the phytoremediation process, the efficiency value of BOD decreases in the different concentrations. It is supposed to be caused by several reasons, among which is water turbidity. The higher the level of turbidity is, the more difficult the *C. demersum* does photosynthesis as the light coming through the water is limited (Remédios et al., 2012). The result of photosynthesis is oxygen. It is the one used by microorganisms to move aerobically.

The thicker concentration of the liquid waste is, the higher the BOD becomes. The high contents of BOD in the treatments would increase the needs of polluting microorganisms in decomposing organic substances aerobically (Safitri et al., 2015). On the contrary, the amount of oxygen resulted from the photosynthesis process would decrease.

The increasing needs of microorganisms influence the amount of oxygen needed by aquatic organisms like *C. demersum* to do their metabolic processes (photosynthesis and cell respira-

Figure 3. Before and after phytoremediation of tannery wastewater using *C. demersum* (A=control, B=7.74 mg/L, C=11.30 mg/L, D=17.00 mg/L, E=23.73 mg/L)

tion). If the metabolic processes are hampered, the plant's ability in remediating BOD weakens.

Water temperature also influences the amount of oxygen needed by microorganisms for the aerobic decomposing process. The rise of temperature would result in the rise of biological activities, and in turn, the needs of more oxygen. The rise of water temperature would decrease the level of oxygen solubility, so that it lowers the aquatic organisms' ability to use available oxygen to sustain the biological processes in the water (Safitri et al., 2015).

Meme et al., (2014) states that the level of BOD have direct relation with the other parameters component of water quality such as water temperature and pH in the water. Temperature influences most biochemical reactions. Biological activities increase as the temperature goes up to 60ºC. Organisms reorganizing organic materials would be able to adapt in the range of 6.8-8.3 acidity. The process of organic waste decomposition occurs through the oxidation process by microorganisms with aerobic bacteria. Organic waste is broken and decomposed into carbon dioxide (CO_2), water and ammonia (NH_3). The resulted ammonia is the one causing bad smell of the water polluted by organic waste.

The levels of chlorophyll-a, chlorophyll-b and total chlorophyll found in *C. demersum* used as phytoremediator of tannery wastewater showed various conditions (Figure 5). Generally, the level of chlorophyll-a was higher than chlorophyll-b and the total chlorophyll in all treated waste concentrations. The highest level of chlorophyll-a as much as 9.88 mg/L was found in the 11.30 mg/L concentration; of chlorophyll-b as much as 8.21 mg/L is found in the 17.00 mg/L concentration; and of total chlorophyll as much as 4.88 mg/L was found in the 17.00 mg/L concentration.

For 14 days, the chlorophyll in *C. demersum* increased although being exposed to an extreme environment. It is because *C. demersum* have the ability to survive extreme condition. Based on a research by Chorom & Jaafarzadeh (2012), the level of chlorophyll and wet weight of *C. demersum* still increased in an extreme condition of 23.73 mg/L nickel-contaminated treatment. However, the result showed that the levels of total chlorophyll decreased in each treatment. Its level in the 100% concentration was totally different from the one in the control. It proves that the higher the concentration of liquid waste is, the lower the level of clorophyll in *C. demersum* becomes. According to Panda and Patra (2002), a plant accumulating heavy metals would experience an oxidative stress, including decreased level of chlorophyll. The decreases of total chlorophyll, chlorophyll-a, chlorophyll-b, and carotenoid happens because chrome has the ability to lower the ∂-aminolevullinic acid dehydratase. It is a very essential enzyme for the biosynthesis process of chlorophyll that it affects the utilization of ∂-aminolevullinic acid (ALA). Besides, chrome-VI is able to change most Mg^+ ions and drain the chlorophyll content.

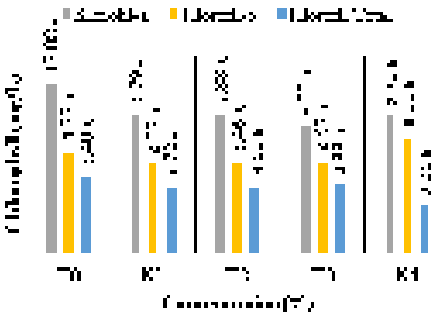

Figure 5. The level of total chlorophyll of *Ceratophyllum demersum* leaves as phytoremediator of tannery wastewater (K0=Control, K1=7.74 mg/L, K2=11.30 mg/L, K3= 17.00 mg/L, K4=23.73 mg/L).

Panda & Patra (2002) also stated that the decreased level of chlorophyll in a heavy metal-accumulated plant is caused by chrome exposure in a micromolar range, which results in ultrastructural change of chloroplast and thylakoid structures that hampers photosynthesis. Chrome also blocks the Hill reaction, affecting the light and dark reactions. Those reactions are essential stages of photosynthesis. According to Panda & Choudhury (2005), *Lemna minor* and *Pistia sp.* with chromium stress experience chlorosis in their new leaves.

Chromium stress is an important factor influencing CO_2 fixation, electron transport, photophosphorylation, and photosynthesis enzyme activities. According to Vazquez et al. (1987), the effects of chrome to the hampered electron transport is caused by the change of enzyme in the Calvin cycle. Chromate is used as the hill reagent by the isolated chloroplast. On the other hand, Bishnoi et al. (1993) pointed out that chrome affects photosystem I (PS I) in the chloroplast activity, so that it gets isolated in photosystem II (PS II). It is proven in their research on peas with high chromium content where the level of chlorophyll decreases drastically. Krupa and Baszynski (1995) describe a number of hypotheses on the possible mechanisms of chromium toxicity in photosynthesis; they are the reduction of carbon

essential for the process, the disorganization of chloroplast ultrastructure, and the obstruction of electron transport as the chrome divertsthem from PS I side to PS II side. The obstruction results in degrading photosynthesis ability as chrome induction occurs. There is a possibility that not all electrons resulted in the photochemical process are used for carbon fixation as shown by the degrading photosynthesis performance.

According to Shanker (2003), chrome toxicity on photosynthesis happened because it is able to shrink the size of peripheral parts of the antenna. The decrease of chlorophyll is also caused by protein destabilization and degradation in the peripheral part. Enzyme inactivation involved in the chlorophyll biosynthesis fascia also affects the decrease of chlorophyll level in plants with chromium oxidative stress.

The phytoremediation potential of *C. demersum* could be used in a bigger scale. The design management could consider the aspects of material volume, the amount of phytoremediator, and the time constraint. The volume of tannery wastewater could range from 25 to 1000 L, by using 30 g × (25-1000) of *C. demersum*, for 14 days. Containers with 25-1000 L capacity or more could be used for the treatment.

CONCLUSIONS

Ceratophylum demersum is good for remediating chrome in tannery wastewater of 7.74 mg/L concentration by using static method. The remediation potential is accompanied by the ability to reduce the levels of water turbidity and BOD. The use of *C. demersum* as phytoremediator of tannery wastewater results in the decrease of its chlorophyll level so that their growth is hampered.

REFERENCES

Al-Ubaidy, H. J., & Rsheed, J. A. (2015). Phytoremediation of Cadmium in river water by *Ceratophyllum demersum*. *World Journal of Experimental Medicine,* 3(1), 14-17.

Bilotta, G. S., & Braizer, R. E. (2008). Understanding the influence of suspended solids on water quality and aquatic biota. *Water Research*, 42(12), 2849-2861.

Bishnoi, N. R., Anita, D., Gupta, V. K., & Shawaney, S. K. (1993). Effect chromium on seed germination seeding growth and yield of peas. *Journal Agriculture Ecology, Ecosystem*, 47(1), 47-57.

Chorom, M. A. P & Jaafarzadeh N. (2012). Nickel Removal by the Aquatic Plant (*Ceratophylum demersum* L.). *International Journal of Environmental Science and Development,* 3(4), 372-375.

Cobbet, C. S. (2000). Phytochelatins and their roles in heavy metal detoxification. *Plant physiology,* 123(3), 825-832.

Fawzy, M. A., Badr, N. E. S., El-Khatib, A., & Abo-El-Kassem, A. (2012). Heavy metal biomonitoring and phytoremediation potentialities of aquatic macrophytes in River Nile. *Environmental Monitoring and Assesment,* 184(3), 1753-1771.

Hazra, B. R., Sarkar S., Biswas & Mandal, N. (2010). Comparative Study of the Antioxidant and Relative Oxygen Species Scavenging Properties in The Extract of The Fruits of *Terminalia chebula, Terminalia belerica* and *Emblica officinalisis*. *BMC Complementary and Alternative Medicine*, 10(1), 2-15.

Hidayati, N. (2005). Fitoremediasi dan Potensi Tumbuhan Hiperakumulator. *Hayati*, 12(1), 35-40.

Kasmiyati, S., Santosa, S., Priyambada, I. D., Dewi, K., Sucahyo, S., & Sandradewi, R. (2016). Growth Response of Sorghum bicolor (L.) Moench. Cultivars to Trivalent Chromium Stress. *Biosaintifika: Journal of Biology & Biology Education, 8*(1), 73-86.

Kementrian Lingkungan Hidup. (2002). *Revitalisasi Sentra Industri Kecil Penyamakan Kulit Berwawasan Lingkungan di Desa Sukaregang Garut*. Garut: Kementrian Lingkungan Hidup

Krupa, Z. & Baszynski., T. (1995). Some Aspect of Heavy Metals Toxycity Towards Photosyntethic Apparatus-direct and Indirect Effect on Light and Dark Reactions. *Acta Physiol Plant,* 17(1), 77-90.

Maryam, F., Najafi, P., & Toghiani, S. (2011). Trace Element Removal from Wastewater by *Ceratophyllus demersum* L. *Journal Applied Science Environment,* 15(1), 197-201.

McGrath, S. P., Zhao, F. J. & Lombi, E. (2002). Phytoremediation of Metals, Metalloids, and Radionuclides. *Advance in Agronomy*, 75, 1-56.

Meme, K. F., Arimoro, F. O., & Nwadukwe, F. O. (2014). Analyses of Physical and Chemical Parameters in Surface Waters nearby a Cement Factory in North Central, Nigeria. *Journal of Environmental Protection*, 5(10), 826-834.

Moral, R. J., Pedreno, J. N., Gomez, I. & Mataiz, J. (1995). Effect of Chromium the Nutrient Element Content and Morphology of Tomato. *Journal Plant Nutritiont,* 18(4), 815-822.

Panda, S. K. & Patra, H. K. (1997). Physiology of Chromium Toxicity in Plants. *Journal Plant Physiology Biochemical,* 24(1), 10-17.

Panda, S. K. & Patra, H. K. (2002). *Chromium Toxicity in Water Stress Simulation Effect in Imtact Senescing Leaves of Greengram (Vigna radiate L.) Advance in Stress Physiology of Plants*. India: Scientific Publisher.

Panda, S. K. & Choudhury, S. (2005). Chromium Stress in Plants. *Brazilian Journal of Plants Physiology,* 17(1), 95-102.

Prasad, M. N. V. & Freitas de Oliveira, H. M. (2003). Metal Hyperacumulation in Plants Biodiversity

Prospecting for Phytoremediation Technology. *Electronic Journal of Biotechnology,* 6(3), 285-321.

Priyanto, B. (2006). Uji Toksisitas Limbah Cair Penyamakan Kulit Menggunakan Metode Penghambatan Pertumbuhan *Lemna sp*. *Jurnal teknik lingkungan*, 7(2), 212-218.

Remédios, C., Rosário, F., & Bastos, V. (2012). Environmental Nanoparticles Interactions with Plants: Morphological, Physiological, and Genotoxic Aspects. *Journal of Botany*, 1–8.

Safitri, R., Priadie, B., Miranti, M., & Astuti, A. W. (2015). Ability of Bacterial Consortium: *Bacillus coagulans, Bacillus licheniformis, Bacillus pumilus, Bacillus subtilis, Nitrosomonas sp.* and *Pseudomonas* putida in Bioremediation of waste water. *AgroLife Scientific Journal, 4*(4), 146-152.

Shanker, A. K., Cervantes, C., Loza-tavera, H., & Avudainayagam, S. (2005). Chromium toxicity in plants. *Environmental International*, 31(5), 739-753.

Shanker, A. K. (2003). Physiological, Biochemical and Molecular Aspect of Chromium Toxicity and Tolerance in Selected Crops and Tree Species. *Thesis*. India: Tamil Nadu Agricultural University, Coimbatore.

Sharma, D. C. & Sharma, C. P. (1993). Chromium Uptake and its Effect on Growth and Biological Yield of Wheat. *Cereal Research Communications,* 21, 317-322.

Teymouri, P., Ahmadi, M., Babaei, A. A., Ahmadi, K. & Jaafarzadeh, N. (2013). Biosorption Studies on Naclmodified *Ceratophyllus demersum* L: Removal of Toxic Chromium from Aqueous Solution. Taylor & Francis Group. *Chemical Engineering Communication*, 200(10), 1394-1413.

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy Metals Toxicity and the Environment. *NIH Public Access*, 101, 133-164

Turner, M. A. & Rust, R. H. (1971). Effect of Chromium on Growth and Mineral Nutrition of Soy Beans. *Journal Soil Sciences for American Programe,* 35(5), 755-758.

Vazquez, M. D., Poschenriender, C. & Barcelo, J. (1987). Chromium (IV) Induced Structural and Ultrastructural Changes in Bush Bead Plant (*Phaseolus vulgaris* L.). *Annals of Botany,* 59(4), 427-438.

Villegas, L. B., Fernandez, P. M., Amoroso, M. J., de Figueroa, L. I. C. (2008). Chromate Removal by Yeasts Isolated from Sediment of A Tanning Factory and A Mine Site in Argentina. *Biometals*, 21(5), 591-600.

Wilbur, S., Abadin, H., Fay, M., Yu, D., Tencza, B., Ingerman, L., … James, S. (2012). *Toxicological Profile For Chromium*. United States of America: US Departmen of Health and Human Services.

Yusuf, G. (2008). Bioremediasi Limbah Rumah Tangga dengan Sistem Simulasi Tanaman Air. *Jurnal Bumi Lestari*, 8(2), 1356-144.

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The Potential of Flora and Fauna as Tourist Attractions in Biodiversity Park of Pelawan Forest, Central Bangka

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Abstract

Indonesia has a high potential for the diversity of flora and fauna species together with their ecosystem. Preservation of natural resources can be done through conservation using the concept of ecotourism. The purpose of this research is to identify the potential of the flora and fauna in Biodiversity Park of Pelawan Forest for tourist attraction. The study was conducted from October 2016 to January 2017 through inventory and in-depth interview. The analysis on the results of this study indicate that there are 41 species of 27 families of plants and 135 species of animals consisting of amphibians (6 species), reptiles (16 species), birds (99 species) and mammals (14 species). This indicates that Biodiversity Park of Pelawan Forest is very competitive for tourism attractions, which is supported by the presence of key species (*Tristaniopsis merguensis*), flagship species (*Cephalopachus bancanus*), and abundance of birds for bird watching. Based on the IUCN red list, several species of flora, such as *Gonystylus bancanus*, and fauna, such as *Setornis criniger*, *Chloropsis sonnerati*, *Macaca nemestrina*, *Nycticebus menagensis*, and *Cephalopachus bancanus*, are vulnerable to extinction. This study on flora and fauna results in the initial data that can be used to support conservation efforts. Moreover, the result of this study can provide an opportunity for visitors to enjoy these tourist attractions, which can benefit the local community.

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INTRODUCTION

Indonesia is a mega-biodiversity country which has a very high diversity of species and their ecosystems. At this time, it estimated that there are 42,584 species of plants (39% endemic) in Indonesia. For vertebrates, there are 720 species of mammals (53% endemic), 1.605 species of birds (20% endemic), 385 species of amphibians (41% endemic), and 723 species of reptiles (31% endemic) (Widjaja et al., 2014). However, the threat of its biodiversity extinction is one of the highest in the world due to the loss of the primary habitat, such as forests (Sutarno & Setyawan, 2015). It is caused by illegal logging, forest fires, and forest conversion or deforestation (Cleary & DeVantier, 2011).

Aside from being a habitat for plants and animals, forests act as lungs of the world. Therefore, conservation to maintain and preserve them is needed. This is stipulated in the Regulation of the Minister of Environment No. 03 of 2012 on Biodiversity Park, which serves as a backup area for local biological resources outside the region which has the function of *in situ* and *ex situ* conservation (BAPPENAS, 2016). Biodiversity Park of Pelawan Forest in Central Bangka, as one of the few biodiversity parks in Indonesia, has a key species of Pelawan trees (*Tristaniopsis merguensis*), which are significant for animals and other plants for their survival in this ecosystem (Akbarini, 2016).

The conservation activity that can be applied to sustainably preserve the diversity of the biological resources is through the concept of ecotourism because it is a clean and environmentally friendly tourism business with economic impacts for communities since it can increase the number of visits without exploiting the natural resources (Kirkby et al., 2011; Hakim et al., 2012).

Exploration of the potential of plant and animal species is necessary for the use of making basic data concerning the condition of the natural environment. The goal is to obtain data about uniqueness, excellence, and benefits that can be used to develop ecotourism, so that in turn, it can be used to formulate a sustainable ecotourism management for environmental changes (Scott, 2011). Therefore, data collection on the diversity potential of the Biodiversity Park of Pelawan Forest should be done to measure its competitiveness for it is development into a tourist attraction.

METHODS

This research was conducted at the Biodiversity Park of Pelawan Forest located in 106°11'00,19 E and 2°22'03,25 S in the Regency of Central Bangka in Bangka Belitung from October 2016 to January 2017. The observation was carried out along the corridor of Pelawan Forest (Figure 1). Biodiversity Park of Pelawan Forest is included in category IV in the list of the United Nations (UN), in which the main purpose of this inclusion is to protect, conserve, and restore its species and habitats (Dudley, 2008).

The data of this study are primary and secondary data. The primary data were obtained directly through observation and recording, especially in plant species, and in-depth interview about the benefits received by the public. The secondary data were obtained from results and reports from previous studies. The identification was done by cross-checking references that support this research.

The data from the analysis on the potential of forestry resources in forms of plant species that can be processed by the community into products of ecotourism attraction will be presented in tables that display family name, species name, local name, and benefits. Animal species will be represented by family name, species name, local name, and state of conservation. The criteria for determining the quality of flora and fauna is very important in identifying the competitiveness of certain species for tourist attractions. The criteria for the quality of flora and fauna diversity are presented in Table 1.

RESULTS AND DISCUSSION

Potential of Flora

Based on the results of inventory and interviews about plant species of Biodiversity Park of Pelawan Forest, there are 41 species of plants from 27 families dominated by the family of *Myrtaceae*, as presented in Table 2.

The family of *Myrtacea*e dominates plant species since this family, in general, is capable and successful to survive in acidic soil conditions and under stressful conditions. Family of *Myrtaceae* is immune to acidic soil because it can produce secondary metabolites such as phenol, one of which is tannin (Henri et al., 2016). It is also associated with the members of *Myrtaceae* family who are able to survive in nutrient-poor ecosystem habitat (Oktavia et al., 2015). Some plant species from the family of *Myrtaceae* can also be used as local plants for post-mining tin revegetation (Nurtjahya et al., 2008).

The results of this study identify 41 species of plants, which means that the species is more

than 31 in the criteria of diversity quality of flora. Thus, it can be interpreted that Biodiversity Park of Pelawan Forest is very good to be developed into ecotourism attractions (Latupapua, 2013). The species in Biodiversity Park of Pelawan Forest has a high degree of diversity. In general, the species in this list are used by the community on the island of Bangka as a climbing pole for pepper tree (37 species), firewood (13 species), building materials (12 species), pharmaceuticals (4 species), and food (4 species).

Based on data from the 41 species of plants, the outline of plant species in Pelawan Forest is still classified as many in its nature, although there are three types of plants whose conservation status are included into the category of Least Concern; i.e. jelutung (*Dyera costulata*), idat (*Cratoxylum arborescens*), and resak (*Vatica rassak*), which means that their extinction risks are still low. In addition, there is a type of plant whose conservation status is included in Vulnerable category; i.e. namang or ramin tree (*Gonystylus bancanus*), which means that the species is facing the risk of extinction in the wild in the future.

According to the people on the island of Bangka, several species of plants, such as a leting tree (*Elaeocarpus nitidus*), rempudong tree (*Symplocos cochinchinensis*), and pelawan tree (*Tristaniopsis merguensis*), are useful as a source of nectar for wild honeybee (*Apis dorsata*). *T. merguensis*, which is considered as a key species with a very large impact on the environment, is able to affect ecosystem. In addition *T.* merguensis (Figure 2), can be used as a host for edible fungus like Pelawan mushrooms (*Heimoporus* sp.) (Akbarini, 2016). This high species diversity has become an attraction for local and foreign tourist because this diversity has a uniqueness that does not exist in other areas.

Potential of Fauna

Bangka Belitung is flanked by large islands, such as Sumatra, Kalimantan, and Java, which makes its biodiversity a combination of the three islands. This is because during the Pleistocene interpluvial time, when most sea levels were very low, the islands became a "bridge" between the larger islands. Therefore, the diversity of its fauna is very diverse ranging from amphibians, reptiles, birds, and mammals.

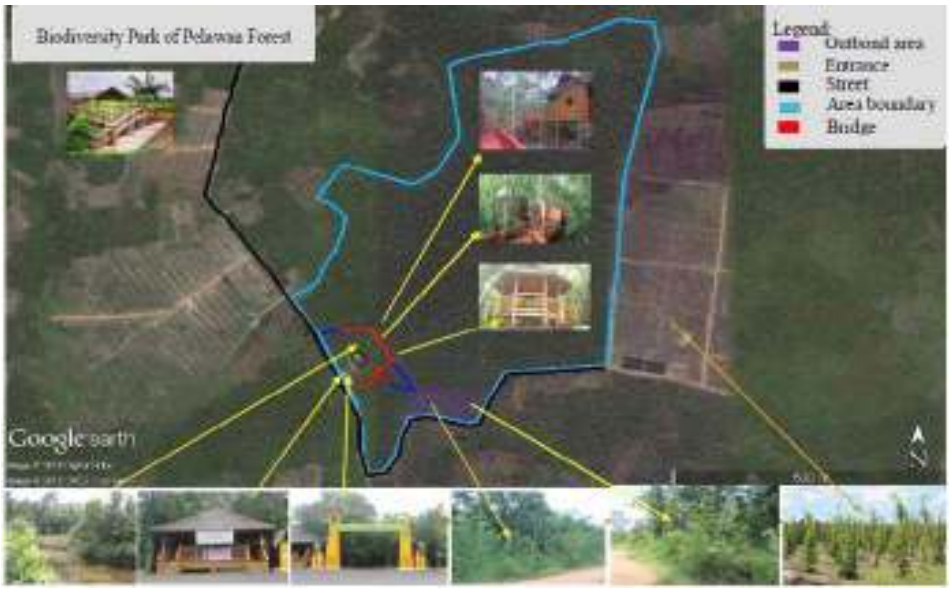

Figure 1. Map of Biodiversity Park of Pelawan Forest and site location

Table 1. Quality criteria of flora and fauna diversity

Scale	Numbers flora species	Numbers fauna species	Quality
1	< 5 species	1-2 species	Very poor
2	6-10 species	3-5 species	Poor
3	11-20 species	6-10 species	Fair
4	21-31 species	11-15 species	Good
5	> 31 species	> 15 species	Very good

Source: (Latupapua, 2013).

Table 2. Flora in Biodiversity Park of Pelawan Forest

Family	Scientific Name	Local Name	Other Potential Benefits*)				
			I	II	III	IV	V
Anacardiaceae	*Campnosperma auriculatum*	Terentang	√				√
	Gluta velutina	Mengkikir	√				
Apocynaceae	*Dyera costulata*	Jelutung	√		√		√
Aquifoliaceae	*Ilex cymosa*	Mensirak	√				
Araliaceae	*Polyscias biformis*	Juluk Antu	√				
Bonnetiaceae	*Ploiarium alternifolium*	Riang-riang	√				
Calophyllaceae	*Calophyllum lanigerum*	Mentangor belulang	√				√
	Calophyllum pulcherrimum	Mentangor prit	√		√		√
Clusiaceae	*Garcinia parvifolia*	Asam kandis				√	
Dilleniaceae	*Dillenia suffruticosa*	Simpur	√				
Dipterocarpaceae	*Vatica rassak*	Resak	√				
Elaeocarpaceae	*Elaeocarpus nitidus*	Leting	√				
Euphorbiaceae	*Hevea brasiliensis*	Karet			√		
Fagaceae	*Lithocarpus blumeanus*	Kabal putih	√		√		
Guttiferae	*Cratoxylum arborescens*	Idat	√		√	√	
Lamiaceae	*Vitex pinnata*	Leban	√	√	√		
Lauraceae	*Litsea firma*	Medang	√				
Melastomataceae	*Pternandra rostrata*	Mengketan	√		√		
Myrtaceae	*Eugenia lepidocarpa*	Samak	√	√			
	Melaleuca leucadendra	Gelam merah	√		√		√
	Rhodamnia cinerea	Merapin	√	√	√		√
	Syzygium attenuatum	Sisel	√		√		
	Syzygium bisulea	Jambu utan	√			√	
	Syzygium decipiens	Isot-isot	√			√	
	Syzygium lineatum	Kebecir	√				
	Syzygium muelleri	Uber	√				
	Syzygium pachyphyllum	Sabar bubu	√				
	Syzygium perforatum	Mengkalai	√		√		
	Syzygium racemosum	Bantui	√				
	Tristaniopsis merguensis	Pelawan	√	√	√		√
Pentaphylacaceae	*Adinandra dumosa*	Pelempang hitam	√		√		√
Phyllanthaceae	*Aporosa microcalyx*	Pelangas	√				√
Proteaceae	*Helicia serrata*	Keratong	√				
Rhizophoraceae	*Gynotroches axillaris*	Mengkelik	√				
Rubiaceae	*Gaertnera vaginans*	Kayu abu	√				
	Timonius flavescens	Kayu ruan	√				
Sapindaceae	*Guioa pubescens*	Pulas	√				
Sapotaceae	*Palaquium rostratum*	Nyatoh					√
Symplocaceae	*Symplocos cochinchinensis*	Rempudong	√				
Theaceae	*Schima wallichii*	Seruk	√		√		√
Thymelaeaceae	*Gonystylus bancanus*	Namang/ramin	√				√

***) :** I: climbing pole for pepper tree; II: pharmaceuticals; III: firewood; IV: food; and V: building materials

Photograph: personal document/by Henri, 2016
Figure 2. Key species (*Tristaniopsis merguensis*). a) stem; b) leaf; c) flower; and d) fruit

Composition diversity of various plant species is the carrying capacity of suitable habitat for various species of animals, such as vertebrates. The composition of this biodiversity can lead to the mutualistic relation in the interaction in the habitat. The results of data processing supported by the secondary data show that various species of vertebrate inhabit Biodiversity Park of Pelawan Forest; they are 3 families of amphibians (6 species), 8 families of reptiles (16 species), 35 families of birds (99 species), and 8 families of mammals (14 species), as shown in Table 3.

Table 3. Fauna in Biodiversity Park of Pelawan Forest

Fauna	Family	Species	(%)
Amphibians	*Bufonidae*	2	33.33
	Ranidae	2	33.33
	Rhacophoridae	2	33.33
	Total	6	100
Reptile	*Agamidae*	2	12.5
	Colubridae	4	25
	Gekkonidae	1	6.25
	Geoemydidae	1	6.25
	Natricidae	1	6.25
	Pythonidae	1	6.25
	Scincidae	4	25
	Varanidae	2	12.5
	Total	16	100
Bird	*Acanthizidae*	1	1.01
	Accipitridae	1	1.01
	Aegithinidae	2	2.02
	Alcedinidae	8	8.08
	Ardeidae	2	2.02
	Campephagidae	3	3.03
	Caprimulgidae	2	2.02
	Chloropseidae	3	3.03
	Columbidae	4	4.04
	Corvidae	1	1.01
	Cuculidae	9	9.09
	Dicaeidae	4	4.04
	Dicruridae	1	1.01
	Eurylaimidae	2	2.02
	Hemiprocnidae	1	1.01
	Laniidae	2	2.02
	Megalaimidae	2	2.02
	Meropidae	2	2.02
	Monarchidae	2	2.02
	Muscicapidae	6	6.06
	Nectariniidae	8	8.08
	Pachycephalidae	1	1.01
	Pellorneidae	1	1.01
	Phasianidae	3	3.03
	Phylloscopidae	1	1.01
	Picidae	5	5.05
	Pittidae	2	2.02
	Pycnonotidae	6	6.06
	Rallidae	2	2.02
	Strigidae	2	2.02
	Sylviidae	2	2.02
	Tephrodornithidae	1	1.01
	Timaliidae	4	4.04
	Trogonidae	2	2.02
	Turnicidae	1	1.01
	Total	99	100
Mammals	*Cercopithecidae*	3	21.43
	Cynocephalidae	1	7.14
	Lorisidae	1	7.14
	Pteropodidae	1	7.14
	Rhinolophidae	2	14.29
	Sciuridae	4	28.57
	Tarsiidae	1	7.14
	Tupaiidae	1	7.14
	Total	14	100

Based on the data of the composition abundance of various species, the number of fauna species in Biodiversity Park of Pelawan Forest is very abundant (>15 species), so that the element of becoming a tourist attraction is in the category of very good and competitive for development (Latupapua, 2013).

The most abundant fauna is bird species, such as the family of *Alcedinidae* (8 species), *Cuculidae* (9 species), *Muscicapidae* (6 species), *Nectariniidae* (8 species) and *Pycnonotidae* (6 species). One species of bird that is endemic to Bangka Island is the green paok (*Pitta sordida*) (Figure 4b). This indicates that some species of birds in the families can live together in one habitat and can form a community so that interaction with other species in the habitat appears. This diversity of birds also indicates that Biodiversity Park of Pelawan Forest can be used as an indicator of ecosystem stability (Susanto et al., 2016). The high abundance of bird species becomes a special priority to determine its conservation indicators, such as endemicity, populations status, and endangered species (Hadiprayitno et al., 2016).

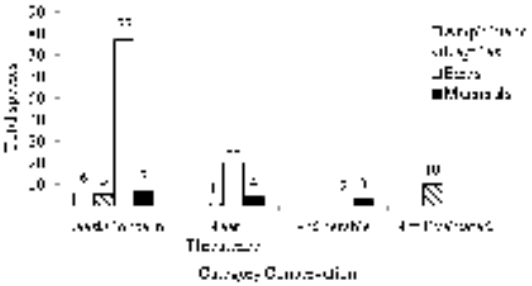

Figure 3. Conservation status of fauna in Biodiversity Park of Pelawan Forest

Based on Figure 3, the status of fauna conservation specified under IUCN (International Union for Conservation of Nature) proves that, in general, the types of fauna in the Pelawan Forest are still included in the Least Concern category, which means that their extinction risks are still low. Several other fauna species are included in the category of Vulnerable, where these species are facing the risk of extinction in the wild in the future. The species are empuloh paruh kait (*Setornis criniger*) (Figure 4a), cica daun besar (*Chloropsis sonnerati*), beruk (*Macaca nemestrina*), kukang bukang (*Nycticebus menagensis*), and krabuku ingkat or mentilin (*Cephalopachus bancanus*) (Figure 4c).

Photograph: by Syahputra.

Figure 4. Fauna Biodiversity Park of Pelawan Forest. a) *Setornis criniger*; b) *Pitta sordida*; and c) *Cephalopachus bancanus*

Fauna on Pelawan forest has a uniqueness in terms of its flagship species. They are krabuku ingkat, or mentilin in local language, with the scientific name of *Cephalopachus bancanus*. This species the identity of Bangka-Belitung. In contrast to its relatives in Sulawesi (krabuku tangkasi), this nocturnal primate is solitary, or lone-living, animal. The conservation status of this species is vulnerable, which means that this species is facing the risk of extinction in the wild life of the future. *Cephalopachus bancanus* can have up to 2-3 hectares territorial area, except during the mating season, where males and females live together for some times. Females usually give birth to the pups after 6 months of pregnancy (Syahputra, 2016).

Biodiversity Park of Pelawan Forest has a high and unique flora and fauna diversity potential such as Pelawan tree a key species (*Tristaniopsis merguensis*) (Akbarini, 2016), as well as flagship species of Bangka Island, i.e. the mentilin (*Cephalopachus bancanus*) (Syahputra, 2016). In addition to key species and flagship species, the high bird abundance of Biodiversity Park of Pelawan Forest can be used as bird watching attraction. Bird watching is one of tourism activities directly related to the nature (Connell, 2009).

This potential has an important contribution to the community through ecotourism development that has a direct role in improving the economy (Adamu et al., 2015). Ecotourism development is not limited to visits from tourists, in terms of improving economic returns. It also reaches preservation of local culture and natural environment (Ahmad, 2014).

The development Biodiversity Park of Pelawan Forest with the involvement of community is the most significant thing in maintaining the nature. Resources and environment are the most important factors in ensuring sustainable tourism development. Tsaur et al., (2006), proposed three tourism management strategy as follows: The primary concern for the development of ecotourism is an urgency to pay attention to the negative impact on the environment, degradati-

on, and destruction. Here does the government have a role to overcome the problem that does not correspond to the use of biological resources by making regulations to support resource protection and conservation strategy; For community and citizens, the primary concern is to protect the livelihood of citizens so as to maintain the attractiveness of the environment. Sustainable tourism needs to add values in involving the community in a fair process; For tourists, let alone making visitors enjoy high-quality tourism activities, managers must provide them with interpretive services by providing environmental education.

This study on flora and fauna results in initial data that support the conservation efforts in Biodiversity Park of Pelawan Forest. Moreover, this potential of flora and fauna can provide an opportunity for visitors to enjoy these tourist attractions, which can benefit the local community. According to Kiper (2013), tourism development can become a sustainable tourism if it focuses on three things: a) quality, improvement on the environmental quality so that the tourist attraction will attract more tourists to revisit; b) sustainability, the development of tourism areas must be accompanied by preservation and regeneration of natural resources; and c) balancing between the needs of tourists and environmental protection by carrying capacity on the tourist attraction. The role of local communities and stakeholders can be mutually beneficial, which is seen as an economic concept of sustainability in increasing long-term economic objectives and environmental stewardship.

CONCLUSIONS

Based on the data of potential, there are 24 plant families (41 species) and 135 species of vertebrates, with the composition of amphibians (6 species), reptiles (16 species), birds (99 species) and mammals (14 species), living in Biodiversity Park of Pelawan Forest. Thus, it can be said that this park is very suitable for ecotourism attraction. This is supported by an identity or a characteristic in a form of the presence of key species, flagship species, and abundance of birds for bird watching that can become a sustainable tourist attraction and destination in Central Bangka. The role of communities and governments should be optimized to preserve this diversity without neglecting environmental sustainability factors.

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REFERENCES

Adamu, A., Yacob, M. R., Radam, A., Hashim, R., & Adam, S. U. (2015). Economic valuation of ecotourism resources in Yankari Game Reserve, Bauchi Nigeria. *Procedia Environmental Sciences*, *30*, 139–144.

Ahmad, A. (2014). The disengagement of the tourism businesses in ecotourism and environmental practices in Brunei Darussalam. *Tourism Management Perspectives*, *10*, 1–6.

Akbarini, D. (2016). Pohon pelawan (*Tristaniopsis merguensis*): Spesies kunci keberlanjutan Taman Keanekaragaman Hayati Namang-Bangka Tengah. *Al-Kauniyah Jurnal Biologi*, *9*(1), 66–73.

BAPPENAS [Badan Perencanaan Pembangunan Nasional]. (2016). *Indonesian biodiversity strategy and action plan 2015-2020*. Jakarta: Kementerian Perencanaan Pembangunan Nasional/BAPPENAS. In: http://www.bappenas.go.id.

Cleary, D. F. R., & DeVantier, L. (2011). Indonesia: Threats to the country's biodiversity. *Encyclopedia of Environmental Health*, 187–197.

Connell, J. (2009). Birdwatching, twitching and tourism: Towards an Australian perspective. *Australian Geographer*, *40*(2), 203–217.

Dudley, N. (2008). *Guidelines for applying protected area management categories*. IUCN (The International Union for Conservation of Nature). Gland, Switzerland. In:https://portals.iucn.org.

Hadiprayitno, G., Mertha, I. G., & Ilhamdi, M. L. (2016). Species richness and birds conservation priority in Mount Rinjani Areas, Lombok. *Biosaintifika: Journal of Biology & Biology Education*, *8*(3), 270–277.

Hakim, L., Soemarno, M., & Hong, S.-K. (2012). Challenges for conserving biodiversity and developing sustainable island tourism in North Sulawesi Province, Indonesia. *Journal of Ecology and Field Biology*, *35*(2), 61–71.

Henri, Fakhrurrozi, Y., & Akbarini, D. (2016). Keanekaragaman tumbuhan yang dimanfaatkan oleh masyarakat Bangka dalam berkebun lada (Studi kasus di Kecamatan Merawang, Kabupaten Bangka, Bangka Belitung). In: *Seminar Nasional Pendidikan dan Saintek 2016* (pp. 299–305).

Kiper, T. (2013). Role of ecotourism in sustainable development. *Advances in Landscape Architecture*. 773–802.

Kirkby, C. A., Giudice, R., Day, B., Turner, K., Soares-Filho, B. S., Oliveira-Rodrigues, H., & Yu, D.

W. (2011). Closing the ecotourism-conservation loop in the Peruvian Amazon. *Environmental Conservation*, *38*(1), 6–17.

Latupapua, Y. T. (2013). Analisis potensi keanekaragaman hayati di Taman Nasional Manusela sebagai daya tarik ekowisata. *Jurnal Agroforestri*, *8*(4), 248–260.

Nurtjahya, E., Setiada, D., Guhardja, E., Muhadiono & Setiadi, Y. (2008). Revegetation of tin-mined land using various local tree species in Bangka Island, Indonesia. In *National Meeting of the American Society of Mining and Reclamation*, 739–755.

Oktavia, D., Setiadi, Y., & Hilwan, I. (2015). The comparison of soil properties in heath forest and post-tin mined land: Basic for ecosystem restoration. *Procedia Environmental Sciences*, *28*, 124–131.

Scott, D. (2011). Why sustainable tourism must address climate change. *Journal of Sustainable Tourism*, *19*(1), 17–34.

Susanto, E., Mulyani, Y. A., & Suryobroto, B. (2016). Bird communities in Seblat Nature Recreation Park (SNRP) North Bengkulu, Bengkulu. *Biosaintifika: Journal of Biology & Biology Education*, *8*(1), 25–32.

Sutarno, & Setyawan, A. D. (2015). Biodiversitas Indonesia: Penurunan dan upaya pengelolaan untuk menjamin kemandirian bangsa. *Pros Sem Nas Masy Biodiv Indon*, *1*(1), 1–13.

Syahputra, R. (2016). Habitat dan populasi mentilin (*Cephalopachus bancanus bancanus*) di Kabupaten Bangka. *Tesis*. Bogor: Sekolah Pascasarjana, Institut Pertanian Bogor.

Tsaur, S. H., Lin, Y. C., & Lin, J. H. (2006). Evaluating ecotourism sustainability from the integrated perspective of resource, community and tourism. *Tourism Management*, *27*(4), 640–653.

Widjaja, A., Rahayuningsih, Y., Rahajoe, J. S., Ubaidillah, R., Maryanto, I., Walujo, E. B., & Semiadi, G. (2014). *Kekinian keanekaragaman hayati Indonesia 2014*. Jakarta: LIPI Press.

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Traditional Usages of Taro (*Colocasia* spp.) by Ethnic Communities in Borneo

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Borneo; Taro; *Colocasia* spp; ethnics

Abstract

Borneo has a a wealth of various flora, including the Aroids (Araceae). Taro is one of the Aroids that cultivated and used as food crop since a long time ago by the people in Borneo. This study aimed to determine the utilization of Taros traditionally by several ethnic communities in Borneo. The research used Survey Explorative Method with Direct Interview Technique in the field. Taro samples were taken from various habitats of Banjar, Dayak, Kutai, Malay, Bugis, Toraja and China ethnic. The results showed that Taro species widely used are : *Colocasia esculenta var. esculenta* and *var. antiquorum, Colocasia affinis* and wild of *Colocasia esculenta*. *C. esculenta* var. *esculenta* and var. *antiquorum* is used as subsistence food crop and vegetables. Besides, it is used as medicine such as high blood pressure lowering and for consumption of diabetics. Parts of the plants consumed include leaves, petiole, corm and stolon. *C. affinis* is used as ornamental plants of the home garden, while wild *C. esculenta* is used as animal fodder. Taro in Borneo have a considerable variation of traditional cultivars and vernacular names. Fifty eight traditional cultivars from 5 different habitat to be used by some ethnics.

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INTRODUCTION

Borneo or Kalimantan is one of the largest archipelagos in the Asia Pacific region. Borneo Island is divided into 3 countries namely Brunei, Malaysia and Indonesia. The largest part of the Island is belongs to Indonesia and is commonly known as Kalimantan (Galapphatie et al., 2014). Borneo Island is inhabited by three native ethnic communities: Dayak, Kutai and Banjar. These people have used resources sustainably based on their traditions and knowledge. In addition, Borneo also has ethnic immigrant people such as Bugis, Toraja, Malay and other ethnicities.

The Island of Borneo has the richest Aroids in the world (Boyce et al., 2010). One of the Aroid (Araceae) groups that is useful as an important food crops of the world is Taro. *Colocasia spp* especially *Colocasia esculenta* (L.) Schott is one of the many widely cultivated Taros in the tropics and sub tropics (Nath et al., 2015 ; Naidoo et al., 2015). In Southeast Asia, Taro was previously a plant widely grown in areas that are currently dominated by rice (Blench, 2012). According to Rao et al., (2010) Southeast Asia region is considered as a place of Taro domestication for the first time.

The highest Taro diversity in the world is presumably found in Indonesia (Lebot & Aradhya, 1991 ; Kreike et al., 2004 ; Prana, 2007) because Indonesia is one of the areas of Taro origin. This diversity can be seen in areas where Taro is widely cultivated as in Java, Sumatra, Sulawesi and Borneo (Prana & Kuswara, 2002). However, the large diversity of Taro has not been supported by the availability of complete information about the utilization by the local community.

There are only few recorded ways of planting and utilizing Taros. Information about the genetic diversity of Taros and their use by farmers in an area is very important to be preserved and necessary for the management of genetic diversity of Taros (Jianchu et al., 2001). According to Liu et al., (2014) the traditional knowledge about *Aroid* plant groups is getting less. The documentation on traditional knowledge and use of Aroid plants including Taro is a very important value.

Knowledge of the plant group of *Aroid* (Araceae) is still needed by community. Although the cultivation of *C. esculenta* has been going on for more than 1700 years, the use of this plant as medicine has not been widely known and limited to some areas (Liu et al., 2014). In Borneo, although traditional Taro cultivation has existed for a long time, the information about Taro utilization is still very limited. In an effort to realize food security and food crop diversification program, Taro is definitely one of the plants to be reckoned with (Prana & Kuswara, 2002). The utilization of Taros is sometimes also closely related to the culture of the population of a region, so this plant is very important for communities life (Walujo, 2011). As stated by Iskandar & Iskandar (2017), it has strong relation between sociocultural aspects, the local community and managing and maintaining biodiversity**.** In the case of a crop like Taro, how farmers use and manage diversity is important for the conservation of its genetic resources. Based on this, it is necessary to do a research to find out the traditional utilization of Taros by some ethnic societies in Borneo.

METHODS

The study was conducted from February 2016 to June 2016. Taro samples were taken from various habitats of Banjar, Dayak, Kutai, Malay, Bugis, Toraja and Chinese ethnic (Figure 1). Those areas include province of North, East, South, Middle and West Borneo.

Figure 1. The location of Taro sampling in Borneo island.

The research used Explorative Survey Method with Direct Interview Technique in field (Walujo, 2004). This method was used to inventory the known biodiversity of the community where the research was conducted. The interviews were conducted involving 75 respondents. The respondence was consisting of 20 peoples of Banjar, 24 peoples of Dayak, 6 peoples of Kutai, 2 peoples of Bugis, 2 peoples of Toraja, 2 peoples of Java, 15 peoples of Malay, 2 peoples of Madura, and 2 Chinese peoples. Two of the respondents were farmers of Taro and 73 were local communities**.**

Field notes were made along the way con-

taining habitat, location using GPS (Global Positioning Satellite), vernacular names, species or traditional cultivar and uses of Taro as well as picture for documentation. Note of the locations used GPS conducted in places where there was a Taro population. All data such as latitude, longitude and altitude were recorded with Garmin 62 Sc. Data analysis was done descriptively based on the field interview result with tables and pictures. Quantitative analysis was done by calculating the percentage of utilization of Taro plant parts.

RESULTS AND DISCUSSION

The spreading of Taro in Borneo is quite wide and is found in most of the province in Borneo. Taros grow in swamp areas, the margin of paddy fields and ditches, home garden, orchard, the edges of the highways and other natural habitats. The condition of Borneo Island located in the tropics allows Taros to grow and develop well. According to Lebot & Legendre (2015) Taros can grow well throughout the wet tropics.

Taros belong to a group of tuber crops that have been known and cultivated for a long time by people living in Borneo. According to MacKinnon (2000) the people of Borneo have known Taro plants since they discovered and could processed iron ore. With tools made from ore, the forest has become easier to be opened, so the people have been able to grow rice and Taros on the land. Tuber cultivation along with the cultivation of fruit crops is considered the oldest form of cultivated plant in Borneo.

Based on the field interviews both men or women have same knowledge about Taro plants. The respondents consisted of 39 woman, 36 man and generally aged 30-60 years. There were housewife, employees, private and farmers.

The people of Kalimantan consume Taro as a subsistence food crops, vegetable crops, medicine or used for other purposes. In Indonesia the presence and expansion of cultivated and food crops such as maize and tubers have been pushing the existence of Taros as a food crop in some areas. This causes Taros currently only to be a subsistence food crop. Chotimah et al., (2011) has stated that a Taro in Borneo is one of indigenous vegetable crops. Indigenous vegetables are often referred to as local vegetables, which are native vegetables of the region and have long adapted and are known by people in a particular location. In many countries according to Matthews et al., (2012) Taro leaves are used as one of the vegetables consumed by the community.

Taro species that widely used in Borneo are *Colocasia esculenta* both variety of *esculenta* and *antiquorum. C.esculenta var. esculenta* is the most widely found. According to Purseglove (1972) and Prana (2007) *C. esculenta var. esculenta* is a variety of Taros that commonly found in Indonesia and tropical region, while *var. antiquorum* is a Taro derived and cultivated in temprate climates such as China and Japan. The tropical cultivars produce large central corms with relatively few side corms, and the temperate cultivars produce many small side corms from a small central corm. The variation of the varieties owned by each type of plants is a priceless source of germplasm for the benefits of development of local food resources and for the development of science (Waluyo, 2011).

Taros in Borneo have a large number of traditional cultivars and vernacular names. In this research fifty eight cultivars from 5 different habitat are used to be by some ethnics (Table 1). According to Lebot et al., (2004) in the worldwide there are thousands of Taro cultivars growing from *C. esculenta var. esculenta* and *var. antiquorum*. The diversity of Taro cultivars can be seen based on variations of morphological characters such as corms, stolons, leaves, and flowers.

In some areas such as in Hulu Sungai Utara and Kapuas Region species of *Colocasia affinis* is found. This Taro is used as ornamental plant in home garden by the Banjar and Dayak ethnic in the provinces of South Borneo and Central Borneo (Figure 2a). According to Matthews & Medhi (2014) *C. affinis* is the result of hybridization of other Taro species. The characteristics of this Taro is a black spot on leaf surface. The wild of *Colocasia esculenta* have been found in Samarinda and Muara Badak (East Borneo), Landak and Sanggau regencies (West Borneo). The parts used are leaves and petioles as animal fodder especially pigs by Toraja and Dayak ethnic (Figure 2b). It is processed by boiling first before mixed with other feed ingredients. The purpose of the boiling process is to reduce the acridity. As state by Bradbury & Nixon (1998) the edible aroids and other genera of Araceae contain needle-like calcium oxalate raphides which have been implicated as a cause of acridity.

Wild Taros are also consumed by the community but with a special treatment to relieve acridity. According to Matthews (2010) wild Taros in Asia and the Pacific have relatively little carbohydrate content, long stolons and acrid. In Indonesia wild Taros have long and many stolon characteristics, relatively small corm and high flowering ability (Prana & Kuswara, 2002). According to Matthews et al., (1992) ; Jianchu et al., (2001) Matthews & Naing (2005) ; the use of wild

Tabel 1. Traditional Taro cultivars and their utilization in Borneo

Ethnicity	Species	Traditional Cultivar	Habitats	Utilization/Part use
Banjar	*C. esculenta*	Keladi Gunung	Orchard	Corm and leaves are consumed (sayur asam)
		Keladi Sulur	Orchard	Stolon are consumed (sayur oseng)
		Keladi Lais	Orchard	Corm are consumed
		Keladi Akar	Orchard	Corm are consumed
		Keladi	Margin	Stolon are consumed
		Keladi	Margin	Corm, leaves, petiole and stolon are consumed
		Keladi Liar	Swamp	Stolon are consumed
		Keladi Putih	Margin	Corm, leaves, petiole and stolon are consumed
		Keladi Liar	Margin	Corm, leaves, petiole and stolon are consumed
		Keladi Telur	Orchard Orchard	Corm, leaves, petiole and stolon are consumed
		Keladi	Orchard Orchard	Petiole and stolon are consumed
		Keladi Hitam Ke-	Margin	Corm are consumed
		ladi Putih Keladi	Home Garden	Corm are consumed
		Hitam	Orchard swamp	Corm are consumed and used for diabetics
		Keladi Hitam	Home Garden	Corm are consumed (sayur santan)
	C.antiquorum C.	Keladi Hutan	Swamp	Cormel, stolon are consumed
	affinis	Keladi Hias	Margin	Ornamental
		Keladi Liar	Margin	Ornamental
Kutai	*C. esculenta*	Talas Sayur	Orchard	Corm, leaves, petiole and stolon are consumed
		Talas Hitam	Orchard	Corm are consumed (sayur asam)
		Keladi Putih	Home Garden	Corm are consumed
Dayak Pampang	*C. esculenta*	Keladi	Home Garden	Corm are consumed
		Talas Malaysia	Home Garden	Corm, leaves, petiole and stolon are consumed
Dayak Benuaq	*C.esculenta*	Keladi	Orchard	Corm are consumed
	C. antiquorum	Keladi Gunung	Margin	Corm, leaves, petiole and stolon are consumed
Dayak Malinau	*C.esculenta*	Talas Ungu	Margin	Corm are consumed
Dayak	*C.esculenta*	Talas	Margin	Corm, leaves, petiole and stolon are consumed
		Talas Putih	Margin	Corm and leaves are consumed
		Talas Liar	Orchard	Animal fodder
	C. affinis	Keladi Hias	Orchard	Ornamental
Dayak Katingan	*C. esculenta*	Talas Sayur	Orchard	Corm, leaves, petiole and stolon are consumed
Dayak Kahayan	*C. esculenta*	Kujang Bawa'	Orchard	Corm, leaves, petiole and stolon are consumed
		Kujang Enyuh	Home Garden	Corm, leaves, petiole and stolon are consumed
		Kujang Gahuri	Margin	Corm are consumed
Dayak Kalteng	*C.esculenta*	Keladi Habang	Orchard	Corm, leaves, petiole and stolon are consumed
Dayak Aje	*C. esculenta*	Keladi Hitam	Orchard	Animal fodder
		Talas Malaysia	Orchard	Corm, leaves, petiole are consumed
Dayak Kenelas	*C.esculenta*	Keladi Cina	Orchard	Corm, leaves, petiole and stolon are consumed
		Keladi Mei	Swamp	Corm, leaves, petiole and stolon are consumed
		Keladi Mei Hitam	Orchard	Animal fodder
Dayak Ahe	*C. esculenta*	Keladi Madura	Swamp	Corm are consumed
		Keladi Sayur	Swamp	Corm, leaves, petiole and stolon are consumed
Melayu	*C. esculenta*	Keladi Tikus		Animal fodder
		Keladi Cina	Orchard	Corm, leaves, petiole and stolon are consumed
			Margin	Petiole for blood pressure (medicine)
		Talas Manis	Margin	Corm, leaves, petiole and stolon are consumed
		Keladi Liar		Corm and stolon are consumed
		Keladi Bangkok	Orchard	Corm, leaves, petiole and stolon are consumed
			Orchard	Petiole for blood pressure (medicine)
		Talas Merah	Orchard	Corm are consumed
		Talas Putih	Home Garden	Corm, leaves, petiole and stolon are consumed
		Keladi Minyak	Margin	Corm, leaves, petiole and stolon are consumed
		Keladi Udang	Margin	Corm and leaves are consumed
		Keladi Liar		Animal fodder
Bugis	*C. esculenta*	Keladi Hitam	Margin	Corm, leaves, petiole and stolon are consumed
			Cultivation	Corm for diabetics
		Talas Malaysia	Cultivation	Corm are consumed
Madura	*C. esculenta*	Talas Hitam	Home Garden	Corm, leaves, petiole and stolon are consumed
		Talas Kelapa	Margin	Corm are consumed
Jawa	*C. esculenta*	Talas Bentul		Corm, leaves, petiole and stolon are consumed
Cina	*C. esculenta*	Talas Liar		Animal fodder
		Talas Hutan/		
		Manis	Orchard	Corm, leaves, petiole and stolon are consumed
		Keladi	Orchard	Corm, leaves, petiole and stolon are consumed
Toraja	*C. esculenta*	Keladi	Margin	Animal fodder

Taros as foodstuff and fodder spreads throughout Southeast Asia and East Asia.

Utilization of Taros by the Banjar ethnic

Taro among the Banjar ethnic is known by the name of *Keladi*. Some traditional cultivars of Taro utilized : Keladi Gunung, Keladi Sulur, Keladi Lais, Keladi Akar, Keladi Hitam, and Keladi Telur. The corm of Keladi Gunung, Keladi Lais and Keladi Akar are consumed as vegetables that cooked as Sayur Asam and Sayur Santan. Besides, Keladi sulur consumed for the stolon that

spread for vegetable food "oseng sulur" while the corms are not eaten because rather itchty (Figure 3a and 3b). A bunch of Keladi Sulur is sold five thousands rupiah in traditional markets known as the night market. This market activity usually occurs at night (Figure 4d). According to Matthews (2010) stolon, in China and Southeast Asia, is generally consumed but only used on a small scale. Keladi Gunung, Lais, Sulur and Akar are cultivated by people in shaded orchard and swamp.

The cultivar of Keladi Gunung is sometimes referred to as "red Taro" because it has pink tuber flesh by Banjar ethnic in Central Borneo. It is usually processed into "sayur asam" mixed with other vegetables (Figure 3c). The vegetable is popular among Banjar ethnic communities in this region and it is usually available everyday at Banjar traditional food stalls in Borneo. In Tambak Anyar Hilir village, the cultivar part of talas Hutan consumed is cormel along with stolons. While the cultivar of Keladi Putih has a characteristic white corm that consumed, Keladi Telur cultivar has a leaf shape like eggs (Figure 2f). All parts of this Taro - corm, leaves, petioles, and stolon can be consumed.

Banjar ethnic communities have traditional cultivar of Talas Hitam used for consumption of diabetics. The used a white tuber for carbohydrate sources. According to Aprianita (2014) Indonesia has many traditional tuber plants that contain carbohydrates and are potentially used in diets to reduce the risk of obesity, heart attack and diabetes.

Utilization of Taros by Kutai ethnic

Kutai communities know Taros as a vegetable food ingredient. Taro is generally processed into Sayur Kuning using a mixture of coconut milk (Figure 3f) and Sayur Talas is mixed with pineapple (Figure 3e). Plant parts used are corm and petioles. Taro cultivars consumed and utilized are Keladi Putih, Keladi Hitam and Talas Sayur. The corms, leaves and petioles of Taro are used as a vegetable and considered as a rich source of carbohydrates, proteins, minerals and vitamins (Sharma, 2008). According to the Kutai ethnic community in the Perian village of West Kutai regency, the corm of Talas Putih has a soft texture and are single corm. In addition to the use of coconut milk, this vegetable is mixed with other vegetables such as eggplants, sour and others (Figure 3d). According to Brennan (2000) along the Pacific Islands, Taro are processed using coconut milk and other spices.

In East Kutai Regency, the people of Kutai ethnic used Talas Sayur that is generally consumed by community if this Taro is available and sold in traditional market. According to information from the community, the leaves, petiole, corm, and stolon can be consumed. As state by Matthews (2004) different cultivars are used in different ways and preserving culinary knowledge is important for preserving genetic diversity in Taro. This Taro grows on the margin of the ditches and on the sides of the highways. Although not cultivated clearly, Taro are widely found and some Taro types are particularly well adapted to difficult land and soil. According to Quero-garcia

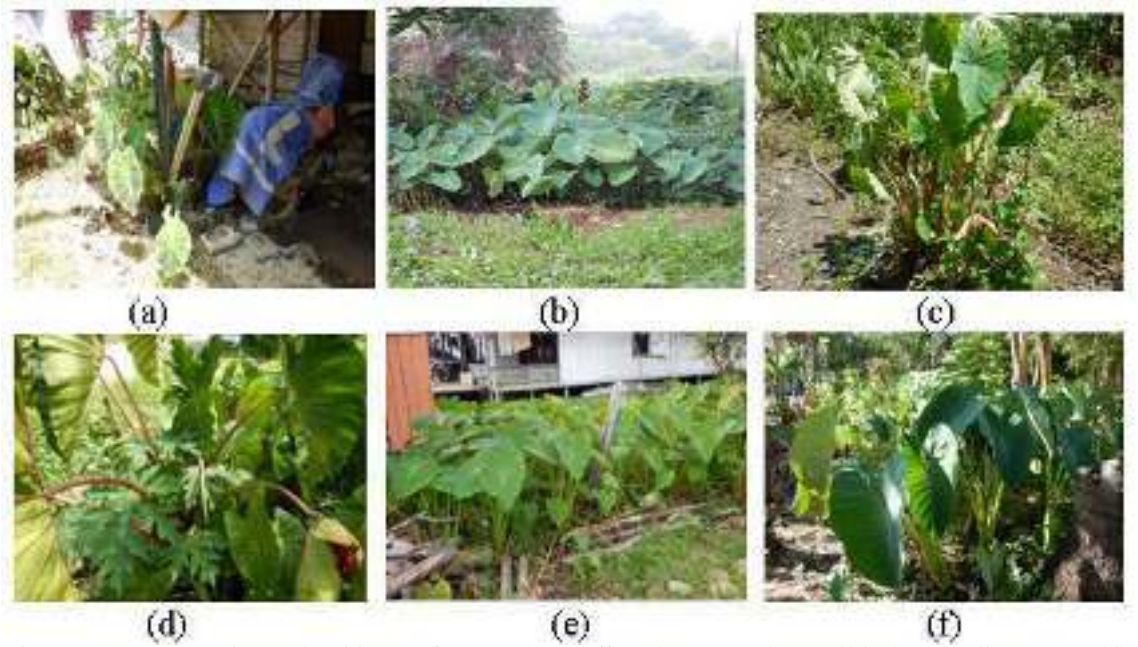

Figure 2. Some species and cultivars of Taro (a). *C. affinis* (b). *C. esculenta* wild (c). Keladi Gunung (d). Keladi Udang (e). Talas Malaysia (f). Keladi Telur

(2010) Aroid plant group includes minor crop plants but are a staple food in tropical developing countries. Taros are cultivated as a home garden plants or simple farming system with few results.

Utilization of Taros by Dayak ethnic

Among Dayak Kahayan ethnic located in Central Borneo, Taro are known as Kujang. The traditional cultivars that are used by this ethnic include Kujang Bawa '/ Buah, Kujang Enyuh and Kujang Gahuri.

Talas "Malaysia" according to ethnic Dayak Pampang in East Borneo comes from Malaysia brought and cultivated by their ancestors in Borneo decades ago (Figure 2e). In Sanggau regency of West Borneo, Talas Malaysia cultivars was brought by workers returning from Malaysia and then planted by residents around the orchard. Ethnic Dayak Aje utilizes Malaysia Taros as "sayur asam pedas". All the parts of Taro can be consumed except stolons and corm have a sweet taste.

Keladi Gunung cultivars used by Dayak Benuaq ethnic morphologically are different from those used by Banjar ethnic (Figure 2c). Keladi Gunung of The Dayak ethnic have a reddish petiole with many corms (*C. esculenta var. antiquorum*), whereas those of Banjar ethnic have green petiole and single corm (*C. esculenta var. esculenta*). In addition, there is also a cultivar Keladi that can be consumed and grown under the house of Dayak people (Figure 4b).

In Central Borneo Dayak Katingan utilizes Talas Sayur cultivar for consumption and stolon is the most delicious part of this cultivar. Meanwhile, Dayak Kahayan knows traditional cultivars of Kujang Bawa ', Kujang Enyuh and Kujang Gahuri. Within one community or country, the two kinds of diversity are not necessarily correlated. A single cultivar can be used in many different ways, and more than one cultivar can be used in the same way (Matthews, 2004). Kujang Gahuri usually consumed during the dry season because the corms are not too watery and taste better. To reduce the acridity that arises when consuming Taros, the people of Dayak Kahayan process it by boiling and the boiling water should first be discarded. According to FAO (1990) the acridity that arises at the time of consuming Taro can be removed by boiling Taros first.

Talas Hitam is one of the traditional cultivars known by the Dayak Kenelas ethnic group (Figure 4f). This Taro can not be consumed and is only used for pig fodder which is kept around the community's residence. According to Matthews (2010) the overall utility of Taros is sometimes unknown by the people who utilize this plant, even in areas where Taro cultivation has long been done. Besides, Dayak Kenelas also recognizes Keladi Cina and Keladi Mei cultivar that can be consumed.

Utilization of Taros by the Bugis ethnic

One of the traditional Taro cultivars utilized by the Bugis ethnic is Keladi Hitam. This Taro grows in the orchard, margin of the rice fields in a relatively dry area. All parts of plants such as leaves, petiole and corm are consumed. The most commonly used part is the corm and has a very tender flavor. The corms are usually consumed and used for diabetics similar to cultivars known by the Banjar ethnic group. The corms was boiled

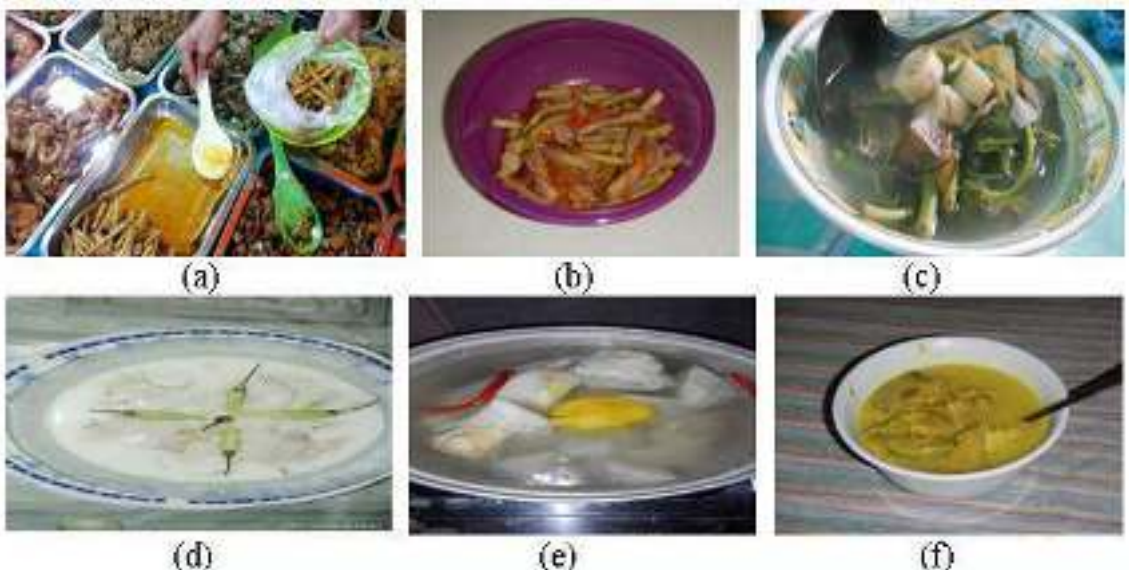

Figure 3. Traditional vegetables food made of Taros (a) and (b). oseng sulur (c). sayur asam (d) sayur santan (e) sayur talas (f) sayur kuning

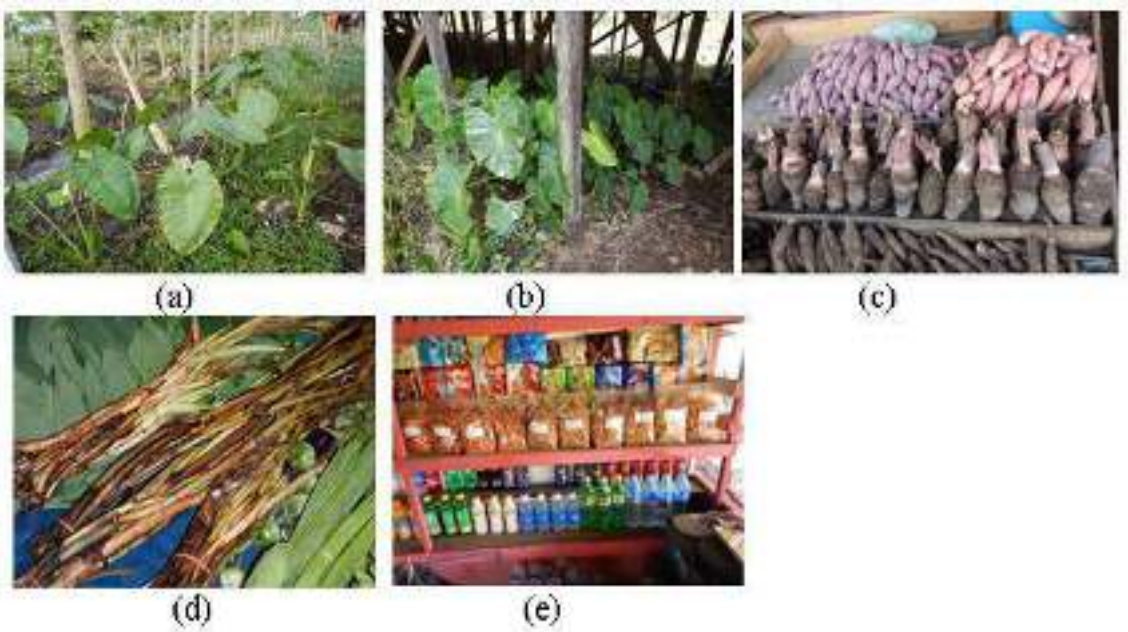

Figure 4. Cultivation, Taro products (a). Cultivation of Talas Kelapa (b). Taro under a house of ethnic Dayak Benuaq (c). Corm (d) stolon in night market (e) snack made from Taro.

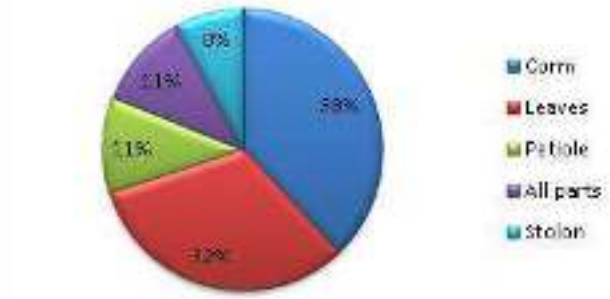

Figure 5. Percentage of utilization of Taro parts by community

first and then consumed as substitute for rice. According to community intensive consumption is still not known for certain and just for substitute rice**.** Englberger (2013) state that Taro has a high carotene content, especially the yellow corms and beneficial to ward off chronic diseases such as cancer, heart attack and diabetes

In North Borneo province, Taro cultivars utilized by the Bugis community are Talas Malaysia. The part consumed is the corm while the other parts are underutilized. According to information obtained Talas Malaysia corms can be consumed if not stepped on by livetock. The corms that have been stepped by livestock are not consumed because they will be itchier.

Utilization of Taro by the Malay ethnic

Traditional Taro cultivars utilized by ethnic Malays include: Keladi Bangkok (Malaysia Taro), Talas Merah, Talas Putih, Talas Manis, Keladi Minyak, and Keladi Udang. The young plants of Keladi Bangkok cultivars are all consumed, but old plants cannot be used because they can cause itching when consumed. According Akpan & Umoh (2004) Taro consumption often causes acridity causing irritation and burning on the skin and mouth. The acridity is thought to be due to the calcium oxalate crystals found on Taros directly in contact with skin (Kaushal, 2015). In addition to food, Keladi Bangkok cultivars can also be used as medicines especially petiole to lower blood pressure. Processing by way of the petiole boiled first and then consumed. In the province of Lampung, the part of Taro plants used as medicines is a leaf that serves as a wound medicine for the community (Utami & Asmaliyah, 2010).

Talas Merah cultivar corms are consumed more than unused petiole because they are rather itchy. This cultivar has morphologically pink tubers, roots, and stolons. The corms are better grown in a wet soil environment, whereas in dry

soil corms have a rather hard texture. Unlike Talas Merah cultivars, Talas Putih cultivars or Talas Cina have white tubers, stolons, and roots. Tubers and young petiole can be consumed. In addition, Keladi Minyak cultivar was discovered. This cultivar has a corm that is tenderer, not itchy and tastier. It is usually cooked by boiling and added coconut milk or made into Sayur Bening. According to Ghani (1982) the most economically important and widely grown variety in Malay Peninsula region is Keladi China, besides, the well-known Taros are Keladi Minyak and Keladi Udang. In Sanggau district, people give the name of Keladi Udang due to the red leaf bone and petiole like shrimp skin (Figure 2d).

In West Borneo, there is a Taro intensive cultivation area on Jl. Soekarno-Hatta. The cultivated Taro is the Talas Kelapa and the Talas Hitam cultivar (Figure 4a). The crops are generally shipped out of areas such as Java and Jakarta. Taro corm commodity prices are relatively high at 15-20 thousand/kg (Figure 4c). The people process them into Taro snack that becomes typical souvenirs of West Borneo (Figure 4e). In the eastern part of Indonesia, the corms are used as a staple food, while the people in the rest of the country use the corms as a raw material for animal feed and snacks (Kumoro et al., 2013). Cultivation of Taro in this area is done by the immigrant community from the island of Java, especially Madurese who become migrant farmers in West Borneo. According to Rao (2010) Taro plants in cultivation are managed by farmers and their genetic resources are maintained under the control of local communities.

Spesies and utilization of Taro parts by ethnic communities in Borneo varies widely and differently (Figure 5). For species of Taro consumed, the community utilizes *C. esculenta* both *var. esculenta* and *var. antiquorum*. The most widely used parts are the corms of 38% and leaves 32%, while the petiole as well as all the Taro parts is as much as 11%. The least utilized part is stolons by 8%. Quero-Garcia (2010) state that Taro are mostly consumed for their corms and cormels but leaves and petioles can also be part of the diet.

CONCLUSIONS

The Taros used by some ethnic communities consist of *C. esculenta* var. *esculenta* and var. *antiquorum, C. affinis* and wild of *C. esculenta*. *C. esculenta* var. *esculenta* and var. *antiquorum* is used as subsistence food crops and vegetables, *C. affinis* is used as ornamental plants, and wild *Colocasia esculenta* is used as animal fodder. There are fifty eight traditional cultivars Taros used by some ethnic in Borneo. Keladi Hitam cultivars are used by diabetics as a source of carbohydrates and Keladi Putih and Keladi Bangkok are used as blood pressure-lowering drugs. The most widely used Taro parts are corm.

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REFERENCES

Akpan, E. J., & Umoh, I. B. (2004). Effect of heat and tetracycline treatments on the food quality and acridity factors in cocoyam [Xanthosoma sagittifolium (L) Schott]. *Pakistan Journal of Nutrition*, 3(4), 240-243.

Aprianita, A., Vasiljevic, T., Bannikova, A., & Kasapis, S. (2014). Physicochemical properties of flours and starches derived from traditional Indonesian tubers and roots. *Journal of food science and technology*, 51(12), 3669-3679.

Blench, R. M. (2012). Irrigated Taro (*Colocasia esculenta*) in the Indo-Pacific Biological, Social and Historical Perspectives, Senri Ethnological Studies 78: *Vernacular Names for Taro in the Indo-Pacific Region: Implication for Centers of Diversification and Spread*. National Museum of Ethnology. Osaka.

Boyce, P. C., Wong, S. Y., Ting, A. P. J., Low, S. E., Low, S. L., Ng, K. K & Ooi, I. H. (2010). The Araceae of Borneo-The Genera. *Aroideana,* (33), 3-73

Bradbury, J. H., & Nixon, R. W. (1998). The acridity of raphides from the edible aroids. *Journal of the Science of Food and Agriculture*, 76(4), 608-616.

Brennan, J. (2000). *Tradewinds & Coconuts: A Reminiscence & Recipes from the Pacifi c Islands.* Boston: Periplus.

Chotimah, H. E. N., Kresnanita, S. & Miranda, Y. (2011). Seminar Nasional Reformasi Pertanian Terintegrasi menuju Kedaulatan Pangan: *Studi Etnobotani Sayuran Indigenous (Lokal) Kalimantan Tengah*. Madura: Fakultas Pertanian Universitas Trunojoyo

Englberger, L., Schierle, J., Marks, G. C., & Fitzgerald, M. H. (2003). Micronesian banana, Taro, and other foods: newly recognized sources of provitamin A and other carotenoids. *Journal of Food Composition and Analysis*, 16(1), 3-19.

Galappathie, S., Palombo, E. A., Yeo, T. C., Ley, D. L. S., Tu, C. L., Malherbe, F. M., & Mahon, P. J. (2014). Comparative antimicrobial activity of South East Asian plants used in Bornean folkloric medicine. *Journal of Herbal Medicine*, 4(2), 96-105.

Ghani, F. D. (1982). The Status of Keladi China *Coloca-*

sia Esculenta (L.) Schott Cultivation in Peninsular Malaysia. *International Symposium on Tropical Root and Tuber Crops*. Philipine.

Iskandar, J. & Iskandar, B. S. (2017). Various Plants of Traditional Rituals: Ethnobotanical Research Among The Baduy Community. *Biosaintifika: Journal of Biology & Biology Education,* 9(1), 114-125.

Jianchu, X., Yongping, Y., Yingdong, P., Ayad, W. G., & Eyzaguirre, P. B. (2001). Genetic diversity in Taro (Colocasia esculenta Schott, Araceae) in China: An ethnobotanical and genetic approach. *Economic Botany*, 55(1), 14-31.

Kaushal, P., Kumar, V., & Sharma, H. K. (2015). Utilization of Taro (*Colocasia esculenta*): a review. *Journal of Food Science and Technology,* 52(1), 27-40.

Kreike, C. M., Van Eck, H. J., & Lebot, V. (2004). Genetic diversity of Taro, *Colocasia esculenta* (L.) Schott. in Southeast Asia and the Pacific. *Theoretical and applied genetics,* 109(4), 761-768

Lebot, V. & Aradhya, K. M. (1991). Isozyme variation in Taro (*Colocasia esculenta* (L) Schott.) from Asia and Oceania. *Euphytica,* 56(1), 55-66

Lebot, V., Prana, M. S., Kreike, N., van Heck, H., Pardales, J., Okpul, T., Gendua, T., Thongjiem, M., Hue, H., & Yap, T. C. (2004). Characterisation of Taro (*Colocasia esculenta* (L.) Schott) genetic resources in Southeast Asia and Oceania. *Genetic Resources and Crop Evolution,* 51(4), 381-392.

Lebot, V. & Legendre, L. (2015). HPTLC screening of Taro hybrids (*Colocasia Esculenta* (L.) Schott) with high flavonoids and antioxidants contents. *Plant Breeding,* 134(1), 129-134

Liu, B., Liu, Y., Cao, W., Zhang, S., Liu, Z., Ni, Y & Li, F. (2014). Ethnobotany of Medicinal Aroids in Xishuangbanna, Yunnan Province, China. *Aroideana*, 37(2), 69-78

MacKinnon, K., Hatta, G., Halim, H. & Mangalik, A. (2000). *The ecology of Kalimantan. The ecology of Indonesia Series III*. Singapore: Periplus Editions (HK) Ltd.

Matthews, P. J. (2004). Genetic Diversity in Taro and the Preservation of Culinary Knowledge. *Ethnobotany Research and Applications,* 2, 55-71

Matthews, P. J. & Naing, K.W. (2005). Notes on the Provenance and Providence of Wildtype Taros (*Colocasia esculenta*) in Myanmar. *Bulletin of the National Museum of Ethnology,* 29(4), 587–615.

Matthews P. J. (2010). Ethnobotany and global diversity of Taro. *In* Rao, V.R., Matthews, P. J., Eyzagulrre, P. B. and Hunter, D. (Eds). *The Global Diversity of Taro* ; *An introduction to the history of Taro as a food*. Biodiversity International. Rome

Matthews, P. J., Agoo, E. M. G., Tandang, D. N & Maludid, D. A. (2012). Ethnobotany and Ecology of Ethnobotany and Ecology of Wild Taro (*Colocasia esculenta*) in the Philippines: Implications for Domestication and Dispersal. *Senri Ethnological Studies* 78, 307-340.

Matthews, P. J & Medhi, D. (2014). Feasibility Study for Field Research: Ethnobotany and Ecology of Wild and Cultivated Aroid in Assam State, Northeast India. *AREIPGR,* 30, 159-183

Naidoo, K., Amonsou, E. O., & Oyeyinka, S. A. (2015). In vitro digestibility and some physicochemical properties of starch from wild and cultivated amadumbe corms. *Carbohydrate Polymers*, 125, 9-15

Nath, V. S., Hegde, V. M., Jeeva, M. L., Misra, R. S., Veena, S. S., Raj, M., & Sankar, D. S. (2015). Morphological, pathological and molecular characterization of *Phytophthora colocasiae* responsible for Taro leaf blight disease in India. *Phytoparasitica,* 43(1), 21-35.

Prana, M. S. & Kuswara. (2002). *Budidaya Talas: Diversifikasi untuk Menunjang Ketahanan Pangan Nasional*. Bogor: Medikom Pustaka Mandiri.

Prana M. S. (2007). Studi Biologi Pembungaan pada Talas (*Colocacia esculenta* (L.) Scott. *Biodiversitas,* 9(1), 63-66

Purseglove, J. W. (1972). *Tropical Crops: Monocotyledons*. London: Longman.

Quero-García, J. (2010). Taro and Cocoyam. *In* Bradshaw. J. E. (Ed). *Root and Tuber Crops*, Handbook of Plant Breeding 7. Springer Science+Business Media.

Rao R. V., Matthews P. J., Eyzaguirre P. B., & Hunter D. (2010). Ethnobotany and global diversity of Taro. *In* Rao, V.R., Matthews, P. J., Eyzagulrre, P. B. and Hunter, D. (Eds). *The Global Diversity of Taro* ; *Ethnobotany and Conversation*. Biodiversity International. Rome

Ridley, H. N. (1905). The Aroid of Borneo. *Journal of the Straits Branch of the Royal Asiatic Society*, 4, 169-188

Sharma, K., Mishra, A. K., & Misra, R. J. (2008). The Genetic structure of Taro: a comparison of RAPD and isozyme markers. *Plant Biotechnology Reports,* 2(3), 191-198.

Utami, S. & Asmaliyah. (2010). *Potensi Pemanfaatan Tumbuhan Obat di Kabupaten Lampung Barat dan Kabupaten Tanggamus Propinsi Lampung*. Lampung: Balai Penelitian Kehutanan.

Walujo, E. B. (2004). *Pedoman Pengumpulan Data Keanekaragaman Flora: Pengumpulan data Etnobotani.* Bogor: Pusat Penelitian Biologi Indonesia

Walujo, E. B. (2011). *Keanekaragaman Hayati Untuk Pangan*. Herbarium Bogoriense Pusat Penelitian Biologi Lembaga Ilmu Pengetahuan Indonesia. Kongres Ilmu Pengetahuan Nasional X. Jakarta.

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The Gonad Maturity of Female *Osteochillus vittatus* in the presence of Ascorbic Acid

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Osteochillus vittatus; ascorbic acid; gonad maturity; reproductive performance

Abstract

The fish's reproduction status is affected by both fed compositions and vitamins intake lead to determine improvement of eggs quality as well as fish production. The presence of Ascorbic Acid (AA) in the cultivation ponds, might accelerate female's gonad maturation and so rematuration. The research aimed to determine: (1) Level gonad maturity of supplementation Ascorbic Acid; (2) Oocyts diameter; (3) Larvae survival rate. The research used experimental methods.The method was a completely randomized design (CRD) of 4 treatments and 4 replications. Treatments were supplementation of AA at different dosages of 0, 600, 1200 and 1800 mg/kg fish-fed ration.The gonad maturity level was analysed descriptively and oocyte diameter and larvae survival rate data were analysed by ANOVA. The result showed that supplementation of AA at a dosage of 1200 mg / kg fish-fed ration accelerated the process of gonad maturity, development of oocyt diameter and larvae survival rate as well as the viability of *O. vittatus* larvae at 90 rearing days. In this case, level IV gonad maturity was reached at 90 days which characterized by completed vitelogenesis process and oocyte diameter of 1.1 mm. Thus, this study is useful for aquaculture science by providing information on utilization of AA as food supplement in fish culture and also for fish farmer who wish to accelerate *O. vittatus* reproduction.

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INTRODUCTION

Gonads maturity of adult female fish is determined by either environmental or genetic aspects. Nutrition as well as fed components like fats, amino acids, minerals, and vitamins might also play significant role in affecting the fish reproduction phase) (Canyurt & Akhan, 2008). Vitamin C (Ascorbic Acid/AA), one among those micro nutrients, supposed to present in the fish fed, since this compound has significant role on fish reproductive status and must be present in the fed since they do not have these in nature. The absence of L-gulonactone oxidase enzyme in teleost fish, which catalyses AA from sugar, causes the fish are not able to synthesis AA. The AA therefore needs to be added on on the feeding rations of this fish (Ibrahem et al., 2010) whereas, the third one received a balanced diet supplemented with vitamin C (500 mg kg-1 group since this component takes a significant role in optimallizing the fish reproductive performance especially during vitellogenesis as well as embryo development. Supplementation of AA on the fish-fed ration during ovum development in the ovarium may increase the ova hatching rate and fish larvae development (Sandnes, 1991).

Several studies on the supplementation of AA on the fish-fed ration related with its effect to the gonad maturitiy of Cod (*Gadus morhua* L) and sperms quality of the Trout (*Oncorhynchus mykiss*) had been reported previously (Canyurt & Akhan, 2008). In the last group of fish, Trout (*Oncorhynchus mykiss*), it was noted that AA addition on fed-ration to be an important factor of its reproductive performance especially when they reached the maturity status (Valdebenito et al., 2015) both male and female, are determined by several factors (age, management, feeding, chemical and physical factors, water quality, etc.. Additonal AA on the catfish (*Clarias gariepinus*) ration accelerated its gonad maturity on level III, i.e.: accelerating the oocytes stadia (Hengky, 2010). Other vitamins, like vitamin C, E an Zn mineral on the Nile tilapia (*O. niloticus*) ration might improve spawning frequency, larvae productions, fecundity, hatching rate, sperm motility and viability (Gamanpilla et al., 2007). During the study of reproductive performance, vitamin C (AA) supplemetned at a dosage of 150 mg kg-1 on the *O.niloticus* fed ration incrase the Gonado Somato Indeks (GSI) (Martins et al., 2016). Gonad maturity is related to egg development. Sandnes reported, that AA supplementation on the fed ration of salmon fish resulted a variable increase of egg diameter (Sandnes, 1991). Different habitat was also noted to cause different egg size, since the diferent habitat may contain different levels of nutrients on their natural fed of *Channa lucius* (Syandri, 2013) and *O. vittatus* (Syandri & Azrita, 2015). Disuniformity of mature egg diameter might be used to determine the spawning frequency as well as spawning period by checking the modus on the eggs diameter (Sharifuddin & Omar, 2010).

Nilem fish *(Osteochillus vittatus*, Cyprinidae) is a native Indonesian fish and has high in economic value (Kottelat et al., 1996). Naturally, the gonad development of this fish group is strongly depending on its habitat leads to a very limited frequency of spawning to twice per annum. Spawning frequency of the nilem, however, might happen monthly though it will reach maximum spawning frequency on a particular month on the year (Subagja et al., 2006). Increasing spawning frequency which does not always depend on this environmental nutrients content, has alredy been applied in the fish-cultivation practices by increasing nutrition on the fish-fed ration and additional supplement toward the female fish. Addition of 30% protein and supplementation of ascorbic acid (AA) reported to improve reproductive performance of *O vittatus* to reach its gonad maturity and rematuraty earlier than normal condition. By doing so, it is expected that spawning frequency of the *O vittatus* might happens more than two times per annuum leads to increase production of fish larvae, especially for fish famers.

The aim of the research was to level gonad maturity of supplementation Ascorbic Acid, oocyts diameter and larvae survival rate.

METHODS

Experimental materials 64 adult individuals of post-spawning females *O.vittatus* taken from the Banyumas Regency Central Java, starch of ascorbic acid (AA), fish-fed ration contain 30% protein, *Tubifex* worms, Neutral Buffer Formalin (NBF) 10% solution, tapioca starch, aquabidest, synthetic hormon (Ovaprim).

All adult individuals of females *O.vitttus* were grown separately in 16 different ponds (1x2x0,5m/pond), each pond was filled with 4 individuals of tagged-fish. The fish were fed by ration supplemented with AA as on the treatment dosages i.e.:3% of their total body size and applied twice per day, morning and afternoon.

Current research was done experimentally by applying a Completely Randomised Design (CRD) with four treatments performed in four replications. Treatments were supplementation

of AA toward adult individuals of post spawning female *O.vittatus* at different dosages of 0, 600, 1200 and 1800 mg/kg fish-fed ration (Hengky, 2010). The observed variables were: gonad maturity, oocyt diameter, and larval viability. The adult female-fish were reared for total of 90 days.

Observation was done by dissecting the adult female fish at the age of 30 , 60 and 90 days of rearing, then observed for its histological appearance. Oocyt's diameter was observed by applying canulation method i.e.: by taking the ovum using a particular hose that fit to the genital size and pulled by a spuit (Setyaningrum et al., 2006). Fifty eggs were observed for their diameter under oculer microscope completed with micrometer. Sampling was done in the interval of 2 weeks, when the oocyt's size reaches 1.1 mm diameter means that the fish has ready to be spawned. Artificial spawning was done by applying synthetic hormon namely ovaprim.

Larvae of the hatched eggs were then grown in a fish aquarium compeleted with aeration system, on each treatment 100 larvaes were reared and performed in four replicates. Larvae were subjectyed to be fed naturally contains tubifex worms, and observed for its viability in 10 days of rearing.

Samples which were gonad maturity observation were taken at the interval for 30 days of rearing for 90 days, but the oocyt diameter was observed every two weeks. Samples were then put in the preparat flasks and added by 10% NBF solution, shook homogently to be ready for histological preparation and some were subjected for measurement of their size under ocular microscope completed with micrometer. The larvae viability was determined by counting total number of dead-larvae after 10 days.

Measurement of ovum diameter = total ocular scales multiplied by calibration value (Effendie, 1979).

Searching for 1 ocular value on the objective scale:

1 ocular x total oculars= total objectives x objective

1 oculars = Larvae's viability: Survival (%) = % (Effendie, 1979)

Data of gonad's maturity were analysed descriptively as done by Hibiya(Hibiya, 1982). Data of oocyt diameter and larvae viability were analysed statistically by applying ANOVA on a one way analysis using an SPSS version 21 software, when there was a significant different ($P<0,05$) analyses was then continued by test of least significant difference (LSD) test for determining oocyt diameter in relation to the the best larval viability.

RESULTS AND DISCUSSION

Gonad Maturity

At the rearing period of 30 days, gonad maturity of *O. vittatus* fish reached level III with two different stages of yolk formations i.e.: previtellogenesis which dominated by appearance of primary oocyte (op) which characterised by globular yolk (yg), and vitellogenesis stage which determined by the formation of secondary oocytes (os) but in small numbers. The OS consists of yolk plate (yp) (Figure 1). In all treatments, the study noted that gonad's maturity was dominated with the presence of primary oocytes and the nucleus which was located in the center of the ovum. Treatments of AA of 1200 mg/kg and 1800 mg/kg fish fed produced many secondary oocytes, and nucleus was in the postition of going to the edge of the cell.

A histological observation of the *O. vittatus* fish gonad after 30 days rearing showed if the both types oocytes (primary and secondary) had started to develop, as determined by the presence of globular yolk (yg) so it can be said that the ovarium was still in a pre-vitelogenesis (level III) stage. At this stage, the primary oocytes were still has its vacuoles and globular yolk, however there were some secondary oocytes appeared to sign that the gonad has almost in mature phase (Syandri & Azrita, 2015); (Ritonga et al., 2016). The finding of high number of primary oocytes shows the early phase of gonad maturity (Mannan, et al, 2010). Dorostghoal et al has as noted that there were three different phases of gonad maturity in the *Barbus grypus* namely: previtellogenic, vitellogenic, and maturation. The first phase was divided into two phases: primary oocytes and globular yolk; the vitellogenic however divided into three different stages; primary, secondary and tertiary globules. The last period, maturation, is characterized by two phases migration of the nucleus and hydration (Dopeikar et al., 2015).

Gonad maturity after 90 days of rearing period was characterized by its ovum which had reached the vitellogenesis where the secondary oocytes (os) were determinant. Apart from that, it was obviously seen if the nucleus almost reached the Germinal Vesicle Breakdown (GVBD) stage and so in periphere site (Figure 2). At either the absence of AA or 600 mg/kg fish-fed ration, current study noted if primary oocytes (op) were still present, however, treatment of AA supplementation at both 1200 mg/kg and 1800 mg/kg fish-fed

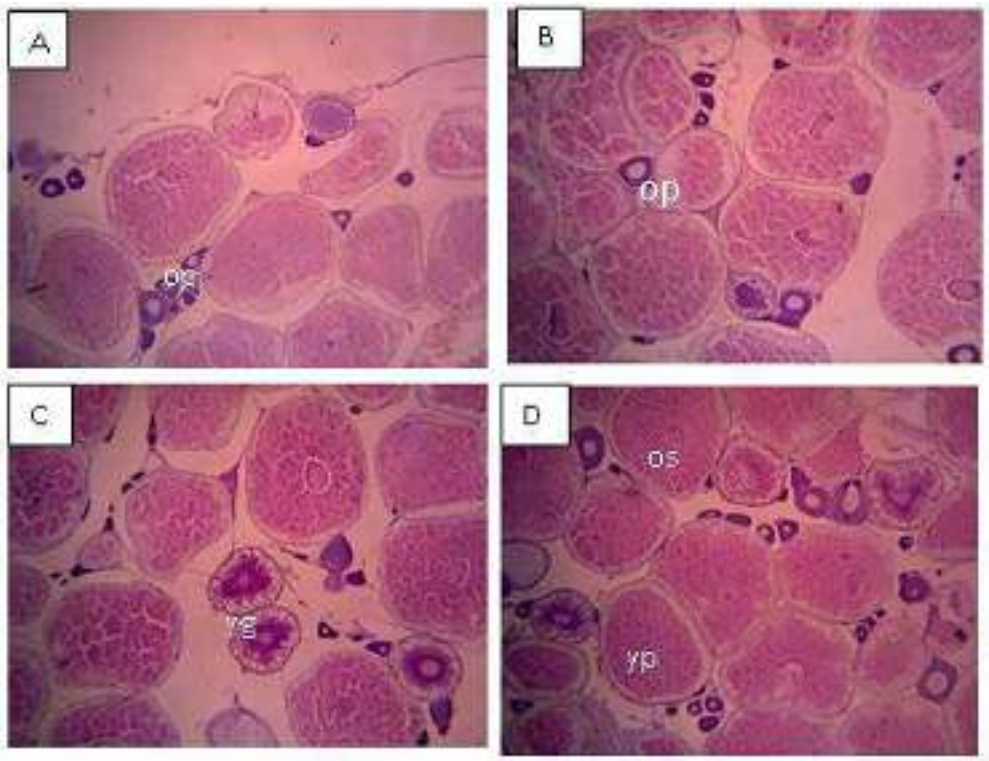

Figure 1. Gonad's maturity of *O. vittatus* at the phase of pre-vitelogenesis and vitelogenesis on 30 days of rearing period; op: primary oocytes; yg:globular yolk, os:secondary oocytes, yp: yolk plate, n: nucleus; A: without AA , B: AA 600 mg/kg fed ration, C: AA 1200 g/kg fed ration, D: AA 1800mg/ kgfed ration.

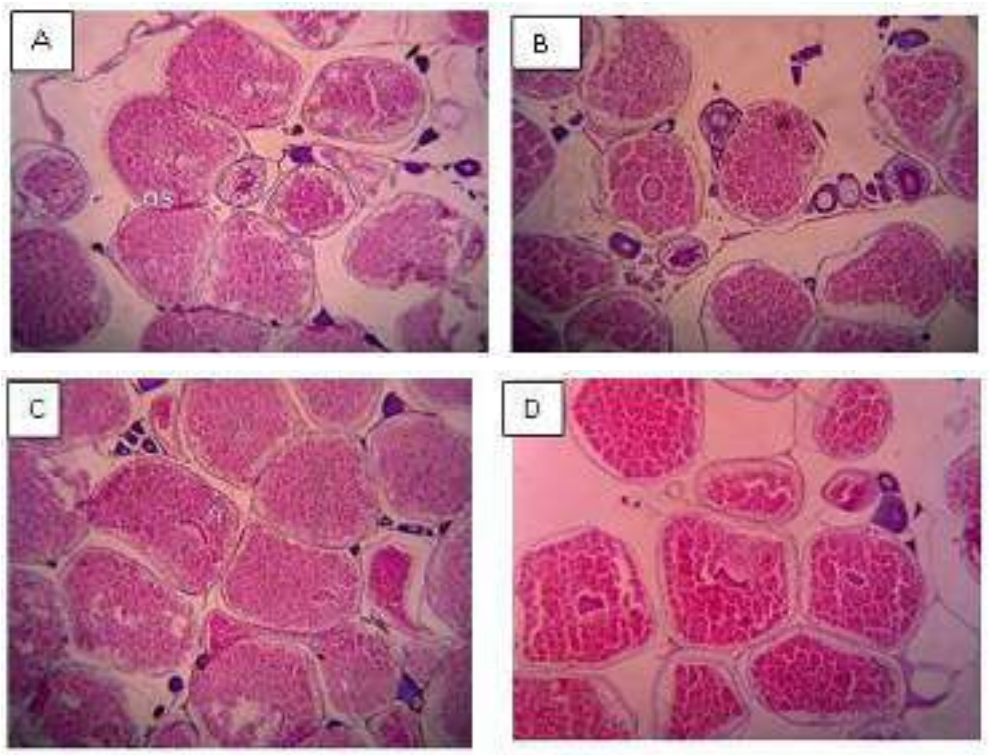

Figure 2. Gonad maturity of *O. vittatus* at vitelogenesis stage performed in 90 rearing-days; op:primary oocytes; os:secondary oocytes, n: nucleus; A: AA absent, B: AA 600 mg/kg fish-fed ration, C: AA 1200 g/kg fish-fed ration, D: AA 1800mg/kg.

rations the presence of secondary oocytes (os) were dominant in numbers though some oocyt primer dan the nucleus of primary oocytes were still obviously present on the periphere. Based on the nucleus position, the ovum were partly ready to enter the stage of gonad maturity i.e.: Germinal Vesicle Break Down (GVBD) but not all ovum were ready to be spawned, though the ovum performance seem like to be ready for it.

Gonad maturity at 90 rearing-days was dominated by the presence of secondary oocytes, especially that of AA supplemented at 1200 mg/kg fish-fed ration and so 1800 mg/kg. The *O. vittatus* ready to enter gonad maturity of Germinal Vesicles Break Down (GVBD) or level IV where nucleus migrated to the periphere and so called as germinal vesicle, from the oocyte periphere to the microphyle (Santos-silva et al., 2015) leads to the position of being ready for spawning. On the other hand, the absence of AA or when AA present at the dosage of 600 mg/kg fish-fed ration, the data showed if the primary oocytes were still present though some ova have already started to enter gonad maturity Level IV. During the development of gonad of the *O. vittatus* there was no atresia found means that all ovum were well develop due to the treatments (supplementation of AA on the fish-ged rations) given to the adult female fish. Sivakumaran reported gonad maturity of carp fish in Victoria, he stated that some of the research's objects had atresia on their ovum caused the ovum failed to develop since there was not enough nutrient available in the nature (Sivakumaran et al., 2003). Another research paper said that in the Lamprey fish, the female gonad contain more ascorbic acid (130-133,8 μg/g) than male ones (79,3-104,1 μg/g), the higher concentration of AA the better quality of eggs would be produced (Moreau & Dabrowski, 1998) it is still unclear from the evolutionary perspective when the ability to synthesize the vitamin first appeared in the animal kingdom and how frequently the trait has been lost. We report here ascorbic acid biosynthesis ability in sea lamprey (Petromyzon marinus). Based on its processes of oocytes development, the *O. vittatus* can be group to the partial spawner fish, i.e.: a type of fish which does not release all eggs simulataneously at once, but only some eggs which related with the oocyt diameter size (Syandri & Azrita, 2015); (Kanta et al., 2009). Current data also noted if the oocyt size was still varied means if these ova were still grown and developed (Hibiya, 1982).

Oocyt diameters

The average size of oocyt diameter at 90 rearing days varied from 0.857 mm when AA was supplemented at 600 mg/kg of the fish-fed ration to the largest of 0.966 mm when AA was supplemented at 1800 mg/kg fish fed ration. These data, however, were not significantly different ($p > 0,05$) (Table 1).

Figure 3 shows the oocyt diameter of each treatment where the diameter increased in every two weeks observation during the rearing period of 90 days. The smallest size of 1.011 mm oocyt diameter was reached when the fish fed did not added with contrastingly when AA was supplemented at 1,800 mg/kg fish fed and reared for 90 days the ovum reached to the size of 1.125 mm.

The oocyt diameter of 90 rearing days of the *O. vittatus* supplemented by AA showed a positive effect where most of the ovum were develop thoroughly in different sizes, along the ovum development this situation led to cause different size of egg diameter. The variation in the ovum diameter was strongly related to spawning process since the *O. vittatus* was a partial spawner and so they do not release all eggs they produce but depends on the oocyt diameter (Sivakumaran et al., 2003). The larger the oocyt the more chance of them to be fertilized and oocyt to be larvae. *O. vittatus* was characterized by variable size of oocyt diameter. Based on this variation in egg sizes, the length period of spawning can be predicted, when all ovum in the ovarium are similar in diameter sizes, the spawning process take shorter time than when the diameter size is the variable. In contrast, when the *O. vittatus* fish spawn in a long time period or even spawn continously, it

Table 1. Relationship between AA supplementation and Oocyt diameter (mm) of *O.vittatus*

Treatments	Observations at 2 week interval						Average
	I	II	III	IV	V	VI	
A	0.700	0.847	0.878	0.925	1.004	1.011	0.894[a]
B	0.613	0.721	0.878	0.899	0.953	1.078	0.857[a]
C	0.848	0.923	0.948	0.944	0.950	1.077	0.948[a]
D	0.824	0.874	0.973	0.988	1.014	1.125	0.966[a]

Values are Means±SD (n = 3) within columns values with different superscripts are significantly different ($p<0.05$).

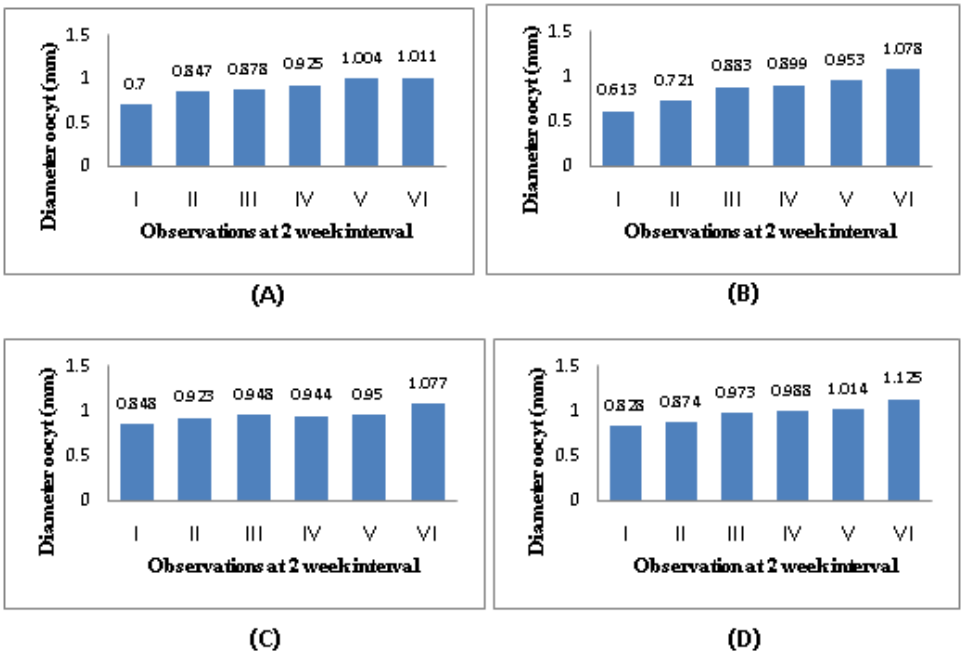

Figure 3. Oocyt Diameter of *O. vittatus* at the supplementation of AA

represents if the eggs in the ovarium are in variable sizes (Sharifuddin & Omar, 2010)(Abedi et al., 2011). The development process of ovum in the ovarium, is strongly related with length and weight of the fish-body, types and age of the fish. The oocyt diameter of the *Toxotes chatareus* and *Toxotes jaculatri* fish for examples, are vary which related with their fecundity and gonadosomatic index (Simon et al., 2012) spawning season, sex ratio, and fecundity. Spawning season was assessed using monthly changes in gonadosomatic index (GSI).

Larvae survival rate

Following to the artificial spawning, the viability of *O. vittatus* larvae observed at 10 rearing-days and fed with tubifex worm were varied where supplementation of AA at the fish-fed ration of 1,200 mg/kg to the adult female showed the highest larvae viability i.e.: 89.25% but only 20% fish larvae were survived when AA did not supplemented on the fed-ration of the adult female's fish. These data were statistically showed a significant different (p< 0,05) (Figure 4 and Table 2).

Remarks = A: AA is absent in the fish-fed ration; B: Fish-fed ration is supplemented with 600mg/kg AA; C: Fish-fed ration is supplemented with 600mg/kg AA; D: Fish-fed ration is supplemented with 600mg/kg AA

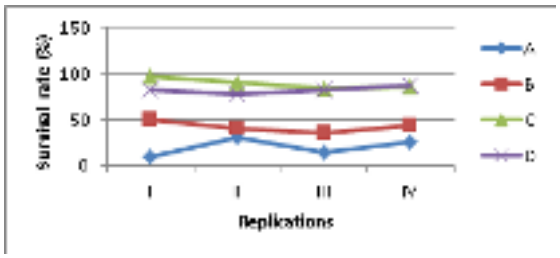

Figure 4. Survival rate (%) of *O.vittatus* after treated with synthetic spawning

Table 2. Survival rate (%) of *O.vittatus* after synthetic spawn treated with synthetic spawning

Treatments	Replications				Average
	I	II	III	IV	
A	10	30	15	25	20^{a}
B	50	40	35	43	42^{b}
C	97	90	84	86	89.25^{cd}
D	82	78	83	88	82.75^{dc}

Values are Means±SD (n = 3) within columns values with different superscripts are significantly different (p<0.05).

Apart from that, the larvae were able to utilize the tubifex worms, to run their own metabolism activities and grow further. Syandri (2013) stated if the *O. vittatus* larvae fed by the tubifex worms resulted in a better growth as well as their

viability, becuase the tubifex worms contain a complete nutritions like water (mg/100 mg fresh weight) 87,19 ± 0,83, rough protein 57 ± 0,58, lipid 13,3 ± 0,06, ash 3,60 ± 0,16 and amino acids 7,18 mg/100 mg dry weight namely: lysine, leusin, arginin, valin, treonin, fenilalanin, isoleusin, tirosin, histidin, methionin, and well digested (Syandri & Azrita, 2015). Muchlisin et al., (2016) has note that survival rate of *Tor Tambra* larvae was increased by papain enzyme supplementation of 27.50 mg / kg and protein 30% because the enzymes able to optimaize protein digestion.

Our result proved that ascorbic acid supplementation accelerated gonad maturity which might leads to increase spawning frequency of the *O vittatus* become more than twice per annuum and result in the increase of larvae production. This will provide high benefit for fish famers.

CONCLUSIONS

Supplementation of AA at the dosage of 1200 mg/kg fish-fed ration could accelerate gonads maturity, development of oocyt diameter, as well as the viability of *O. vittatus* larvae at 90 rearing days. Rematuration was also run in a faster period than other treatment groups.

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REFERENCES

Abedi, M., Shiva, A. H., Mohammadi, H., & Malekpour, R. (2011). Reproductive biology and age determination of Garra rufa Heckel, 1843 (Actinopterygii: Cyprinidae) in central Iran. *Turkish Journal of Zoology*, *35*(3), 317–323.

Canyurt, M. A., & Akhan, S. (2008). Effect of Ascorbic Acid Supplementation on Sperm Quality of Rainbow Trout (Onchorynchus mykiss). *Turkish Journal of Fisheries and Aquatic Science*, *8*(November 2003), 171–175.

Dopeikar, H., Keivany, Y., & Shadkhast, M. (2015). Reproductive biology and gonad histology of the Kura Barbel, Barbus lacerta (cyprinidae), in bibi-sayyedan river, tigris basin. *North-Western Journal of Zoology*, *11*(1), 163–170.

Effendie, M. (1979). *Metode Biologi Perikanan* (II). Bogor: Yayasan Dewi Sri.

Gammanpila, M., Yakupitiyage, A., & Bart, A. N. (2010). Evaluation of the effects of dietary vitamin C, E and Zinc supplementation on reproductive performance of Nile tilapia (Oreochromis niloticus). *Sri Lanka Journal of Aquatic Sciences*, 12, 39–60.

Hengky, S. (2010). Kandungan Vitamin C pada Ovarium Ikan Lele (Clarias gariepinus) Saat Siklus Reproduki. *Jurnal Perikanan dan Kelautan*, *6*(3), 120–124.

Hibiya, T. (1982). *An Atlas of Fish Histological Features*. Tokyyo: Kodansha Ltd.

Ibrahem, M. D., Fathi, M., Mesalhy, S., & Abd El-Aty, A. M. (2010). Effect of dietary supplementation of inulin and vitamin C on the growth, hematology, innate immunity, and resistance of Nile tilapia (Oreochromis niloticus). *Fish and Shellfish Immunology*, *29*(2), 241–246.

Kottelat, M., & Whitten, T. (1996). *Freshwater fishes of Western Indonesia and Sulawesi: additions and corrections*. Hong Kong: Periplus Editions.

Mannan, M. M., Maridass, M., & Thangarani, S. (2010). Gonad developmental cycle of Puntius filamentosus. *International Journal of Biotechnology*, 1(2), 69-77.

Martins, T. P. A., Gomides, P. F. V., & Navarro, R. D. (2016). Vitamin C supplementation on growth performance and gonadal development in Nile tilapia. *Acta Scientiarum. Technology*, 38(4), 477.

Moreau, R., & Dabrowski, K. (1998). Body pool and synthesis of ascorbic acid in adult sea lamprey (Petromyzon marinus): an agnathan fish with gulonolactone oxidase activity. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(17), 10279–10282.

Muchlisin, Z. A., Afrido, F., Murda, T., Fadli, N., Muhammadar, A. A., Jalil, Z., Yulvizar, C. (2016). The Effectiveness of Experimental Diet with Varying Levels of Papain on The Growth Performance, Survival Rate and Feed Utilization of Keureling Fish (Tor tambra). *Biosaintifika: Journal of Biology & Biology Education*, *8*(2), 172–177.

Ritonga, L., Soeprijanto, A., & Kilawati, Y. (2016). The effectiveness of gonad extract of Yellowfin tuna fish (Thunnus albacares) on increase of reproduction factor of nilem carp (O. hasselti). *International Journal of ChemTech Research*, *9*(7), 435–442.

Saha, B. K., Islam, M. R., Saha, A., & Hossain, M. A. (2009). Reproductive Biology of the Mola Carplet Amblypharyngodon mola (Hamilton) (Cypriniformes: Cyprinidae) from Netrakona Water. *Bangladesh Journal of Scientific and Industrial Research*, 44(3), 377-379.

Sandnes, K. (1991). Vitamin C in fish nutrition – a review. *Fiskeridir Skrift Ser Ernær*, *4*(1), 3–32.

dos Santos-Silva, A. P., de Siqueira-Silva, D. H., Ninhaus-Silveira, A., & Veríssimo-Silveira, R. (2016). Oogenesis in Laetacara araguaiae (Ottoni and Costa, 2009)(Labriformes: Cichlidae). *Zygote*, 24(4), 502-510.

Setyaningrum, N & Nuryanto, A. (2006). Penjinakan

dan Budidaya Ikan Brek (Puntius orphoides) Sebagai Upaya Menuju Diversifikai Usaha Tani. *Jurnal Pembangunan Pedesaan,* 6(1), 25-31.

Sharifuddin, & Omar, A. (2010). Aspek reproduksi ikan nilem , Osteochilus vittatus (Valenciennes, 1842) di Danau Sidenreng, Sulawesi Selatan Jurnal Iktiologi Indonesia. *Jurnal Iktiologi Indonesia*, *10*(2), 111–122.

Simon, K. D., Bakar, Y., Mazlan, A. G., Zaidi, C. C., Samat, A., Arshad, A., … Brown-Peterson, N. J. (2012). Aspects of the reproductive biology of two archer fishes Toxotes chatareus, (Hamilton 1822) and Toxotes jaculatrix (Pallas 1767). *Environmental Biology of Fishes*, *93*(4), 491–503.

Sivakumaran, K. P., Brown, P., Stoessel, D., & Giles, A. (2003). Maturation and reproductive biology of female wild carp, Cyprinus carpio, in Victoria, Australia. *Environmental Biology of Fishes*, *68*(3), 321–332.

Subagja, J., Gustiano, R., & Winarlin, L. (2006). Pelestarian Ikan Nilem (Osteochilus hasselti C.V) Melalui Teknologi Pembenihannya. *Lokakarya Nasional Pengelolaan Dan Perlindungan Sumber Daya Genetik Di Indonesia: Manfaat Ekonomi Untuk Mewujudkan Ketahanan Nasional*, 279–286.

Syandri, H., & Azrita, J. (2015). Fecundity of Bonylip barb (Osteochilus vittatus Cyprinidae) in different waters habitats. *International Journal of Fisheries and Aquatic Studies*, 2(4), 157-163.

Syandri, H. (2013). Fecundity, Egg Diameter and Food Channa lucius Cuvier in Different Waters Habitats. *Journal of Fiheries and Aquaculture*, *4*(3), 115–120.

Valdebenito, I. I., Gallegos, P. C., & Effer, B. R. (2015). Gamete quality in fish: evaluation parameters and determining factors. *Zygote*, *23*(2), 177–197.

Yulghi, S. (2013). Relationship between Biological Egg Characteristics and Female Brood Fish in Stellate Sturgeon, Acipenser stellatus Pallas, 1771 (Pisces: Acipenseridae) in the Southeast Caspian Sea, Iran. *Caspian Journal of Environmental Sciences*, 11(2), 195-203.

Yones, A. M. M., Metwalli, A. A., & Al-Jilany, S. S. . (2016). Effect of Artificial Diets on Growth Performance, Body Compostion and Gonad Maturation of Mullet (Liza ramada). *International Journal of Fiseries and Aquaculture Research*, *2*(2), 28–49.

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The Relationship of Body Length and Ratio Pappilla with Sex in Gobi Fish (*Sicyopterus macrostetholepis*)

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papilla; *S. macrostetholepis* Blkr.; sex, body length

Abstract

Research about the relationship of body length and ratio papilla with sex in goby fish (*S. macrostetholepis* Blkr.) has been done at Animal Structure and Developmental Laboratory, Biologi Department, Faculty of Matematics and Natural Sciences, Andalas University, Padang, which purposed to analyse the relationship of body length and ratio papilla with sex of goby fish (*S. macrostetholepis* Blkr.). The samples were taken in wild stream area at Batangkuranji river, Padang City. This research used descriptive method and data were analyzed by qualitatively and quantitatively. The results of investigation showed that in several goby fishes (*S. macrostetholepis* Blkr.) with different sex had the same of body length and the same of ratio papilla. So, there was not relationship between of body length and ratio papilla with sex. Goby fishes (*S. macrostetholepis* Blkr.) it belongs to the hermaphrodite protogini, which the androgynous young females, while in adulthood, it would change sex to male. The results of this study are expected to add to the treasures of knowledge and information about reproductive gobies (*S. macrostetholepis* Blkr.) in the preservation and development of fish farming.

How to Cite

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INTRODUCTION

Fish are members of cold-blooded vertebrates (*poikilotermik*) which live in the water, moving with fins, the body has scales and breathing with gills. The fish has a number of species diversities with more than 27.000 in the world. The fish can be found in all of he puddles like in freshwater, brackish water or salt water at variation depth. The fish also has a high economic value either as a food ingredient or as a pet. In Indonesia, most of the utilization of fisheries is still rooted in fishing from nature. The arrests were carried out continuously causes decreasing of fish population.

Decreasing of fish population caused by fishing activities which are not controlled, it can lead to overfishing and the destruction of habitat, thus It will effect the environment and the existence of biological activity in these waters, which could threaten the sustainability of the source itself. It is estimated that the rare fish in Indonesia is increasing, with the increasing of illegal fishing activities and exploitation being offset by conservation activities. Therefore, to maintain fish populations still in high population and sustainable, it needs cultivation.In cultivation, Biology aspect of fish reproduction is very important. At least, it consist thre types of fish reproduction. One of them is hermaphrodite. According to (Effendi, 2002); (Victor, 2010) Elacatinus rubrigenis, is described from Utila in the Bay Islands of the Gulf of Hon- duras (Western Atlantic a hermaphrodite is an interesting phenomenon in fish reproduction. An individual is said to be a hermaphrodite fish when the body is having ovarian tissue as a determinant of individual females and testicular tissue as a determinant of individual males. One group of fish that includes hermaphroditic fish is from the family Gobiidae.

Gobies are the largest tribe widespread fish in the waters of temperate and tropical region (Zamroni, 2016); (Larson et al., 2016). In addition, these fish are also a lot of mangrove forests in encounter. (Fahrian et al., 2015) states that found 6 glodok fish in magroveat Mororejo village, Kendal Regency. These fishes can be found in the waters of salty, brackish and freshwater, as well as public waters such as lakes and rivers. According (Devi, 2012), (Larson et al., 2016) a special feature of this fish has pelvic fins together and a from of suction disc which allows them to remain in position in the fast-flowing waters. Small, but thick with thinning tail shape, sirip- thick fins by having two dorsal fins. According (Victor et al., 2010); (Victor, 2010) Elacatinus rubrigenis, is described from Utila in the Bay Islands of the Gulf of Hon- duras (Western Atlantic, some kind of family Gobiidae, it belongs to the hermaphrodite protogini, which the androgynous young females, while in adulthood, it would change sex to male.

To distinguish males and females can be seen from the primary and secondary sexual characteristics. Primary sexual characteristics of fish is characterized by the organ that is directly related to the reproduction process that ovarian veins and vessels for females and testes with veins and vessels for the male fish. Secondary sexual characteristics, they can be seen on signs of the primary sexual characteristics, such as shape, color or other organs (Peristiwaldy, 2006). By Ghufron (2005), based on the length of the fish's body supposedly can be determined by gender differences in fish. To distinguish the sex of male and female fish, it can be done by observing the physical form of the body fish with body length sightings. If the physical characteristics can not be done, the gender distinction can be made by observing the fish genital organs (papilla).

Research on the papilla observations have been made by Burhanuddin & Genisa (1984), the gobies *Periophthalmodonschlosseri* and *Boleophthalmusboddarti* live in the waters of the estuary of the Banyuasin (Palembang). The male fish, its papilla is in long form, while the female fish, its papilla is spherical shape. But the gobies *S. Macrostetholepis* Blkr that live in the waters of Batangkuranji river, Padang City, particularly in females who undergo sex change, until now there is no information about it. Therefore, research on the relationship of the body length and the papilla with sex ratio needs to be done in order to add information about the reproductive biology of gobies, as well as assist in developing the breeding gobies programs.

From the description above, it can be formulated that problems which need to be answered from this study. It is how long the relationship of the body and papilla ratio gobies (*S. macrostetholepis* Blkr.). The purpose of this study was to determine the relationship of the body length and the papilla ratio gobies (*S. macrostetholepis* Blkr.) with sex. The results of this study are expected to add to the treasures of knowledge and information about reproductive gobies (*S. macrostetholepis* Blkr.) in the preservation and development of fish farming.

METHODS

This research was descriptive method. Body length measurements and observations of the ratio (length and width) papilla are associated with gonads histological structure examination, as obtained in sampling. The tool is used in this study the stereo zoom binocular with motic image plus program, and the light microscope with a magnification of 40X100. A set of surgical tools, petridishes, tissue, paper labels, bottles films, ring case for embedding, lights spritus, Microtome American Optical with a thickness of 8 μm, the dyebath for 16 units, glass objects, glass covers, the incubator temperature to 50^0 C, Caliper varnier with a precision of 0.01 mm. Fishing gear centrums (Electrical Snatcher) and stationery. The materials used papilla and gonad gobies. Physiological solution, bouin, alcohol series 70-100%, paraffin hard, aquadest, xilol, hematoxylin, eosin, poly l-lysine, and entelan.

Sampling was carried out while gobies in their reproductive years. The samples were obtained by 196 tails using fishing gear centrum (Electric Snatcher) on the fast-flowing areas in the waters of Batangkuranji river, Padang City. Geographically located at 0^0 48' – 0^0 56' and 100^0 21' LS-100^0 33' BT, with a long stream of approximately 17 km with area 22.149.32 ha (Bapedalda Padang City, 2004). The measured parameters such as length and width papilla which starts from the edge of the anus until the hole papilla (Rodgers, 2005). Measurements papilla used Stereo zoom binoculars. Stereo zoom binoculars are equipped with a camera and connected directly to the computer so that the image can be directly extracted and measured. The program used software Motic Image Plus.

Preparation of histological gonadin order to know the sex of gobies. Gonad gobies that have taken the data from it morfometrik, then it is isolated for histology preparations which made semi-sheer follow Paraffin method. The gonads were washed with physiological solution, fixed with fixative solution (Bouin) for 24 hours, then dehydrated in alcohol series 70-100% for 1 hour, purification with xilol for 1 hour, infiltrated with paraffin and planted on the beam cutter. Then, it sliced crosswise using a microtome (American Optical) with pasting on the slide, and continued thickness of approximately 8 μm with the coloring process haematoksilin eosin staining and deparafinisasi with xilol.

Mixture is examined under a light microscope with a magnification of 4x100 to see its histology structure. From observation, the sex cells are found, the epitelial layer, connective tissue, gonad developmental stages to find out the sex. Furthermore, the fish are grouped by gender. Subsequent histological is preparations which represents photographed.

To determine the relationship between body length and ratio papilla *S. macrostetholepis* Blkr., it is analyzed using simple linear regression, with the formula Sudjana (2016) as follows:
Simple Linear Regressi on formula: $Y = a \pm bX$

RESULTS AND DISCUSSION

Characteristics of *S. macrostetholepis* Blkr.

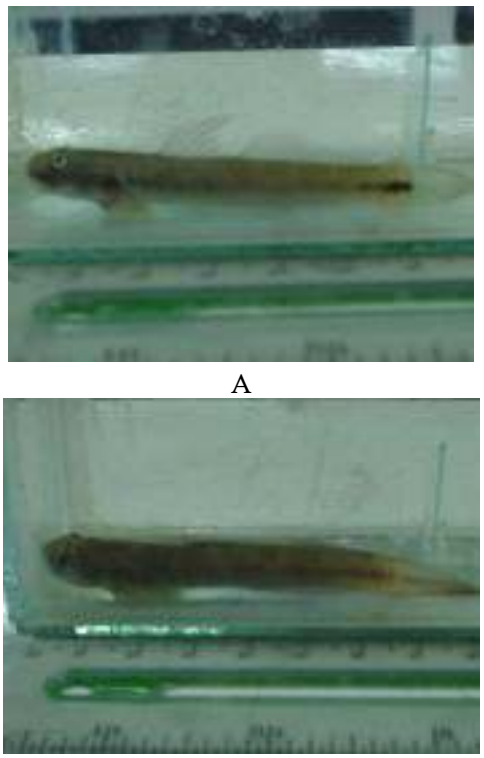
Number of *S. Macrostetholepis* Blkr. caught from Batangkuranji river, Padang that are used for sample are 196 tails. Having observed their morphological characteristics, theycould be divided into males and females since *S. Macrostetholepis* Blkr. is dimorphism. According to (Victor et al., 2010); (Victor, 2010) Elacatinus rubrigenis, is described from Utila in the Bay Islands of the Gulf of Hon- duras (Western Atlantic; (Rodgers, 2005), sexual dimorphism is a morphological characteristic that can be used to distinguish males and females.

Characteristics of morphological *S. macrostetholepis* Blkr. female individually are fat and slow. It makes slow in swimming. In the body, there is a black stripe look like ribbons which numbering 4-8 pairs, and a dark brown body color, (Figure 1a). Meanwhile, in the male individual body has color brighter than the females, which is mauve with streamers 6-8 line pairs and caudal fin has orange color which is surrounded by a black line on the rim (Figure 1b). According Agromedia (2002), the male fish brightly colored than the female fish.

Lestrel (2000) states the male is smaller and slimmer than the females which is elliptical. From the results, the male is smaller than the female fish. This body shape is necessary in order to move swiftly, especially while doing the activity. These fishes move in the mud by immersing pectoral fins simultaneously and push it backward when his straight forward and rigid. Elongated dorsal fin has an orange thorn, when breathing the throat of young male fish has an orange color (Peristiwaldy, 2006).

From observations that have been made, the shape of the papilla of the female is generally elongated with a small base section towards the widened end and rounded as split in half like the fans (Figure 2a), while in males, it elongated widened towards the end and a small rounded (Figure 2b). Burhanuddin & Genisa (1984), stating

that the shape of male fish papilla are elongated and rounded end portions like an inverted triangle, while the female papilla elongated shape with two split ends. According to (Victor et al., 2010); (Victor, 2010)Elacatinus rubrigenis, is described from Utila in the Bay Islands of the Gulf of Honduras (Western Atlantic, papilla in males fish is as genital and papilla in female fish is as the form of holes which serve as an estuary of urine and sperm or eggs.



A

B

Figure 1. Photo of *S. macraostetholepis*. A = Gobi fish female. B = Gobi fish male

Relationship with Body Length Ratio papillae

The measurement results refers to the observations of the characteristics *S. macrostetholepis* Blkr. by observing the characteristics of fish, it is obtained 131 female and 65 male fish tail. It is Based on measurements of body length and papilla ratio of female fish and male fish that live in the waters of Batangkuranji river, Padang City, it can be seen in Table 1.

Based on Table 1, it can be seen, the average body length of the female 59.18 ± 9.06 mm, whereas in males 56.93 ± 9.01 mm. Papilla ratio of females has an average of 1.83 ± 0.36 and 0.81 ± 0.15 for male fish. From the above data, female body length has greater than the length of the male body. This condition also occurs in papilla ratio, the ratio of female papilla is larger than the males. Hutomo & Naamin (1982), states that in gobies (*Periophthalmuskoelreuteri*) males are smaller than females.To see the connection and the length of fish body papilla ratio, it is done by using simple linear regression. The test results can be seen in Figures 3 and 4.

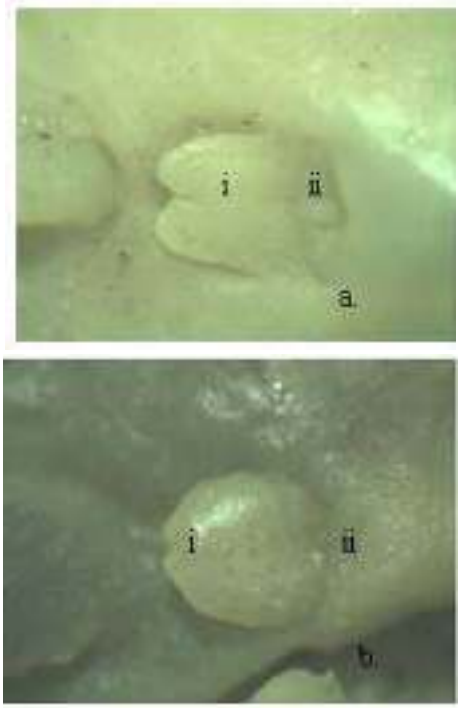


Figure 2. Photo of papilla *S. Macraostetholepis* Blkr. a = female papilla. b = male papilla. i = papilla, ii = anal find.

Table 1. The Average body length and ratio papilla *S. macrostetholepis* Blkr.

Measurement	Body Size Length (mm)		Ratio papillae	
	Females	Males	Females	Males
Average	59.18	56.93	1.83	0.81
Stdv	±9.06	±9.01	±0.36	±0.15

The relationship between body length and the ratio of the papilla on *S. macrostetholepis* Blkr. can be shown in the equation Y = 2.0643 - 0.0039X with r = 0.098 and it is readable, each increasing in body length 2.0643 units, the papilla ratio decreased 0.0039 units for female fish. In the male fish, body length each increased 0.5388 units, the papilla ratio increased 0.0048 units, the equation Y = 0.5388 + 0,0048X and r = 0.248. Results showed that there was no effect on the body length ratio of the papilla, which increase the body length in females and males. It did not occur accretion papilla ratio.Although in some

the length of the body male fish,it also showed the increase of the ratio of the papilla. Genital papilla fish is a bulgeerectile act as a conduit of sperm or eggs (Burhanuddin & Genissa, 1984). Then from the observations that have been made, there are some fish of different sexes having a body length range and papillae same ratio. Gender on the fish has nothing to do with the length of the body and the ratio of the papilla.

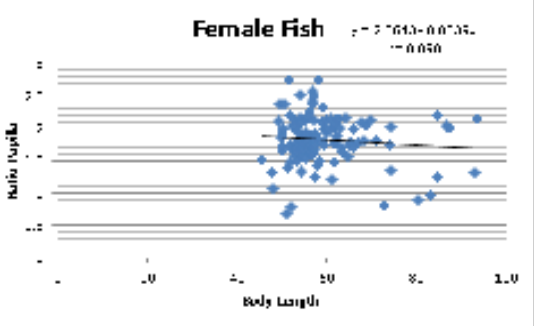


Figure 3. Regression graph of body length and Papilla ratio female fish

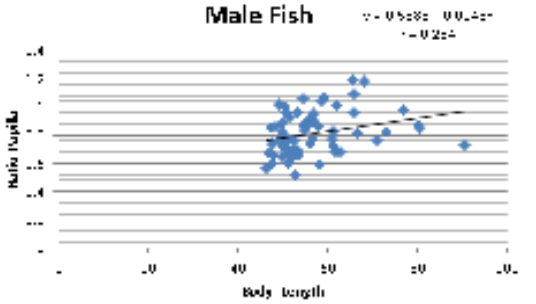


Figure 4. Regression Graph of body length and papilla ratio male fish.

The length of the body in fish is influenced by various factors, physical factors (temperature), chemical factors (dissolved oxygen and acidity of water) and biological factors (amount and type of food) (Kusmawati, 2009). Ghufron, (2005) states that in nature of he existencegenetic variation in the growth potentialfish for the same type on different populations, they are usually influenced by environmental factors such as temperature and food availability.

The Analysis of Histology gonads *S. macrostetholepis* Blkr.

To strengthen the data about gender differences based on the length of the body and the ratio of the papilla on *S. macrostetholepis* Blkr. Then observation of histological structureof fish gonad are needed to be done. The measurement data were then grouped based on histologic observations of the gonads. The histologic observations showed that there was different stages of gonad development in females, adult females and intersex females of 131 females consisting of adult females of 83 and 48 females intersex.

Adult females

In observation of histological preparations gonads, mature female fish showed that it almost has the same structure in all samples that observed, where the tunica albugenia is thin which built by serous layer. In the medulla area, it looks a lamella-lamella. They are drawn up by the oocyte. Oocytes dominated tertiary relatively equal, while the primary oocytes are not so many, and interstitial tissue is narrow. This is in accordance with the opinion of Suherman (2001) that the gonads are in a stage of development characterized large-sized oocytes. The increasing size of the oocyte cause interstitial tissue narrowing.

Agency yolk (vitellogen) already meets some of the oocyte. It is found some irregular nucleus. It indicates the condition of the oocyte in a state of degeneration (atresia). According to Suherman (2001) that the formation of the yolk (vittellogenesis) oocytes can degenerate.

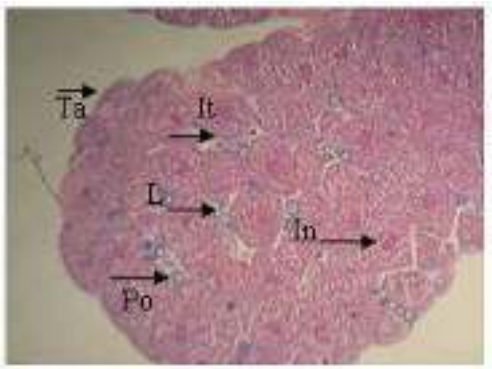


Figure 5. Histology gonad *S. macrostetholepis* Blkr. adult females. Magnification of 10 x 100. Ta = Tunica albugenia, It = intertitial tissue, L = lumen, Op = primary oocytes, In = irregular nucleus.

Female Intersex

Histological observations of gonad female fish stocks intersex are showed at different stages of development, but they show further structures. It is marked by the presence of sperm and the seminiferous tubules. It looks interstitial tissue thickening and lumen clearly visible. In the cortex, the tunica albugenia is visible thickening and fibrous connective tissue with collagen fibers are arranged very dense, which continue to enter between the oocyte. As a result, there is formation of cavities between the lamella and ovaries and oo-

cytes. Some oocytes are degenerated. Suherman (2001) states that the degeneration of oocytes began in the core area, in the central part of the core are masterial (unknown). It is enabled splitting into two which become four, four become eight seemed to occupy all parts of the oocyte.

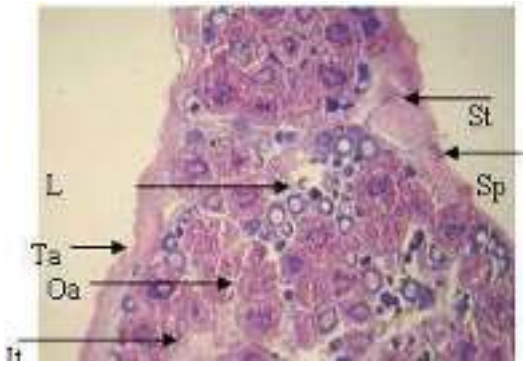


Figure 6. Histology gonad *S. macrostetholepis* Blkr. female intersex. Magnification of 4 x 100. Ta = Tunica albugenia, Tbs = Seminiferous tubules, Sp = Spermatocyte, L = lumen, Oa = Degenerate of oocyte, It = intertitial tissue.

Male

At this stage of gonad, the development of male fishare difficult to observe because the network is very small (Figure 7). According Fujaya (2004), at the rate of testicular development is dominated by primary spermatocytes. In preparation histology male gonads, it can be seen tubular semineferus which surrounding the sperm. It has been in the lumen. While tubular semineferus, there are spermatids at different stages. Spermatids in inside the tubules semineferus will undergo metamorphosis (without undergoing the cell division). It develops into spermatozoa that are functional.

Relations Body Length and Ratio papillae On Adult Females Fish and Fish female intersex.

Histological observation results showed the differences in the female gonad and developmental stages. It is namely adult females and female intersex. Based on observations of the length, the body and the ratio of the papilla are on the second phase of the data obtained which shown in.

From the table above, it can be seen that the average adult female body length is greater than the females intersex where the average adult female body length is 9.21 ± 59.83 mm, while females intersex 58.06 ± 8.79 mm. Something similar, it happened on the ratio of the papilla, where the average adult female ratio is 1.85 ± 0.37 and 0.34 ± 1.79 female intersex.

To see the connection body length of the fish and the fish papilla ratio of adult females and female intersex, it do simple linear regression. The test results in Figure 8 and 9.

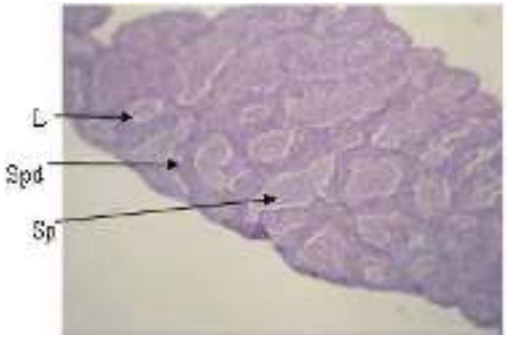


Figure 7. Histology gonad *S. macrostetholepis* Blkr. Male. Magnification of 4 x 100. Spd = spermatid, L = lumen, Sp = Sperm

Table 2. The Average length of the body and the ratio of fish papilla adult females and female intersex.

Measurement	Body Size Length (mm)		Ratio papillae	
	Adult female	Female intersex	Adult Female	Female Intersex
Average	59.83	58.06	1.85	1.79
Stdv	±9.21	±8.79	±0.37	±0.34

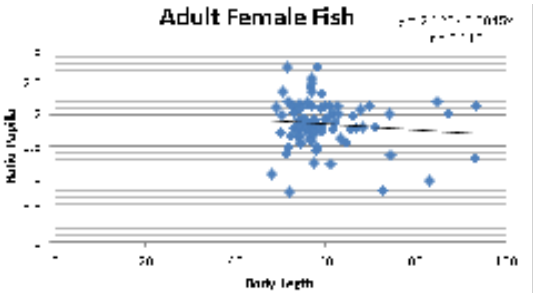


Figure 8. Graph of body legth regression and Papilla ratio adult female fish.

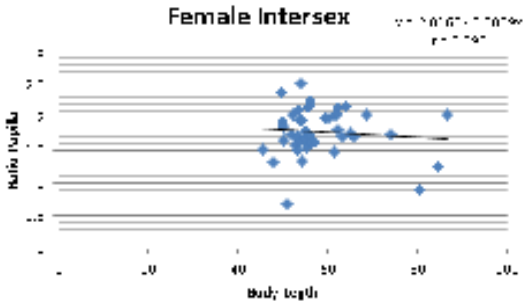


Figure 9. The visible size of the papilla ratio remains despite a growing body length.

Every increasing in body length by 2.1254 units, the papilla ratio decreased by 0.0045, where the regression equation Y = 2.1254 - 0.0045X and r = 0.110. As for the female fish intersex, the regression equation is Y = 2.0165 - 0.0039X and r = 0.098. From these results,it illustrates that the length of the body did not affect the ratio of the papilla, which increase the body length in adult female and female fish intersex. It did not happen accretion papilla ratio. A sex change in fish had nothing to do with the length of the body and the ratio of the papilla.

A sex change on *S. macrostetholepis* Blkr. does not occur in a particular body length, as well as the ratio papilla. A sex change from female to male in *S. Macrostetholepis* Blkr., it believed that occur spontaneously. It is influenced by various factors. One of the factors that can cause changes gender in a social factor. According to Rodgers (2005), the social group of fish in the wild, a male came to power peak charge a minimum of seven fish females. Protogini hermaphrodite fish is in the colony, as males leave the colony then the tail of the female fish will turn out to be male and to the top.

Gobi fish is one type of the hermaphrodite protogyny fish. Hermaphrodite protandre is a group of hermaphrodite fish, which in one life cycle, there is a process of gonad differentiation from male to female phase. (Victor, 2010) Elacatinus rubrigenis, is described from Utila in the Bay Islands of the Gulf of Hon- duras (Western Atlantic, priyono, has explained that based on the development of ovarian tissue and testes present in an individual will determine the kinds of hermaphroditism, synchronous, protogyny and protandre. Hermaphrodite synchronous is a group of fish which in one life cycle consisting female sex cells and male sex cells that can fuse together. (Ratna & Abdulgani, 2012) hermaphrodite protogyny is a group of hermaphrodite fish which in one life cycle posses a process of gonad differentiation from the female phase to the male. While Hermaphrodite protandre is a group of hermaphrodite fish that in one life cycle posses a process of gonad differentiation from male to female phase.

Hermaphrodite protandre and hermaphrodite protogynyare often called as consecutive hermaphrodite. It is when young fish have gonads that are organized into two kinds of sex, where there are tissue testes and ovaries in one of them that has not been developed well (Effendi, 2002). According to (Victor et al., 2010), in succession or other terms known as "Sex Reversal"changes thefunction of female individuals into thefunction of males (protoginus) or vice versa.This is closely related to the work of hormones in addition of influenced by the environment.

The investigation into the gonads of S. macrostetholepis Blkr. can be used as targets to determine the actual sex and the succession process ovaries into testes or vice versa. In addition, this research can be the additional information onbiological reproduction of gobies (S. macrostetholepis Blkr.) in the way of preservation and development of fish farming. Since, Gobi fish not only has high economic value, but also has a good taste to be consumed (Sulistino, 2012); (Willis, 2012).

CONCLUSIONS

From the research has been done on the relationship of the body length and the papilla with sex ratios at gobies (*S. macrostetholepis Blkr.*). It lives in the waters of Sungai Batangkuranji, Padang City, it was concluded that, The length of the body does not affect the ratio of the papilla, both females (adult females and female intersex) and male fish.The body length gobies (*S. macrostetholepis Blkr.*). Females has an average 59.18 ± 9.06 mm, and the average ratio of the papilla is 1.83 ± 0.36, while the average length of the body the male fish is 56 , 93 ± 9.01 mm, with an average ratio of 0.81 ± 0.15 papilla.In female fish, it is found in different stages of development, namely adult females and females intersex (the average length of an adult female body are 9:21 ± 59.83 mm, while the average length of the female body intersex is 58.06 ± 8.79 mm. The average adult female papilla ratio is 1.85 ± 0:37 and the average female intersex is 1.79 ± 0.34).

REFERENCES

Agromedia, R. (2002). *Koi si Ikan Panjang Umur.* Jakarta: Agromedia Pustaka.

Bapedalda, P. (2004). *Data Analysis Report*. Research and Testing Water Quality River in Padang.

Burhanuddin & Genisa, A.S. (1984). Forms of Papillae Fish Gelogok as Secondary Sex Characteristics. Jakarta: *Mangrove Ecosystem Seminar II.*

Devi, L. I. (2012). Identifikasi postlarva famili gobiidae dari muara sungai kedurang, bengkulu melalui dna barcode. *Skripsi.* Bogor: Departemen Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Institut Pertanian Bogor

Effendi, M. I. (2002). *Fisheries Biology*. Yogyakarta: Yayasan Pustaka Nusatama

Fahrian, H. H., Putro, S. P., & Muhammad, F. (2015). Potensi Ekowisata di Kawasan Mangrove, Desa Mororejo, Kabupaten Kendal. *Biosainti-*

fika: Journal of Biology & Biology Education, *7*(2), 104–111.

Fujaya, Y. (2004). *Fisiologi Ikan Dasar Pengembangan Teknik Perikanan*. Jakarta: Rineka Cipta.

Ghufron, M. (2005).*Budidaya Ikan Laut di Keramba Jaring Apung*, Jakarta: Rineka Cipta

Hutomo, M. & Naamin, N. (1982). Preliminary Observations on Mudskipper Fish Behavior, *Beleophthalmus Boddarti Pallas*. A Short Note About *Periophthalmus Koelreuteri* (PALLAS). *In Prossing Seminar II.Mangrove Ecosystems*.

Kusmawati, R. (2009). Pola Pertumbuhan Ikan Gogi (Sicyopterus macrostetholepis.Blkr) di Perairan Sungai Batang Kuranji, Kota Padang. *Skripsi.* Padang: Universitas Andalas.

Larson, H. K., Jaafar, Z., & Lim, K. K. P. (2016). An updated checklist of the gobioid fishes of Singapore. *Raffles Bulletin of Zoology*, 7600(34), 744–757.

Lestrel, E. P. (2000). *Merphometrics For The Life Sciences*. Singapore: Word Scientific.

Peristiwaldy, T. (2006). *Ikan-Ikan Laut Ekonomis Penting di Indonesia,Petunjuk Identifikasi*. Jakarta: LIPI Press.

Ratna, P., & Abdulgani, N. (2012). Aspek Reproduksi Ikan Kerapu Macan, *Jurnal Sains dan Seni ITS,* 1(1), E27-E31

Rodgers, E. W., Drane, S., & Grober, M. S. (2005). Sex reversal in pairs of Lythrypnus dalli: behavioral and morphological changes. *The Biological Bulletin*, 208(2), 120-126.

Sulistino. (2012). Reproduksi ikan beloso (Glossogobius giuris) di Perairan Ujung Pangkah, Jawa Timur Reproduction of tank goby (Glossogobius giuris) in Ujung Pangkah Waters, East Java Penelitian dilaksanakan di Perairan Ujung, 11(1), 64-75.

Suherman, E. (2001). Hubungan Panjang Tubuh-Perubahan Struktur Histologis Gonad Pada Belut Sawah (*Monoptresusalbus* Zuiew.). *Skripsi.* Padang: Universitas Andalas.

Sudjana. (2016). *Metoda Statistika*. Bandung: Tarsito.

Victor, B. C. (2010). The Redcheek Paradox: the mismatch between genetic and phenotypic divergence among deeply-divided mtDNA lineages in a coral-reef goby, with the description of two new cryptic species from the Caribbean Sea. *Journal of the Ocean Science Foundation*, 3(1), 1-16.

Victor, B. C., Vásquez-Yeomans, L., Valdez-Moreno, M., Wilk, L., Jones, D. L., Lara, M. R., ... & Shivji, M. (2010). The larval, juvenile, and adult stages of the Caribbean goby, Coryphopterus kuna (Teleostei: Gobiidae): a reef fish with a pelagic larval duration longer than the post-settlement lifespan. *Zootaxa*, 2346, 53-61.

Willis, S. (2012). Materi yang digunakan dalam penelitian ini ada- lah ikan gelodok yang terdapat di daerah pertam-. *AQUASAINS Jurnal Ilmu Perikanan dan Sumberdaya Perairan*.

Zamroni, Y. (2016). Short Communication: Conservation of mangrove gobies in Lesser Sunda Islands, Indonesia. *Biodiversitas, Journal of Biological Diversity*, *17*(2), 553–557.

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Diversity and Distribution of Myrmecophytes in Bengkulu Province

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H. Formicarum; M. Tuberosa; light intensity; plant biodiversity; temperature; random distribution

Abstract

Myrmecophyte is a common medicinal plant used by local people in Indonesia for treating various diseases especially in Papua. Bengkulu province is one of the Myrmecophyte habitats, but there has no report on its identity and distribution. The objectives of this research were to identify the diversity and analyze the Myrmecophytes distribution as well as factor affecting its presence. This study used purposive sampling method by exploring the area where Myrmecophytes commonly found. The Myrmecophyte distribution based on host tree was analyzed using *Morishita index* and the autecological analysis of abiotic factors was performed using *Principal Component Analysis* (PCA) generated from Minitab 16. The results of this research showed that there were two species of Myrmecophytes in Bengkulu province, namely *Hydnophytum formicarum* and *Myrmecodia tuberosa*, as well as two variants of *M. tuberosa* i.e. *M. tuberosa* 'armata' and *M. tuberosa* 'siberutensis'. The distribution of Myrmecophytes based on host tree was mostly randomly scattered in Central Bengkulu regency, Seluma, North Bengkulu, South Bengkulu, and Kaur. Their distributions were affected by light intensity and temperature. The data of this research can be used as basic information for carried out conservation efforts in Bengkulu province. The abundance of Myrmecophytes is also used as a source of additional income for local people in Bengkulu province.

How to Cite

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INTRODUCTION

Traditional treatment plant-based materials have been widely used by Indonesian people. Various studies on certain parts of tribe to examine the utilization of plants such as the Sakai tribe in Duri (Irawan et al., 2013) and Anak Dalam tribe in Jambi (Mairida et al., 2016). Root, tuber, stem, bark, leaves, and fruit are part of plants used for medicine. One of herbs with the potential to be developed is a Myrmecophyte.

Myrmecophyte is an epiphyte plant. It is called Myrmecophyte because the inner part of its tuber is inhabited by ants and serves as their nest. Each Myrmecophyte is inhabited only by one species of ant (Soekmanto et al., 2010). Myrmecophyte is a member of *Rubiaceae* family. Only five genera have tuber i.e. *Anthorrhiza, Myrmecodia, Hydnophytum, Myrmephytum,* and *Squamellaria* (Huxley & Jebb, 1991). The tuber, its caudex, comes from the swollen hypocotyl formed a network of death tissues among the tubers cavities caused by phellogen (tissue cork) that developed between parenchyma cells (Huxley & Jebb, 1991). Many Indonesian people used the tuber for medicine. Myrmecophytes used as medicine belong to *Hydnophytum* and *Myrmecodia* genera. Phytochemical analysis showed that Myrmecophyte tubers contain of flavonoids and tannins which have the properties of antiproliferative (Senawong et al., 2013), anticancer (Abdullah et al., 2010), antimicrobe (Ahmad et al., 2010), antioxidant (Dirgantara et al., 2013), antiinflammation, antiparasite, antimalarial, and antifungal (Musman et al., 2015).

Myrmecophytes are known with various local names which is differred in each region. This plant is called *Periok hantu, Peruntak,* and *Sembuku* in Malaysia; *By ki nan, Ki nam gai*, and *Ki nam kin* in Vietnam; *Nongon, Lokon,* and *Suhendep* in Papua New Guinea; *Angkis* in Kalimantan; *Urek-urek* and *Ulek-ulek polo* in Java; *Kepala beruk* and *Rumah semut* in Sumatra (Soekmanto et al., 2010). It is called *Simbagh utak* in Bengkulu.

Myrmecophytes grow in mangrove forests on the edge of the beach up to an elevation of 2400 mdpl. They are distributed in the tropical forests of the eastern part, and the highest diversity located in Papua New Guinea. *Hydnophytum* spreads wider than *Myrmecodia* (Huxley & Jebb, 1991). *Myrmecodia tuberosa* found in Kalimantan, Ambon, West Sumatra, and North Sulawesi (Gunawan et al., 2009). *Hydnophytum* believed to grow in South Bengkulu, but its identity has not been known (Ernis 2013).

Previous studies conducted in the province of Bengkulu reveal many benefits of Myrmecophyte, such as decreasing cholesterol level, blood sugar, healing malaria, and uric acid (Ernis 2013). People use it as medicine for curing headache, stomachache, tumor, and cancer. Distribution pattern of plant species is a characteristic of one that lives in a certain habitat. Distribution of a plant closely related to biological and environmental factors of a species (Sofiah et al., 2013). Unfortunately, Myrmecophyte located in the Province of Bengkulu has never been recorded its species diversity, distribution, and environmental factors that influence it.

Considering the important role of Myrmecophytes as one of the potential medicinal plants to be developed in health sector, the research topic on the species diversity, distribution, and factors affecting the availability of Myrmecophyte in the Province of Bengkulu had been carried out. The objectives of this research were to identify the species identity, diversity, distribution, and factors affecting the presence of Myrmecophytes. The information can be used as basic information for carried out conservation efforts as well as further research. The abundance of Myrmecophytes is also used as a source of additional income for local people in Bengkulu province.

METHODS

This research was carried out in July 2015 until May 2016. Samples were collected from six districts, Bengkulu Province, the Central Bengkulu regency (village Tabalagan), Bengkulu city (village Dusun Besar, Lingkar Timur), Seluma (village Tangga Batu, Kembang Tanjung), North Bengkulu (village Kemumu, Batu Roto, Pagar Banyu), South Bengkulu (village Suka Jaya, Sukarami, Suka Negeri, Nanjungan), and Kaur (Tanjung Betung Village). The samples were identified in Laboratory of Ecology and Plant Resources, Department of Biology, Faculty of Mathematic and Natural Sciences, IPB.

Plant materials studied consisted of 233 individuals of Myrmecophytes, and 51 host trees found in the study site.

Data were retrieved using purposive sampling method by exploring the area where Myrmecophytes are easily found (Sutomo & Mukaromah, 2010). The collected samples were all individuals of Myrmecophytes found.

On a location where Myrmecophyte found is made 51 observation plots of (10x10) m^2. The data recorded on each plot were the number of individual Myrmecophytes found on a host, the Myrmecophyte morphology, and the number

and the identity of host tree. Several supporting data were also recorded. They consisted of four microclimate conditions, light intensity, relative humidity, temperature, and wind speed that were recorded using a *digital 4-in-1*. The elevation level and the geographical position of each location were determined using a *Garmin GPS* 60 at 9:00 am to 1:00 pm.

Myrmecophyte identity was defined using five literatures, *The ant-plant Myrmecodia and Hydnophytum (Rubiaceae), and the relationships between their morphology, ant occupants, physiology and ecology* (Huxley, 1978), *Taxonomy and tuber morphology of the Rubiaceae ant-plant* (Jebb, 1985a), *Taxonomy and tuber morphology of the Rubiaceae ant-plants Volume 2 figures and illustration* (Jebb, 1985b), *The tuberous epiphytes of the Rubiacea 1: a new subtribe - the Hydnophytinae* (Huxley & Jebb, 1991), and *The tuberous epiphytes of the Rubiacea 5: a revision of Myrmecodia* (Huxley & Jebb, 1993). The identity of host trees was determined using *Flora of Java* (Backer & Bakhuizen van den Brink, 1965).

The distribution pattern of Myrmecophytes was analyzed using *Morishita* index (Morishita, 1959). The influence of abiotic factors on Myrmecophyte distribution was analyzed using *Principal Component Analysis* (PCA) that were performed using *Minitab* 16.

RESULTS AND DISCUSSION

Myrmecophytes were only found in 8 villages of 13 visited villages. They were found at 13 sampling points among 51 observed host trees. As many as 233 Myrmecophytes were found in all sampling locations (Table 1). The species found were *Hydnophytum formicarum* and *Myrmecodia tuberosa* . The species of *M. tuberosa* are consisted of two variants i.e. *M. tuberosa* 'armata' and *M. tuberosa* 'siberutensis'.

Number of Myrmecophytes growing on a host tree ranged from 1 to 22 individuals. There was a tree hosted 10 individuals of Myrmecophytes grown in a rubber plantation owned by local residents in Pagar Banyu Village. In contrast to that village, there was only one Myrmecophyte individual found in a tree in Tanjung Betung Village. In Pagar Banyu Village, there were found as many as 92 individuals of Myrmecophytes living on 22 host trees in a rubber plantation, but there was only found 3 individuals of Myrmecophytes on 3 host trees in Tanjung Betung Village.

Table 1. Myrmecophyte species collected at each sampling point

Locations (Villages)	Point Coordinates Number on the Location	Species Name	Σ Ind/ Host	Σ Host	Σ Total of Myrmecophyte		Habitat of Myrmecophyte
					Large	Small	
Tanjung Betung	1	*Hydnophytum formicarum*	1	3	3	0	Rubber plantation
Suka Jaya	2 3 4 5	*Myrmecodia tuberosa*. *Myrmecodia tuberosa* *Myrmecodia tuberosa* *Myrmecodia tuberosa*	2-12	9	43	13	*Durio* plantation
Kemumu	6 7	*Hydnophytum formicarum* *Myrmecodia tuberosa* 'armata' *Hydnophytum formicarum*	1-22	6	47	0	natural tourism Forest (waterfall)
Sukarami	8	*Myrmecodia tuberosa*	5-7	2	8	4	natural tourism Forest (waterfall)
Suka Negeri	9	*Myrmecodia tuberosa* *Myrmecodia tuberosa* 'siberutensis'	1-3	4	7	2	*Durio* plantation
Tangga Batu	10	*Hydnophytum formicarum*	1-10	3	13	0	*Durio* and Cempedak plantation
Tabalagan	11	*Hydnophytum formicarum*	2	2	3	1	Rubber plantation
Pagar Banyu	12 13	*Hydnophytum formicarum* *Hydnophytum formicarum* *Hydnophytum formicarum* *Hydnophytum formicarum*	1-10	22	42	50	Rubber plantation
8	13	16	1-22	51	233		

The Myrmecophytes in Bengkulu Province are mostly found in a plantation. Previous study in Papua also reported that many Myrmecophytes, 388 individuals/ha, found in a plantation area around Nature Reserve of Wasur, Merauke, Papua (Parinding, 2007). They also found in area of mixed farms (Gunawan et al., 2009). They grow on many trees of *Hevea brasiliensis*, *Artocarpus integer, Myristica fragrans, Mangifera indica, Syzygium aromaticum, Syzygium aqueum, Lansium domesticum*, and *Durio zibethinus* (Parinding, 2007; Susanti, 2016), which are commonly found in plantation areas. As epiphytic plants, the Myrmecophytes grow on a various tree species that serve as their host and rely on the micro-climatic conditions of forest stands. Some Myrmecophytes also coexist with orchids on the same tree. Both epiphytic plants only use the host as a place to attach to, hang on, and support their live (Febriliani et al., 2013). In addition, the epiphytes usually grow on host trees with thick, streaked, stringy, and tough bark (Nawawi et al., 2014). Myrmecophytes found in various positions of trees, on the height of tree ranges from 3 to 16 m and located on the main branches or trunks of host trees. Myrmecophytes also live on dead host tree (Parinding, 2007).

The Myrmecophytes of *Hydnophytum* were found scattered around the village of Kemumu, Pagar Banyu, Tabalagan, Tangga Batu, and Tanjung Betung. While, the genus *Myrmecodia* was found in the village of Kemumu, Sukarami, Suka Jaya, and Suka Negeri (Figure 1). The Myrmecophytes was not found in the region of Bengkulu city, Batu Roto village, Nanjungan, and Kembang Tanjung. Many the Myrmecophyte individuals were located in mixed gardens (*Durio* sp. and *Artocarpus integer*), settlement of citizens, natural tourism waterfalls, *Hevea brasiliensis* plantation, *Durio* plantation. In the Kemumu village, two species of Myrmecophytes found on the same tree, a host of 22 individuals of Myrmecophytes.

Based on Figure 1, most individuals of *H. formicarum* were found in the Pagar Banyu village. People in this village do not know the benefits of Myrmecophytes. They let them to live in their garden, but they do not harvest them. In this village, many individuals of Myrmecophytes were found, but they have smaller tuber sized (< 10 cm) compared to that of the other villages. The Myrmecophytes in this village were suspected newly developed plants that grow spreading in the rubber plantation. *Myrmecodia tuberosa* was found in the village at Suka Jaya. Some people already cultivated it. They put this species on trees that grows in their yard by adding a moist of coconut coir as media at the bottom of the tuber of Myrmecophytes.

Hydnophytum formicarum and *M. tuberosa* were found in the Kemumu village. The Myrmecophytes in the village were found in the forest near some natural waterfalls and rivers. Since Myrmecophytes prefer habitats closed to water sources (Parinding, 2007), so it is suspected to be the cause of both species found in the village. Very small quantities of *H. formicarum* were found in the Tabalagan Village due to excessive exploitation. People who know these plants sell them in the traditional markets. They harvest them and it reachs a total of 20 to 25 kg once, so at this time Myrmecophytes in the village is getting hard to be found. People in Bengkulu used Myrmecophytes of *M. tuberosa* and *H. formicarum* as a medicine. Both species had been proven its efficacy as a medicine.

Myrmecodia tuberosa has a cylindrical tuber, sometimes grooved, spiny, greenish and black color, with narrow cavity. Its stem is mostly unbranched, thorny, with clear clypeoli, but sometimes unlike clypeoli. This species has simple leaves with oval to oblanceolate in shape, green, 9-21 cm long, margin entire, pinnately veined and white petiole. Its flower has tubular form and white color with 4 petals, 0.8-1.2 cm long, anthers and stigma easily found at the mouth of the tube, style 0.6-1.0 cm long. Its fruit is ovate, green to orange, fleshy, 0.5-1.0 cm long, with 4-5 pyrene and seeds.

Myrmecodia tuberosa found had two variants namely *M. tuberosa* ' armata' and *M. tuberosa* 'siberutensis''. Previous studies reported that *M. tuberosa* 'siberutensis' found in Sumatra were

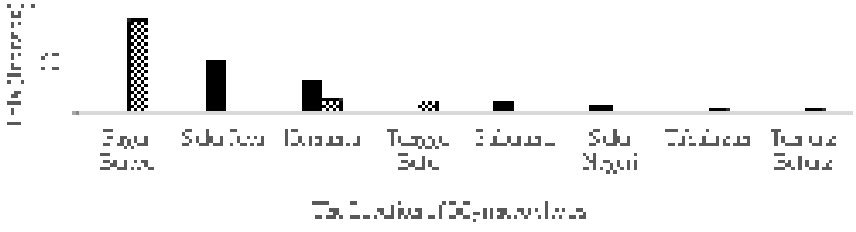

Figure 1. Comparison the number of Myrmecophytes of *M. tuberosa* and *H. formicarum* found at each location. ■= *M. tuberosa*; ▨= *H. formicarum*

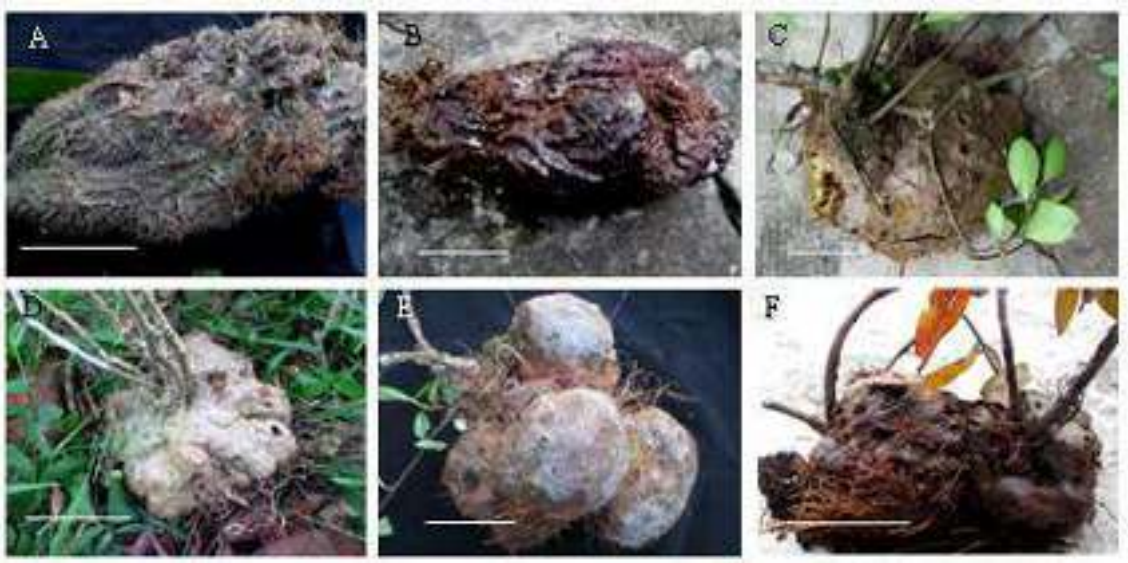

Figure 2. Myrmecophytes tuber variations. *M. tuberosa*: A= *M. tuberosa* 'armata'; B= *M. tuberosa* 'siberutensis'. *H. formicarum*: C= ball; D= sprawl; E= ball with 3 grooves; F= cylinder. Bar 10 cm

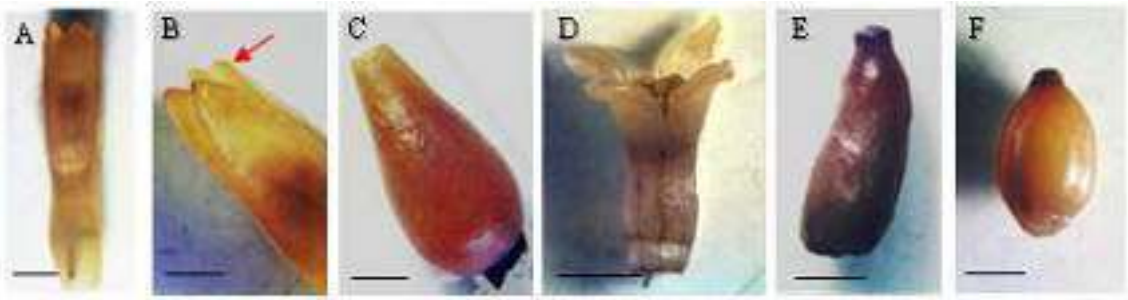

Figure 3. The generative organ of Myrmecophyte. *M. tuberosa*; A= flowers; B= end of flowers (arrow); C= fruit. *H. formicarum*: D= flowers; E= oval-formed fruit; F= ovate-formed fruit. Bar 1 mm

located in Mentawai Islands, Batu, and Siberut, whereas *M. tuberosa* 'armata' found in Payakumbuh and Asahan (Huxley & Jebb, 1993). Both variants are differed in shape and color of tuber, as well as the presence of thorns on the stem and tuber (Figure 2 A-B). The flowers and fruits (Figure 3 A-C) of *M. tuberosa* 'armata' are the same as *M. tuberosa* fruit, meanwhile, *M. tuberosa* 'siberutensis' flower could not be observed because there was no fruit and flower found. Huxley & Jebb (1993) reported that variants of *M. tuberosa* were only given informal name, and could not be categorized as subspecies or varieties because they do not have specific morphological and geographical characteristics.

Hydnophytum formicarum has a sprawl tuber, ball-like, cylindrical, and sometimes cylinder with irregular grooves, no spiny, greenish and brown color, with wide cavity. Its stem has 2 to 7 branches, no thorny, without clypeoli, but its position is sometimes irregular. It has single leaves with oval and oblanceolate blade shape, green, 3 to 12 cm, margin entire, pinnately veined, and green petiole. It has tubular flowers, white, 0.4 to 0.7 cm long, 4 petals, anthers and stigma at the mouth of the tube, style of 0.3 to 0.6 cm long. Fruit is ovate to oval, green to orange, fleshy, 0.5 to 0.8 cm long, with 1 to 2 pyrene and seeds.

The tuber of *H. formicarum* found in Bengkulu has variation of tuber shape: ball-like, cylinder, and sprawl. They also have distinct color (greenish and brownish) and cavity (Figure 2 C-F),but, the generative organs, flowers and fruits, (Figure 3 D-F) of *H. formicarum* are same.

The animals found in the tubers of *H. formicarum* grown in the village of Tanjung Betung was ant *Crematogaster* sp. which is not found in other species of Myrmecophyte in the province of Bengkulu. In contrast to our result, previous studies reported that *H. formicarum* was not only inhabited by *Crematogaster* ants, but also inhabited by *Camponotus, Technomyrex,* and *Iridomyrmex* (Lok & Tan, 2009), *Anoplolepis, Pedomyrna, Pheidole, Polyrachis, Monomorium, Turneria, Vollenhovia*, and *Ocheletellus* as well (Huxley, 1978). These ants do symbiotic mutualism with nearby plants.

Table 2. The distribution pattern of Myrmecophytes based on found host tree at 8 locations in Bengkulu province

Locations (Village)/District	Myrmecophyte genera	Degrees of *Morishita* dispersion (Ip)	Distribution pattern
Tanjung Betung/Kaur	*Hydnophytum*	0.0000	*Random*
Suka Jaya/South Bengkulu	*Myrmecodia*	0.0067	C*lumped*
Kemumu/North Bengkulu	*Myrmecodia*	0.0000	R*andom*
	Hydnophytum	0.0024	C*lumped*
Sukarami/South Bengkulu	*Myrmecodia*	0.0000	*Random*
Suka Negeri/South Bengkulu	*Myrmecodia*	0.0000	*Random*
Tangga Batu/Seluma	*Hydnophytum*	0.0000	*Random*
Tabalagan/Central Bengkulu	*Hydnophytum*	0.0000	*Random*
Pagar Banyu/North Bengkulu	*Hydnophytum*	0.0008	*Clumped*

Plants provide food and ants will help pollinate the plants (Harrison, 2014).

Distribution of Myrmecophytes

Based on *Morishita* index (Morishita, 1959) both genera of Myrmecophytes found on host tree in this study were mostly randomly (77.8%) scattered in each location, and as much as 22.2% were dispersed in a cluster (Table 2). These results are consistent with that of previous studies that reported Myrmecophytes commonly showed clustered or clumped distribution patterns (Dali, 2014), forming uneven patterns, and irregularly scattered (Gunawan et al., 2009). This pattern is believed to be related to environmental factors (microclimate) of its habitat and reduction of their habitat. Their habitat were turned into settlements and plantations. Myrmecophytes like habitat with slope topography and area near a river. The presence of these plants were also related to a water source as humidity resource (Gunawan et al., 2009). The intensity of exploitation activity can also affect the spread of Myrmecophytes. The overexcess of Myrmecophytes collection may lead to their extinction if some conservation efforts are not performed.

The Myrmecophytes were scattered in five districts in the province of Bengkulu were Kaur, South Bengkulu, Seluma, Bengkulu central, North Bengkulu. The distribution of *H. formicarum* was wider than *M. tuberosa*. *Hydnophytum formicarum* was distributed in Kaur district, Seluma, Bengkulu central, North Bengkulu, and was found at 25 to 629 m altitude. *Myrmecodia tuberosa* was found only in South Bengkulu and North Bengkulu, and grew in an altitude ranged 205 to 351 m altitude (Table 3). *Hydnophytum formicarum* found in locations having cold warm temperatures ranged from 25.0 to 36.0°C and 65.6 to 82% humidity. Compared to *H. formicarum*, *M. tuberosa* prefers hotter (28.4 to 33.8°C temperature) and drier area (54.7 to 78.4% humidity). *Hydnophtyum formicarum* individuals were found in various microclimate conditions, and they are able to adapt to the various environment.

The Myrmecophytes was found in Kemumu village at 377 m altitude with 29.2 to 30.6°C temperature, while in the Tangga Batu village was found at 25 m altitude with temperature ranged 32.1 to 33.3°C. The Myrmecophytes in the Kemumu village were found near a waterfall and a river with cool weather. In contrast to the Kemumu village, in Tangga Batu village, Myrmecophytes were found in rice fields with hot weather and got directly sunlight. Myrmecophytes found in the Kemumu village were *H. formicarum* and *M. tuberosa* 'armata', while in the village of Tangga Batu it was found only *H. formicarum*. Thus, the microclimate of each village affected the diversity of Myrmecophytes found.

Microclimate and ecological conditions of Myrmecophytes habitat in Bengkulu Province were different from South Kalimantan. The Myrmecophytes in South Kalimantan grown at range 23.0 to 26.8°C temperature with relative humidity of 78.0 to 82.0%, and light intensity of 570.0 to 870.0 lux (Gunawan et al., 2009). In South Kalimantan forests, Myrmecophytes grew in the mountains, while in Bengkulu Province they were found in lowlands and plantations. Myrmecophytes were found in Bengkulu Province namely *H. formicarum*, *M. tuberosa*, and two variants of *M. tuberosa* (*M. tuberosa* 'armata' and *M. tuberosa* 'siberutensis'), whereas in South Kalimantan only found a species, *M. tuberosa*. *Myrmecodia tuberosa* is suspected to have capability living in the mountain forests and lowlands. Thus, not only the microclimate of each Myrmecophytes

Table 3. Some microclimate parameters around the Myrmecophytes

Village/ District	Location	Microclimate				
		Altitude (m dpl)	Humidity (RH%)	Temperature (°C)	Light Intensity (Lux)	Wind Speed (Km/h)
TB/KAUR	1	629	82.6	25.0	5170.0	1.2
SJ/BS	2	219	63.6-78.4	28.8-31.8	1157.0-1234.0	0.0-1.0
SJ/BS	3	249	57.4	36.1	1192.0	2.0
SJ/BS	4	290	63.8-64.7	30.5-32.0	1463.0-1977.0	2.2-3.4
SJ/BS	5	270	63.0-76.5	29.3-33.8	1353.0-1643.0	0.0-2.4
KM/BU	6	351	74.4-77.0	30.0-30.3	4690.0-6020.0	0.0-1.1
KM/BU	7	377	76.1-76.3	29.2-30.6	3870.0-5260.0	0.0-0.9
SKR/BS	8	211	75.1	28.4	4650.0	0.8
SKN/BS	9	205	71.5-77.3	32.2-32.6	1763.0-3410.0	0.0-1.7
TB/SELUMA	10	25	71.9-73.6	32.1-33.3	5070-5810.0	2.6-2.7
TBL/BT	11	54	76.0-76.9	33.6-34.3	4020.0-5090.0	0.0-0.7
PB/BU	12	133	66.8	35.4	6670.0	1.2
PB/BU	13	132	65.6	36.3	3490.0	1.4

Note : TB= Tanjung Betung; SJ= Suka Jaya; KM= Kemumu; SKR= Sukarami; SKN= Suka Negeri; TB= Tangga Batu; TBL= Tabalagan; PB= Pagar Banyu; BS= Bengkulu Selatan; BT= Bengkulu Tengah; BU= Bengkulu Utara

location, but also the ecological conditions of Myrmecophytes habitat affected the diversity of Myrmecophytes.

Myrmecodia tuberosa was found in the seashore near Jurong and Bukit Timah at Singapore, but they were disappeared in the early 1990s, and *H. formicarum* was categorized into endangered species. *Hydnophytum formicarum* was found in lowland, mangrove forests along the northern coastline of Pulau Tekong and Pawai (Lok & Tan, 2009). In addition, both species were found in lowlands, plantations, beaches, mangrove forests, swamps, and savanna in Papua (Parinding, 2007; Susanti, 2016). *Hydnophytum formicarum* and *M. tuberosa* were allegedly able to adapt to various microclimate conditions. Both species have wide distribution from Sumatra, Kalimantan, North Sulawesi and Papua, and Singapore. The highest diversity and abundance of Myrmecophyte is in Papua.

The diversity of Myrmecophytes found in Bengkulu province was relatively low when compared to that in Papua province and North Sulawesi. The Myrmecophytes found in the Nature Reserve of Wasur Merauke Papua, were 4 species of *Hydnophytum* and 14 species of *Myrmecodia* (Parinding, 2007), whereas in Fakfak, West Papua discovered 7 species of *Hydnophytum* and 2 species of *Myrmecodia* were found (Susanti, 2016). Three species, *Hydnophytum formicarum*, *M. pendans*, and *M. tuberosa* also found in the nature reserve of Gunung Ambang sub region East Mongondow Bolaang, North Sulawesi (Dali, 2014). The light intensity and temperature measured were also different in both locations. The nature reserve of Gunung Ambang sub region East Mongondow Bolaang, North Sulawesi had light intensity of 600 to 870 lux and temperature of 23 to 28°C where Myrmecophytes found (Dali, 2014). The light intensity in Fakfak is 1010 to 1764 lux and temperature ranged 27.5 to 31.9°C (Susanti, 2016). So it is suspected that the diversity of Myrmecophytes affected by environmental conditions where the plant grows.

Based on *Principal Component Analysis* (PCA), it was known that light intensity and temperature factors were contributed to the population size of Myrmecophytes (Figure 4). Some locations where *M. tuberosa* and two variants of *M. tuberosa* 'siberutensis', *M. tuberosa* 'armata' and *H. formicarum* found in Bengkulu had the same microclimates.

The first major component is temperature. It contributed of 54.8% to the presence of Myrmecophytes. The 2nd major component is light intensity of which contributed of 21.9%. The total of major components is 76.7%. Myrmecophytes in Bengkulu Province grew at above 1000 lux light intensity and the temperature reached 36.3°C, while in the mountainous regions of South Kalimantan and Nature Reserve Gunung Ambang Bolaang Mongondow Eastern

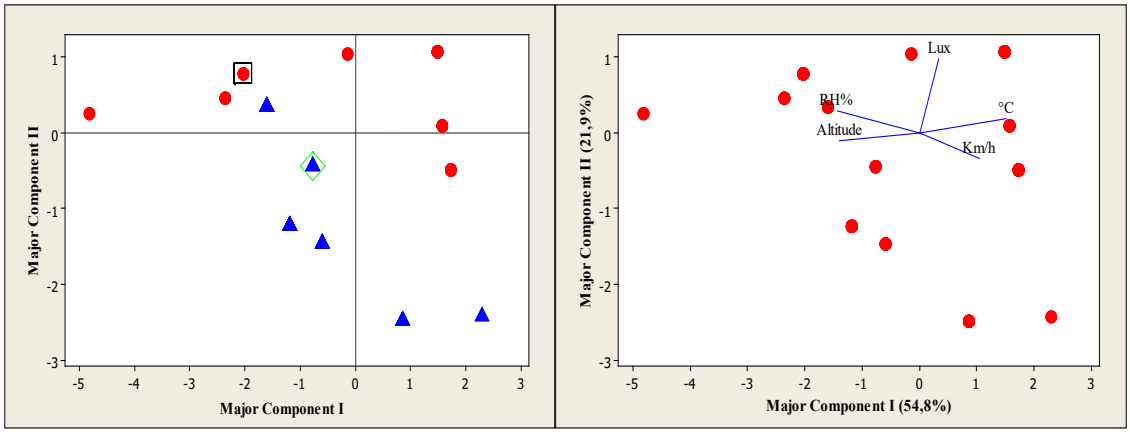

Figure 4. The major components analysis of environmental factors: A. the distribution of Myrmecophytes spesies; B. Environmental factor correlation contributed to the distribution of Myrmecophytes; Altitude= the altitude of the place; %RH= humidity; C°= temperature; Lux= light intensity; Km/h= wind speed; = *H. formicarum*; = *M. tuberosa* 'armata'; = *M. tuberosa* 'siberutensis'; = *M. tuberosa*

(North Sulawesi) they grew in an area that had low light intensities, below 1000 lux (Dali, 2014) and are not able to grow at above 27°C temperatures (Gunawan et al., 2009). Myrmecophytes require high light intensity. One individual tree can be a host of 22 individuals of Myrmecophytes that tend to attach on branches, at canopy, and in the main stem of trees to meet their needs to light. Light is the energy source used by plants to perform photosynthesis, stomata conductance and resistance, and the chlorophyll synthesis (Buntoro et al., 2014).

Myrmecophytes found in Bengkulu Province were *H. formicarum*, *M. tuberosa* and two variants of *M. tuberosa* namely *M. tuberosa* 'armata' and *M. tuberosa* 'siberutensis'. Both species had been proven its efficacy as a medicine and used to people in Bengkulu for medicinal plant. The benefit of this research is to can be used as basic information for carried out conservation efforts (in situ and ex situ) in the area of Bengkulu province to prevent the extinction of this species. The abundance of Myrmecophytes in Bengkulu province is also used as a source of additional income for local people.

CONCLUSIONS

Myrmecophytes found in Bengkulu Province were *H. formicarum*, *M. tuberosa* and two variants of *M. tuberosa* namely *M. tuberosa* 'armata' and *M. tuberosa* 'siberutensis'. Myrmecophytes were distributed in North Bengkulu, Central Bengkulu, Seluma, South Bengkulu, and Kaur. Based on found host tree, most Myrmecophytes (77.8%) were scattered in random distribution pattern and the highest variations of their distribution is in South Bengkulu district. Myrmecophytes in Bengkulu Province was found in altitude of 25 to 629 m altitude, wind speed of 0.0-3.4 km/h, relative air humidity of 57.4 to 82.6%, light intensity of 1157.0 to 6670.0 lux, and temperature 25.0 to 36.3°C.

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REFERENCES

Abdullah, H., Pihie, A. H. L., Hohmann, J., & Molnar, J. (2010). Anatural compound from *Hydnophytum formicarium* induces apoptosis of MCF-7 cells via up-regulation of Bax. *Cancer cell international,* 10(1), 14.

Ahmad, R., Mahbob, E. N. M., Noor, Z. M., Ismail, N. H., Lajis, N. H., & Shaari, K. (2010). Evaluation of antioxidant potential of medicinal plants from Malaysian *Rubiaceae* (subfamily *Rubioideae*). *African Journal of Biotechnology*, 9(46), 7948-7954.

Backer, C. A. & Bakhuizen van den Brink, R. C. (1965). *Flora of Java* (*Spermathopyte only*). Groningen: NVP Noordhoft.

Buntoro, B., H., Rogomulyo, R., Trisnowati, S. (2014).

Pengaruh takaran pupuk kandang dan intensitas cahaya terhadap pertumbuhan dan hasil temu putih (*Curcuma zedoaria* L.). *Vegetalika*, 3(4), 29-39.

Dali, R. (2014). Keanekaragaman dan pola penyebaran tumbuhan sarang semut di kawasan Cagar Alam Gunung Ambang sub kawasan Kabupaten Bolaang Mongondow Timur. *Skripsi*. Gorontalo: Universitas Negeri Gorontalo.

Dirgantara, S., Nawawi, A., & Insanu, M. (2013). Uji aktivitas antioksidan tiga spesies tanaman sarang semut (Famili: *Rubiaceae*) asal Kabupaten Merauke, Papua. *Jurnal Biologi Papua,* 5(1), 10-16.

Ernis, G. (2013). Pengaruh ekstrak umbi "*Simbagh utak*" (*Hydnophytum* sp.) terhadap kadar asam urat *Mus musculus* jantan dan karakterisasi hasil isolasi menggunakan FTIR. *Skripsi*. Bengkulu: Universitas Bengkulu.

Febriliani, Mallombasang, S. N., & Muslimin. (2013). Analisis vegetasi habitat Anggrek di sekitar Danau Tambing kawasan Taman Nasional Lore Lindu. *Warta Rimba*, 1(1), 1-9.

Gunawan, Suyanto, Hafizianor, & Hamidah, S. (2009). Inventarisasi komposisi jenis dan potensi tumbuhan sarang semut *(Myrmecodia* sp.) berdasarkan karakteristik ekologis habitatnya di kawasan hutan pegunungan Meratus Kalimantan Selatan. *Jurnal Hutan Tropis Borneo*, 25, 71-85.

Harrison, R. D. (2014). Ecology of a fig ant plant. *Acta Oecologica*, 57, 88-96.

Huxley, C. R. (1978). The ant-plants *Myrmecodia* and *Hydnophytum* (Rubiaceae), and the relationships between their morphology, ant occupants, physiology and ecology. *New Phytologist*, 80(1), 231-268.

Huxley, C. R., & Jebb, M. H. P. (1991). The tuberous epiphytes of the *Rubiaceae* 1: A new subtribe – The *Hydnophytinae*. *Blumea*, 36(1), 1-20.

Huxley, C. R., & Jebb, M. H. P. (1993). The tuberous epiphytes of the *Rubiaceae* 5: a revision of *Myrmecodia*. *Blumea*, 37(2), 271-334.

Irawan, Y. R., Fitmawati, Herman. (2013). Pengetahuan Tumbuhan Obat Dukun Sakai Desa Sebangar Duri Tiga Belas dan Desa Kesumbo Ampai Duri Kabupaten Bengkalis. *Biosaintifika: Journal of Biology & Biology Education,*, 5(1), 30-35.

Jebb, M. H. P. (1985a). Taxonomy and tuber morphology of the Rubiaceae ant-plant. *Thesis*. Oxford: Postgraduate, University of Oxford.

Jebb, M. H. P. (1985b). Taxonomy and tuber morphology of the Rubiaceae ant-plant. *Thesis*. volume 2 figures and illustration. Oxford: Postgraduate, University of Oxford.

Lok, A. F. S. L., & Tan, H. T. W. (2009). Tuberous, epiphytic, rubiaceous *Myrmecophytes* of Singapore. *Nature in Singapore*, 2, 231-236.

Mairida, D., Muhadiono, M., & Hilwan, I. (2016). Ethnobotanical Study of Rattans on Suku *Anak Dalam* Community in *Bukit Duabelas* Nasional Park. *Biosaintifika: Journal of Biology & Biology Education*, 8(1), 64-72.

Morishita, M. (1959). *Measuring of the Dispersion on Individual and Analysis of the Distributial patterns*. Jepang: Kyushu University.

Musman, M., Karina, S., Defira, C. N., Fadhillah, N., Kayana, A., Hasballah, N., Faunanda, A. R., & Putra, R. (2015). Phytofungitoxic Agent from wild plants. *International Journal of Sciences: Basic and Applied Research*, 21(1), 78-85.

Nawawi, G. R. N., Indriyanto, Duryat. (2014). Identifikasi jenis epifit dan tumbuhan yang menjadi penopangnya di blok perlindungan dalam kawasan Taman Hutan Raya Wan Abdul Rachman. *Jurnal Sylva Lestari*, 2(3), 39-48.

Parinding, Z. (2007). Potensi dan karakteristik bioekologis tumbuhan sarang semut di Taman Nasional Wasur Merauke Papua. *Tesis*. Bogor: Sekolah Pascasarjana, Institut Pertanian Bogor.

Senawong, T., Misuna, S., Khaopha, S., Nuchadomrong, S., Sawatsitang, P., Phaosiri, C., Surapaitoon, A., & Sripa, B. (2013). Histone deacetylase (HDAC) inhibitory and antiproliferative activities of phenolic-rich extracts derived from the rhizome of *Hydnophytum formicarum* Jack.: sinapinic acid acts as HDAC inhibitor. *BMC Complementary and Alternative Medicine*, 13(1), 232.

Soekmanto, A., Subroto, M. A., Wijaya, H., & Simanjuntak P. (2010). Anticancer activity test for extracts of Sarang semut plant (*Myrmecodia pendens*) to HeLa and MCM-B2 cells. *Pakistan Journal of Biological Sciences,* 13(3), 148-151.

Sofiah, S., Setiadi, D., & Widyatmoko, D. (2013). Pola penyebaran, kelimpahan, dan asosiasi bambu pada komunitas tumbuhan di Taman Wisata Alam Gunung Baung Jawa Timur. *Berita Biologi*, 12(2), 239-247.

Susanti, E. (2016). Inventarisasi tumbuhan sarang semut di Kabupaten Fakfak Papua Barat. *Skripsi*. Bogor: Institut Pertanian Bogor.

Sutomo, & Mukaromah, L. (2010). Autekologi Purnawijaya (*Euchresta horsfieldii* (Lesch.) Benn. (Fabaceae) di sebagian kawasan Hutan Bukit Tapak Cagar Alam Batukahu Bali. *Jurnal Biologi*, 14(1), 24-28.

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Effect of Seed Maturity and Storage Duration on Germination of Sambiloto (*Andrographis paniculata*)

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Andrographis paniculata; germination; maturity; seed; storage

Abstract

Seed maturity and its storage are one of problems on propagation and developing of medicinal plants such as sambiloto (*Andrographis paniculata* (Burm.f.) ex Nees). The research aimed to determine effects of seed maturity and storage duration on seed germination of sambiloto was conducted in a green house of Purwodadi Botanical Garden from November 2014 to November 2015. The experiment was done by completely randomised design with two treatments namely seed maturity and storage duration. The seed was classified into immature seed (0.061 g/100 seeds), semimature seed (0.113 g/100 seeds) and mature seed (0.166 g/100 seeds). The seed's storage duration was classified into seed was not stored, seed was stored for six months and seed was stored for twelve months. Each treatment combination was replicated five times. The results showed that there were significant interactions between the treatments on percentage and rate of seed germination. The highest percentage of seed germination was the treatment of mature seed and stored twelve months (98.50%). On the contrast, the lowest seed germination percentage was the treatment of immature seed and stored twelve months (4.25 %). The fastest seed germination rate was the treatment of mature seed and stored six months (3.88 days), whereas the slowest seed germination rate was the treatment of immature seed and without stored (28.58 days). This study is expected to be applied to improve genetic and cultivation of medicinal plant as well as increasing plant growth and yield.

How to Cite

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INTRODUCTION

Plant propagation and cultivation play important roles to develop, conserve and produce medicinal plants including sambiloto (*Andrographis paniculata* (Burm.f.) Nees). It is an urge to fulfil the need of medicinal plant materials for public consumption and industrial sustainably. The need of herbal medicine tends to increase at this time. The trade value of world herbal medicine about 12-15 billion USD in 2001 then increased to 60 billion USD in 2011, including export of herbal medicine from Indonesia about IDR 10-11 trillion (Kadarusman, 2011).

Plant propagation by seed is an important stage of medicinal plant cultivation to produce high quality of plant seeds for increasing plant growth and yield. It is also important to genetic improvement of plant species which is achieved by selection, first from population in fields that continued unique genotype fixed by vegetative propagation (Surya et al.,2016). Maturity and storage of seed need to be attended correlating to seed quality for seed propagation. This is often to become a problem for developing and cultivation of the medicinal plant.

Seed quality can be obtained by propagation with high seed quality, which is characterised by high viability, uniform, pure, free of pests and diseases. A good quality seed can increase plant yield by 15-20% (Ambika et al., 2014). The viability of seed was influenced by seed maturity and weight at harvested (Takač et al., 2015; Negasu, 2015). Seed maturity could be seen visually by the seed and fruit colour. The mature seed of sambiloto was produced by ripe fruit which has purple or brown fruit skin and brown seed. Whereas the immature seed was produced by unripe fruit which has green peel and cream or light yellow seed (Solikin, 2008; Solikin, 2016). Zhang et al (2013) also reported that the colour of over mature and immature seed of *Brassica napus* was black and light brown respectively. The dry weight of mature seed was higher than those of immature seed, as reported Negasu (2015) that the mature seed weight of castor bean (*Jatropha curcas* L.) was about 537.57 to 592.10 g / 1000 seeds; whereas the immature seed about 416.16 g / 1000 seeds. The mature seed has full mature embryo with maximum weight and size also greater germination percentage and faster germination rate than immature seed which not fully mature embrio and low seed viability, even not capable to germinate (Solikin, 2016).

Seed storage was commonly conducted for the plant cultivation, germplasm conservation, delivery and economy. Storage associated with the management of seed moisture content, temperature and humidity of room to maintain seed viability for long time. Longevity of seed storage is influenced by the characteristics of each type of seed and environmental condition. Recalcitrant seed cannot be stored longer because of its rapid declining viability. In contrast, orthodox seed can be stored or saved until a few years duration as reported by Van Treuren et al., (2013) that the seed of *Spinacea oleracea* L. was able to germinate well (about 90.3%) after being stored for four years. Storage of orthodox seeds was also important to solve the problem of physiological dormancy after harvesting so that the seeds can germinate optimally. Solikin (2014) reported that the seed germination of *Stachytarpheta jamaicensis* was increased from 56.8% when the seeds directly planted into 96.8% after stored for eight months after harvesting.

This study aimed to know the effect of seed maturity, storage duration and its interaction of these treatments on the seed germination of sambiloto (*Andrographis paniculata* (Burm.f.) Nees) to develop and to improve cultivation of medicinal plant in nursery and field.

METHODS

Seeds were harvested simultaneously from fruits of sambiloto grown in Purwodadi Botanical Garden in October 2014. The fruits (different stages of maturity with green to purple or brown fruit skin) were inserted into a plastic bag for sun drying and to split the seeds for a week. This was conducted to prevent the seeds scattering around and facilitating to collect seeds. Seeds had been sorted from fruit skin and dried under direct sunlight for two days, then they were selected into three categories (treatments) as shown in Table 1 (Solikin, 2016).

The experiment was conducted in a green house of Purwodadi Botanical Garden from November 2014 to November 2015 at altitude of 300 m above sea level. Minimum and maximum temperatures in the garden were 20.5 ^{o}C and 30.3^{o}C respectively with average temperature 27.6^{o}C and relative air humidity 72.9% during the experiment. The experiment used completely randomised design with two treatments (factorial) namely seed maturity and storage duration. The seed maturity was classified into the immature seed (0.061 g/100 seeds), semimature (0.113 g/100 seeds) and mature seed (0.166 g/100 seeds). The seed storage duration was classified by seed was not stored, stored for six months and stored for

Table 1. The seeds description of sambiloto for experiments (Solikin, 2016)

Maturity	Colour	Seed colour (RGB Color Codes Chart)	Weight/100 seeds (g)
Immature	Cream	*Pale golden rod* (EEE8AA); *light golden rod yellow*(FAFAD2); *light yellow*(FFFE0)	0.061
Semimature	Light brown	*Peru* (CD853F); *golden rod*(DAA520)	0.113
Mature	Brown	*Saddle brown* (8B4513; *seinna* (A0522D	0.166

Note: Seeding media used in this experiment was river sand which had been screened by 2mm sieve

twelve months. Each treatment combination was replicated five times with 100 seeds for each replication.

Seeds were sowed into furrow on media in polybags 13x7 cm as depth as 0.5 cm under media surface. Smooth river sand was used as media in the experiment. The polybags (15 polybags) had been put into a seeding box 38 x 28 x 15 cm before sowing the seeds. The seeding box was covered by transparent plastic and black paranet with 9.14% light penetration on the media surface. Watering had been done by hand sprayer after sowing the seeds and twice a week after that.

The observation was conducted on variables of seed germination percentage, seed germination rate, beginning and end of seed germination. The seed germination percentage was calculated by (germinated seed number/total tested seed) X 100 %; and the seed germination rate was calculated by $(N_1T_1+N_2T_2 + ... N_XT_X)$/ total number of seed germinating, where N is the number of seed germinating between beginning of the test and the end of the particular interval of measurement (Sutopo, 1988).

Data was analysed by analysis of variance (ANOVA) using MINITAB 16 software ($p<0.05$). Data was transformed in arching before analysed. Average values of the variables from all the treatments were compared using Duncan's Multiple Range Test (DMRT) at $\alpha= 0.05$.

RESULTS AND DISCUSSION

The results showed that there was significant interaction between treatment of seed maturity and storage duration on percentage and rate of seed germination of sambiloto (Table 2). The interaction between the treatments was caused by mean values of seed germination on various seed maturity to the storage duration treatment was different. This was also showed by Figure 1 that the seed germination of immature seed declined steadily by increasing storage duration from 9.75%, (without stored) to 4.25% (stored for twelve months) with trend line $Y = -5.20\ln(x) + 9442$; $R^2 = 0.94$ (Figure 1). Table 2 showed that the seed germination of this treatment were 9.75%, 5.00 % and 4.25% (without stored, stored for six and stored for twelve months respectively). This was caused by the immature embryo and least food reserve in the immature seed among the other treatments (mature and semimature). On the contrast, the seed germination on semimature and mature seeds continued to rise from 35.80% and 71.00% (without stored) up to 64. 5% and 98.50% (stored for twelve months) respectively (Table 2, Figure 1). The trendline response of seed germination on the semimature and mature seed which were stored for twelve months were $Y = 27.50\ln(x) + 37.82$, $R^2 = 0.906$ and $Y = 25.87\ln(x) + 72.29$, $R^2 = 0.956$ respectively (Figure 1). The seed germination percentage of the mature seed was 71.00%, 93.8% and 98.5% (without stored, stored for six and twelve months respectively) (Table 2). Increasing seed germination on the mature seed during the storage was also reported by Solikin (2014) on the seed of *Stachytarpheta jamaicensis* (L.) Vahl that its germination continued rising up to the storage for eight months.

Increasing seed germination during storage could be caused by the increasing physiological seed maturity (after ripening) during storage to mitigate dormancy and improve the seed germination such as showed at Figure 2 for the mature and semimature seeds. It was commonly happened on orthodox seeds such as seed of sambiloto. The continuing seed maturity after harvesting (after ripening) was also reported by Hartman et al., (2002) that some types of seed required storage time in dry condition for several days, months or years to be able to germinate optimally as a genetic trait of seed to adapt to the environment. This is a natural mechanism for the seeds of certain plant species to adapt their life cycle with the environment in order to remain viable and prevent extinction, particularly for wild plants. Ekpong (2009) proved that the seeds germination of *Cleome gynandra* which was directly sown the lowest among 1,2,3,4 and five months of storage.

Declining seed germination on immature seed after being stored was caused by the immature seed embrio and fewer food reserves used as germination energy than those in the mature or

Table 2. Percentage and rate of seed germination of sambiloto (*Andrographis paniculata* (Burm.f.) Nees) on the treatment of seed maturity and storage duration

Treatment	Germination percentage (%) *)	Germination rate (day)*)
Immature seed and without stored	9.75[a]	21.66[c]
Immature seed and stored six months	5.00[a]	8.22[a]
Immature seed and seeds stored twelve months	4.25[a]	9.34[b]
Semimature seed and without stored	35.80[b]	28.58[d]
Semimature seed and stored six months	62.50[c]	5.57[a]
Semimature seed and stored twelve months	64.50[c]	7.09[a]
Mature seed and without stored	71.00[c]	10.82[b]
Mature seed and stored six months	93.80[d]	3.88[a]
Mature seed and stored twelve months	98.50[d]	5.60[a]

Note : Numbers followed by the same letters in the same columns were no significantly different by *Duncan's Multiple Range Test* (DMRT) at α= 0.05.
*) : There was significant interaction among the treatments

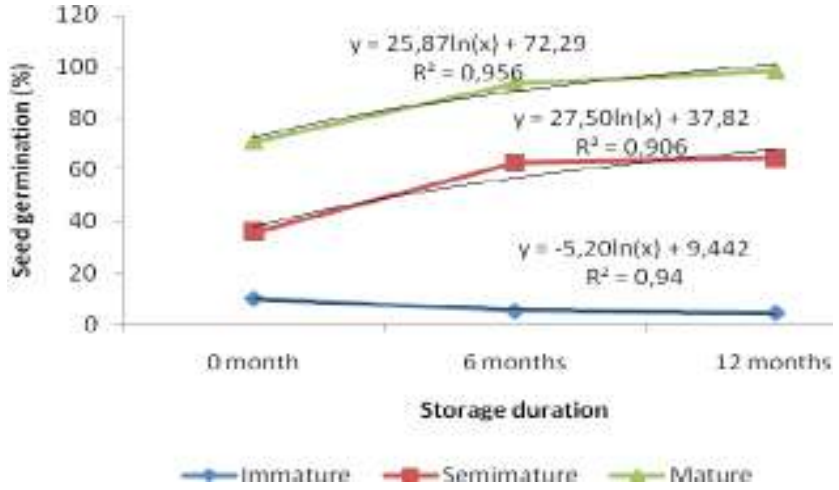

Figure 1. Seed germination of sambiloto (*Andrographis paniculata* (Burm.f.) Nees) on the seed maturity and storage duration

the semimature seed, so most of the immature seeds failed to germinate such as germination on immature seed of groundnut (*Arachis hypogaea*) that its germination lower than those on mature seed (Nautiyal et al., 2010).

Negasu (2015) reported that there was positive correlation between the content of the food reserve (weight) in the seed and the seed germination of castor bean (*Jatropha curcas* L.). Negasu (2015) also proved that germination of the mature seed of castor beans was 90%, whereas the immature seed was 65.67%. Seed coat of mature seed also contained higher calsium than immature seed (Nautiyal et al., 2010) which can increase preventing seed from diseases attacking. Nevertheless the mature seed still required storage after the seeds harvested to mitigate physiological dormancy and increase seed germination as shown in Table 2.

The seed germination rate at treatment of mature seed (the heaviest seed) and stored for six months was the fastest among the other treatments (3.88 days). Figure 2B showed that the seed number which germinated at the mature seed reached 90% in 3-6 days. This might be caused by the optimal seed physiological maturity after being stored for six months. The slowest seed germination rate was the treatment of semimature seed and without stored i.e. 28.58 days (Figure 2A) which showed that the seed germination continue to rise until 55 days. It might be caused by most of the seed maturity at harvesting still requiring a longer time (ripening) to embryo within the seed to germinate. Nevertheless, the seed germination percentage of the semimature seed was higher than those of immature seed. (Table 2).

The beginning time of seed to germinate

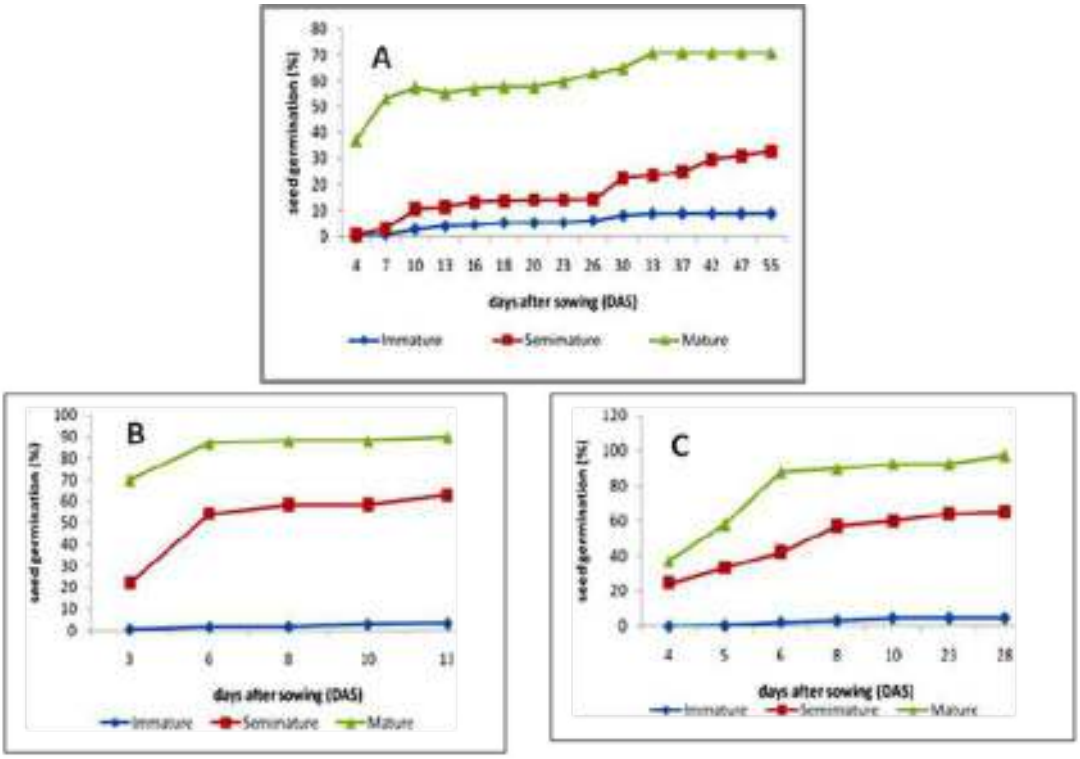

Figure 2. Seed germanination of sambiloto (*Andrographis paniculata* (Burm f.) Nees) on the treatment of seed maturity and without stored (A); stored for six months (B); store for twelve months.

was faster when the seed was stored than the seed without stored such as showed at Table 3 and Figure 2A that the beginning time of seed to germinate on the seed stored for twelve months was the quicker (3.67 days) whereas on the seed without stored was the slower (6.17 days). It was indicated that the time needed of the seed to start germinating on orthodox seed such as seed of *Andrographis paniculata* decrease when the seed stored until twelve months. This was consistent with the results of research by Solikin (2014) that the seed germination rate of *Stachytarpheta jamaicensis* without stored was slower (15.20) days than those of the seed stored two months (5.53) days. Gardner et al., (2008) also stated that some orthodox seeds required a certain period of time in order to germinate optimally.

Seed maturity of plant species has correlated to the change of seed coat (Atis et al., 2011) such as the seed coat of *Brassica napus* change from light colour to blackish colour following seed maturity from immature to over mature (Zhang et al., 2013). Seed colour of sambiloto also changed from cream or light yellow to brown or blackish brown from immature to mature seed (Figure 3). The seed maturity also correlated to the chemical compound(sugar, starch, oil and protein) contained in the seed. Zhang et al., (2013) reported that sugar and protein contain in seed of *Brassica napus* declining by increasing seed maturity, however the oil contain increase by increasing seed maturity; (Vera et al., 2007) also reported that the seed oil contain of canola was increase by increasing seed maturity. Increasing organic compounds which were accumulated in seed during maturity process will be followed by increasing weight as reported by Negasu (2015) at castor bean. Table 1 and Figure 3 showed that the weight of immature seed was 0.036 g/100 seeds whereas mature seed was 0.116 g/100 seeds.

Lipids containing in seed was very important to seed germination as source of germination energy (Hu et al., 2009) so the mature seed of sambiloto which may contain high lipids compound has greater germination energy and faster to begin germinate than immature seed (Table 4). Table 4 shows that the beginning time of seed germination of the mature and semi mature seed was faster (4.55 and 3.25 days respectively) than immature seed (6.25 days). This might be caused by lipids content in mature seed was greater than those of immature seed so its germination was faster than immature seed. This was also showed in Figure 1 that the seed number germinating at mature and semimature in 3-4 th days after sowing was more than immature seed, whereas immature seed starting germinated in 6-7 the days after sowing. Zhang et al., (2013) also proved that

Table 3. The beginning and end time of seed germination of sambiloto (*Andrographis paniculata* (Burm.f.) Nees) on the storage duration treatment

Treatment	The beginning of germination (day)	The end of germination (day)
Without stored	6.17[b]	41.67[b]
Stored six months	4.41[ab]	9.58[a]
Stored twelve months	3.67[a]	17.75[a]

Note: Numbers followed by the same letters in the same columns were no different significantly by *Duncan's Multiple Range Test* (DMRT) at α=0.05

Table 4. The beginning and end time of seed germination of sambiloto (*Andrographis paniculata* (Burm.f.) Nees) on the seed maturity treatments

Treatment	The beginning of germination (day)	The end of germination (day)
Immature seed	6.25[b]	21.58[a]
Semimature seed	3.25[a]	20.00[a]
Mature seed	4.75[a]	20.42[a]

Note: Numbers followed by the same letters in the same coloums were no different ignificanly by *Duncan's Multiple Range Test* (DMRT) at α=0.05

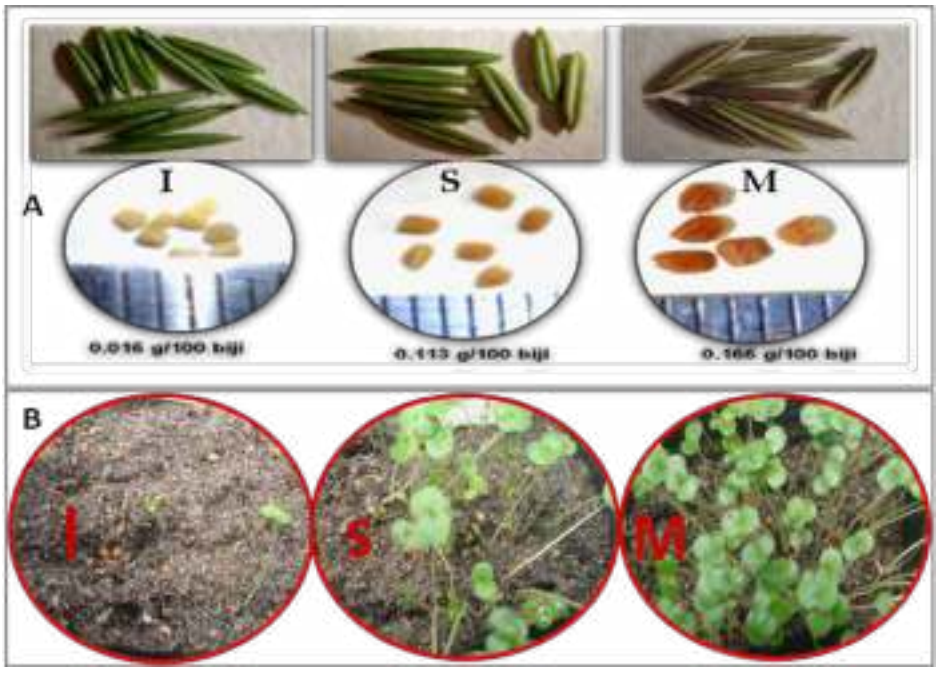

Figure 3. Sources of seeds **(A)** and their germination **(B)**: Immature(I),Semimature (S), Mature (M)

mean germinating time of mature seed of *Brassica napus* was lower (2.4 days) than immature seed (4.2 days). Statistically, the beginning time seed germination of the semimature and mature seed on sambiloto no significant different.

The end of seed germination of all maturity seed stages was no different statistically (Table 4) i.e 20.00 – 21.58 days. It was indicated that effect of seed maturity on the initial germination was determined in the first week, especially at the 1st - 3rd days. Starting of seed germination in this time was influenced by the lipids as or germination energy in seed and seed embrio maturity. After this stage, seed germination occured or continued normally as reported by Zhang et al., (2013) that the seed germination rate of *Brassica napus* at or after 7 th days was no significantly different. It was also showed in this experiment that the end of seed germination was no significantly different i.e. 20.00 -21.58 days.

This experiment indicate that seed maturity at harvesting is important to get high seed quality for sambiloto cultivation and development to increase plant production. Fruits of sambiloto was splitted and seeds scattered when the fruits over rippen so it is a problem for seed collection

in the field. By this research, mature seed can be collected from pinkish green – brown fruits coat to ensure and guarantee that the fruit coat no broken when harvesting or picking. Mature seed can also collected by sorting seeds if fruits harvesting conducted totally.

CONCLUSIONS

The highest percentage of seed germination of sambiloto (*Andrographis paniculata* (Burm.f.) Nees) was obtained from mature seed which was stored for twelve months (98.50%). The immature seed was not appropriate for propagation of sambiloto (*Andrographis paniculata* (Burm.f.) Nees) and indicated by the lowest seed germination percentage (4.25-9.75%). The fastest seed germination rate (3.88 days) was obtained from the mature seed which was stored for six months. This study is expected to be applied to improve plant genetic by seed propagation as well as increasing plant growth and yield of medicinal plant.

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REFERENCES

Ambika, S., Manonmani. V. & Somasundaram, G. (2014). Review on effect of seed size on seedling vigour and seed yield. *Research Journal of Seed Science*, 7(2), 31-38.

Atis, I., Atak, M., Can, E. & Mavi, K. (2011). Seed coat color effects on seed quality and salt tolerance of red clover (*Trifolium pratense*). *International Journal of Agriculture & Biology*, 13(3), 363–368

Ekpong, B. (2009). Effect of Seed Maturity, Seed Sto rage and Pregermination Treatments on Seed germination of cleome (*Cleome gynandra* L.). *Scientia Horticulturae,* 119(3), 236-240.

Gardner, F. P., Pearce, R. B., & Mitchell, R. L. (2008). Fisiologi Tanaman Budidaya. Diterjemahkan H. Susilo & Subiyanto. UI-Press. Jakarta.

Hartman, H. T., Kester , D. E., Davies, F. T. Jr. & Geneve, R. L. (2002). Plant Propagation: Principles and Practices. 7th edition. New Jersey: Prentice- Hall Inc.

Hu, Z. Y., Wang, X. F., Zhan, G. M., Liu, G. H., Hua, W. & Wang, H. Z. (2009). Unusually large oilbodies are highly correlated with lower oil content in *Brassica napus*. *Plant cell reports,* 28(4), 541-549.

Kadarusman, L. K. (2011). Peningkatan Kualitas dan Kuantitas Bahan Baku Jamu untuk Terapi Kedokteran Modern. *Simposium penelitianBahan Obat Alami XV*. Solo, 9-10 November 2011, 5-11.

Nautiyal, P. C., Misra, J. B., & Zala, P. V. (2010).Influence of seed maturity stages on germinability and seedling vigor on groundnut. *Journal of SAT Agricultural Research,* 8, 10 pp.

Negasu, G. B. (2015). Effect of harvesting *Jatropha curcas* L.seeds at different fruit maturity levels on germination, oil content and seed weight. *Net Journal of Agricultural Science,* 3(3), 74-80.

Solikin. (2008). Pertumbuhan generatif tanaman sambiloto (*Andrographis paniculata* (Burm.f.) ex Nees) Prosiding Seminar Tumbuhan Obat Indonesia XXXV. Pusat Penelitian Kimia LIPI. Ristek PUSPITEK,Serpong 2008, 155-159.

Solikin. (2014). Pengaruh lama penyimpanan terhadap perkecambahan biji *Stachytar pheta jamaicensis* (L.) Vahl. *Berita Biologi*, 13(1), 65-70.

Solikin. (2016). Perkecambahan biji sambiloto (*Andrographis paniculata* (Burm.f.) ex Nees) pada Beberapa warna dan berat biji. *In* : Hayati A. et al. Prosiding Seminar Nasional Biodiversitas VI: Biodiversitas untuk Pembangunan Berkelanjutan. Surabaya, 2 September 2016. Dapertemen Biologi. FMIPA Unair, 199-205.

Sutopo, L. (1988). *Teknologi Benih*. Jakarta: PT Rajawali.

Surya, M. I., Ismaini, L., Destri, D. & Normasiwi, S. (2016). An Effort of Mutation Breeding by Oryzalin and Gamma Rays on Wild Raspberry (Rubus spp.) in Cibodas Botanical Garden. *Biosaintifika: Journal of Biology & Biology Education*, 8(3), 331-335.

Takač, A., Popović, V., Glogovac, S., Dokić, V., & Kovač, D. (2015). Effects of fruit maturity stages and seed extraction time on the seed quality of eggplant (*Solanum melongena* L.). *Ratarstvo i povrtarstvo*, 52(1), 7-13.

Van Treuren, R., de Groot, E. C., & van Hintum, T. J. (2013). Preservation of seed viability during 25 years of storage under standard genebank conditions. *Genetic resources and crop evolution*, 60(4), 1407-1421.

Vera, C. L., Downey, R. K., Woods, S. M., Raney, J. P., McGregor, D. I., Elliott, R. H., & Johnson, E. N. (2007). Yield and quality of canola seed as affected by stage of maturity at swathing. *Canadian journal of plant science*, 87(1), 13-26.

Zhang, J., Cui, Y., Zhang, L., Wang, Y., Li, J., Yan, G., & Hu, L. (2013). Seed coat color determines seed germination, seedling growth and seed composition of canola (Brassica napus). *International Journal of Agriculture & Biology,* 15(3), 535–540.

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Alteration of Gills and Liver Histological Structure of *Cyprinus carpio* Exposed to Leachate

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Cyprinus carpio; gill; histological alteration; liver; leachate

Abstract

One of the main problems in the waste management in Indonesia is the treatment of leachate, which mostly dumped to the river This research is aimed to obtain information of histological alteration in gills and liver of *C. carpio* L. exposed to leachate. Measurements on the water quality parameters comprised water temperature, pH, and dissolved oxygen (DO). This research was conducted by exposing leachate to *C. carpio* for 96 hours. The concentration of leachate were 0 ppm, 80 ppm, and 100 ppm. Histological preparation were made on the gills and liver using 10% fixative Neutral Buffered Formalin and Ehrlich Hematoxylin-Eosin staining with qualitative observation descriptive analyses for discussion. The result showed that increasing water temperature is directly proportional to the leachate concentration in the aquaria, while the value of pH and DO inversely proportional to the leachate concentration. Damages on the gills with 80 ppm leachate concentrasion were identified as follows: fusion of secondary gill filaments and hyperplasia of epithelial cell, along with karyorrhexis and hydropic degeneration on the liver. Damages on the gills of fishes exposed to leachate with 100 ppm concentrasion were identified as follows: fusion of secondary gill filaments, hyperplasia of epithelial cell, congestion, and edema along with karyorrhexis, hydropic degeneration and *melanomacrophage centre* (MMC) found on the liver. The results of this study can be used as an overview of the impact of an environmental pollution by leachate as indicated from histological damage to the gills and liver of *C. carpio*, thus contribute significan information to aquaculture sector and endorse better waste management

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INTRODUCTION

Based on data from the Central Statistics Agency (BPS) since 2000 to 2015, the number of Indonesia's population has increased by 0.8% from ± 205.1 million to 255 million. This increasing number of the population is indirectly proportional to the increasing number of private consumption, which considerably contributes to the high volume of waste products (garbage). In Indonesia, 60% of the total waste being transported to the final disposal (TPA) is processed in the landfill (waste management in an open field), due to the easy and relatively inexpensive operation. This is the reason why TPA method has widely been used. However, this waste management gives a negative impact to the environment, one of which is the contamination of the waters due to the leachate (Susanto et al., 2004).

Leachate can be interpreted as liquid waste arising from the entry of water into the garbage heap and dissolving soluble materials contained in the heap (Hartati, 2007). Generally, leachate contains organic and inorganic substances with high concentration, such as ammonia, heavy metals, pathogenic or non-pathogenic bacteria, and microbial parasites (Susanto et al., 2004; Hartati, 2007). The entry of contaminants in water can result in alteration on community structure, decline on biomass or productivity, changes in behaviour, decreasing growth rate, disruption of reproductive system, and the effects, which can disrupt the balance of the aquatic ecosystem (Riani, 2012).

One place deemed potential for leachate production on a large scale is TPA Sarimukti, District Cipatat, West Bandung regency. TPA Sarimukti has a land area of ± 25.2 hectares with the volume of waste reaching to 1.200 tons per day. Sarimukti landfill exloads leachate into Citarum river that flows to the north (Suganda et al., 2012). The use of water from the river to fulfil the population's needs in daily lives is still dependeing on the surrounding community, one of which is for fishing culture activity. Resulting leachate contamination of the river by the living and farmed fish can accumulate pollutants in their bodies, which surely is not safe for consumption.

Fish can be used as one of the test animals in assessing biological effects of contaminants and environmental quality. This is due to low response to toxic substances (Neelima et al., 2015). According to Price (1979), assessment of toxicological effects of some chemical pollutants in the environment can be tested by using a species that lives in waters with pollution. One species of fish generally used as test animals is the common carp (*Cyprinus carpio* L.).

Histopathological changes have widely been used as a tool of biomonitoring health status of fish exposed to a mixture of chemicals on a laboratory scale research and investigation in the field (Stentiford et al., 2003; Boran et al., 2012). The gills are important organs in terms of respiration, guard acid-base balance, osmoregulation and excretion of residual nitrogen in fish (Evans et al., 2005). The occurrence of direct contact between the gills with outside environment makes the gills have high sensitivity, including in terms of stress or pressure, pollutants, and changes in the environment (Palar, 2004). In addition to the gills, liver can also be used as one of the objects of the pollution indicators. Fish liver plays an important role in vital functions in basic metabolism and serves as the main organ of accumulation, biotransformation, and excretion of contaminants in fish (Authman et al., 2013). Most of the toxic substances that enter the body after being absorbed by the cells will be carried to the liver by the portal vein liver. This is what causes the liver accumulate toxic substances in large quantities (Setyowati et al., 2010).

This research was conducted as a result of exposure biomonitoring leachate from the landfill Sarimukti to examine any changes of histological structure in gills and liver of *C. carpio*, and also to determine the concentration of exposure that can cause the greatest changes in histological structure. This research is expected to provide an overview and scientific information on the changes in gill and liver histology *C. carpio* exposed leachate so that it can serve as consideration for further study.

METHODS

Leachate samples obtained from landfill Sarimukti West Bandung regency, the reservoirs have already received treatment and are flowing towards the river (outlet). Leachate was first sterilised before being used in the experiments to avoid microbial activity during the experiments.

This study used carp fish (*Cyprinus Capio* L.) healthy male with an average weight of 90-100 grams as test animals. *C. Carpio* obtained from a fish pond Cileunyi, West Java. Before treatment, the fish were acclimated beforehand for 3 days at room temperature (25°C) and fed twice a day.

After acclimatisation, test animals were divided into three groups treatments in separated aquaria. The fish were exposed by water added by leachate with concentration of 0 ppm, 80 ppm and 100 ppm for 96 hours (4 days). At the end

of the experimental period, organs like gills and liver were isolated for the preparation of permanent incision histology (Karthigayani et al., 2014). Supporting physical and chemical parameters used in this study is the water temperature, acidity (pH), and dissolved oxygen (DO).

Gills and liver of *C. carpio* were isolated and then fixed in a solution of 10%-NBF (Neutral Buffered Formalin). Samples in NBF were removed from the fixative after 48 hours and then washed with water, then dehydrated by placing samples in rising concentrations of ethanol (70%, 80%, 90%, 95% and 100%), cleared using xylene, embedded using paraffin, sliced using a microtome with a thickness of 4-5 µm, and stained using Hematoxyline-Eosin (H&E) (Humason, 1967). The parameters observed in this study was the structure change of the gills and liver organ histologic of *C. carpio* and analysed both qualitatively and descriptively.

RESULTS AND DISCUSSION

The parameters used for the measurement of water quality in this research were the water temperature, acidity (pH), and dissolved oxygen (DO). These parameters were measured twice, namely at the time of acclimatisation and after 96 hours of administration of the leachate for each different concentration. The results of measurements of water quality parameter are available in Table 1.

The measurement results in each water temperature in this study were included in the category of optimal range of the living *C. carpio* based on the classification of Costa-Pierce et al. (1990), which is in the water temperatures ranging between 25-27 °C. Based on the data in Table 1, it can be seen that there was an increase in the water temperature in greater exposure concentrations of leachate. The higher the pollutant concentrations, the higher the water temperature became. The addition of pollutants to the water column also triggered the fish stress. It can be seen from the increase of the movement in swimming speed and operculum.

One of the causes of temperature change from the normal state becomes hotter or colder in the waters is the entry of pollutants like sewage into the waters. High and low temperature changes are depending on the magnitude of the number of triggers that appear or are contained in the water (Mamangkey, 2011). According to Campbell (2002), changes in water temperature are great or even 1 °C alone and sudden in onset, can be felt and affect the adaptation of fish. The influence of these changes can be seen from the changes in the activity of the body, swimming speed, and nerve stimulation.

Fish acclimatised to a relatively high temperature will increase their respiration rate, which can be observed from the changes in the movement of the fish operculum. High water temperature can lead to reduced DO gas, resulting in fish accelerating the movement of the operculum to get oxygen gas as quickly as they need the respiration process. In addition, the increase in water temperature can also cause increasing solubility of toxic substances such as crude oil pollutants and pesticides, as well as increasing toxicity of heavy metals (Fardiaz, 1992).

The DO was lower than the value of DO during acclimatisation time and keep decreasing during treatment obviously on aquaria with leachate concentration of 100 ppm. Decreased oxygen levels in the water may be due binding of oxygen with the chemical compounds and organic material contained in the leachate. This is aligned with Mamangkey (2011) that some of the factors affect the concentration of DO in water include chemical compounds, organic substances, stirring period of water, temperature, flow, and water depth.

Based on the classification of Costa-Pierce et al. (1990), DO value obtained at the time of acclimatisation and leachate concentration of 0 ppm can be categorised as the optimal environmental conditions for the life of *C. carpio*, namely above 6 mg/L. While the aquaria with leachate concentrations of 80 and 100 ppm, based on the grouping Swingle (1969) are included in the category of environmental conditions that are not

Table 1. Measurement Results of Temperature, DO, and pH During Acclimatisation and After 96 hours of Treatments

Parameter	Unit	During acclimatisation	Leachate concentration in the water		
			0 ppm	80 ppm	100 ppm
Water Temperature	°C	25	25	25.5	26.5
DO	mg/L	8.7	8.3	5.5	4.4
pH	-	7	7	6.5	6.5

favourable for *C. carpio* and therefore can cause physiological disorders, namely the DO value ranging from 4 to 5.5 mg/L.

Acidity of water for aquatic animal becomes one important factor in maintaining a state of homeostasis of animals, with the increase or the decrease which can cause acid-base balance, in regulation of ion and ammonia excretion (Wood, 2001). In general, leachate pH are ranging from 5.8 to 8.5 (Renou et al., 2008). A decrease in pH occuring in this study may be due to the chemical contents of the leachate from organic and inorganic materials, especially heavy metals, ammonium, and biological activity of microbial. Decreasing pH to 6.5 will cause stress on the fish, which can be seen from the increase of their swimming speed and body respiration. Fish can experience stress at pH ranging from 4.0 to 6.5 and from 9.0 to 11.0. In general, a pH of 7.0 to 8.5 is the optimum pH for fish life as according to biological productivity. Death of fish can occur at pH of less than 4.0 or more than 11.0 (Bhatnagar & Devi, 2013).

Based on the identification of preparations, gills exposed to leachate with a concentration of 0 ppm showed the result of a normal gill structure that is based on the book Atlas of Fish Histology by Genten et al. (2009). In the book, there was a description of some *C. carpio* gill compiler structures that are branching of the gill filaments (hemibranch), the primary lamella composed by chondrocytes, calcified cartilage, arteriolar blood and epithelium, as well as secondary lamella arranged by arteriolar blood, empty lumen, pillar cells, and epithelial cell layer that surrounds it. This gill histological structure can be seen in the Figure 1.A.

The identification results presented leachate gill preparations with a concentration of 80 ppm, obtained in the form of secondary lamella fusion and hyperplasia epithelial cell. As for the identification preparations, damages were found on the gills exposed to leachate with a concentration of 100 ppm in the form of lamella secondary fusion, hyperplasia epithelial cell, edema and congestion. Gill damage in the form of secondary lamella fusion and hyperplasia epithelial cell can be seen in the Figure 1.B, whereas edema in the

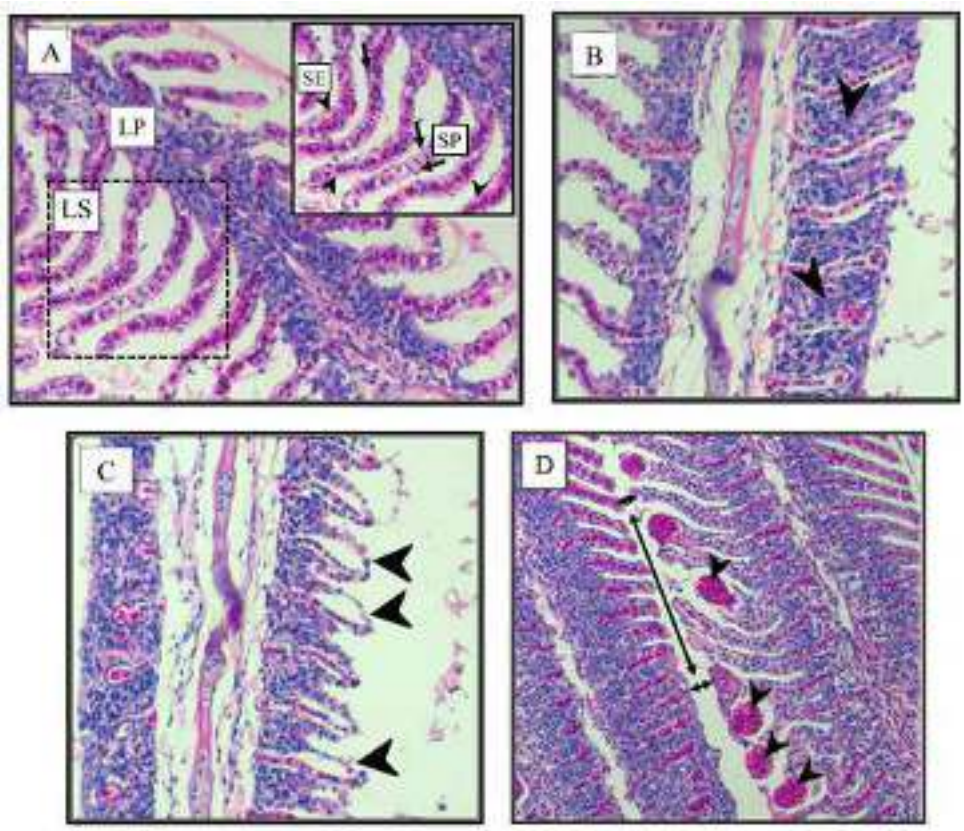

Figure 1. Histological Alteration in gills of *C. carpio* after leachate treatment. **[A]** Gill histology with no leachate exposure (control), showed primary lamella (LP), secondary lamella (LS), epithelial cell (SE), and pillar cell (SP) (Scale: 1,56 cm = 5,524 μm); **[B]** Hyperplasia epithelial cell (arrow) that causes fusion of secondary lamella (Scale: 1,56 cm = 5,524 μm); **[C]** Edema that causes epithelial lifting (arrow) (Scale: 1,56 cm = 5,524 μm); **[D]** Constriction between lamella (two arrows) and Congestion (arrow) (Scale: 1,56 cm = 5,524 μm).

Figure 1.C and congestion in 1.D.

Based on the comparison of damages of the gills histology preparation, it is known that the greatest damages happened in the leachate exposured to a greater concentration, i.e., 100 ppm. This is in line with the statement of Siregar et al. (2012), Setiawan et al. (2013), as well as Kavidha & Muthulingam (2014), that the magnitude of accumulated toxicant found in fish organs is related to the concentration and length of exposure to the toxicated fish. It will be directly proportional to the level of organ damage.

In this study, fusion lamella observed from the primary or secondary lamella was fused with another lamella. Chezhian et al. (2012) and Devaraj et al. (2014) reported the closer move of gill epithelium (via cell hypertrophy) is sometimes considered as one indicator of cell degeneration and eventually signs of early necrosis. The pooling caused the gill surface area reduce the respiration process, so that the respired oxygen decreased. Fusion and hyperplasia of the gill lamella can be triggered by the effects of the toxins that alter cell glycoproteins including mucus, thereby affecting the negative charge of the epithelium and thus supporting the adhesion to the adjacent lamella (Ferguson, 1989). Camargo & Martinez (2007) stated that change such as fusion secondary lamella is one example of a self-defense mechanism. Basically, the increase in the distance between external environment and blood, would be a barrier to the entry of excessive contaminants, but the distance change between the lamella can lead to reduced capability in capturing oxygen that can be transmitted by blood.

Hyperplasia is a state of increasing number of cells in primary lamella due to the division of cells' excessive chloride. In this study, the alleged contents of organic and inorganic materials in leachate, especially for heavy metals and ammonium, are considered as the cause of the hyperplasia of epithelial cell. The same histological changes have also been reported in some studies of *C. carpio* describing ammonium (Devaraj et al., 2014; Chezhian et al., 2012) and the exposure of lead (Pb) (Natalia, 2007). Chemical pollutants and heavy metals that mostly cause hyperplasia are Cadmium (Cd), Cuprum (Cu) and Zinc (Zn) (Saputra et al., 2013). Due to the lamella hyperplasia in primary cause of secondary lamella space between the drains and the enclosed space, mucus production leading to the intake of oxygen into the blood is reduced and creates impaired immune regulation.

Edema was observed in the individuals exposed to leachate concentration of 100 ppm. Based on the results, change of gills with edema is characterised by a white membrane, which does not contain any fluid (former swelling), while visible basement membrane began to stretch off. Edema is swelling of the cell that occurs due to excessive accumulation of fluid in the tissues and it cause the separation between epithelial layer and the underlying systems mast cells that could lead to the destruction of its secondary lamella structure (epithelial lifting). It is a form of physiological adaptation of the fish when experiencing interference from the environment. This damage causes function deficiencies and breathing difficulty in the gills, so that the metabolism began to fail (Robert, 2001; Fitriawan, 2010). In this study, the occurrence of edema could be predicted due to the toxicants of the leachate that have penetrated the gills resulting in cell irritation. Ploeksic et al. (2010) reported that edema often occurs as a result of chemical pollutants exposure, such as heavy metals, metalloids, pesticides, and the use of therapeutic substances (formaldehyde and H_2O_2). Similar observations were reported by Susanah et al. (2013) with *Chanos chanos* (Milkfish) exposed to factory waste in the village pond site Tapak Area Tugu- Tugurejo district of Semarang. Toxic substances in this pond water entered to the gills and made the cells become irritative so that they will cause swelling.

Congestion or blockage of blood vessels can be triggered by the breakdown of cell structure pillars resulting in increasing blood flow in the lamella. Congestion obtained in this study can be caused by metal contents in leachate. This is consistent with the results from Hadi & Alwan (2012) using *Tilapia zillii* exposed with aluminium, a metal that affects the permeability of cell membranes and lead to the occurrence of resistance in the ion exchange system, which in turn lead to the disruptions in the fluid transport into and out of cells. Similar observations were reported by Authman et al. (2013) with *Clarias gariepinus* in heavy metal exposure and also by Salim (2014) with *C. carpio* exposed to heavy metals, pesticides, fertiliser waste, and debris from the river in Kharatrad Gramat Ali river, Basra province.

In the observation of liver histology *C. carpio* some structures making up the liver, the hepatocytes, central venous, and sinusiod were found and did not reveal any changes in the cells. On *C. carpio* liver histology images, sinusiod coated with endothelial cells formed a thin sheet. Cell nuclei elongated and protruded into lumen sinusoidal. The endothelium has small pores (Genten et al., 2009). Incision results of liver histology *C. carpio*

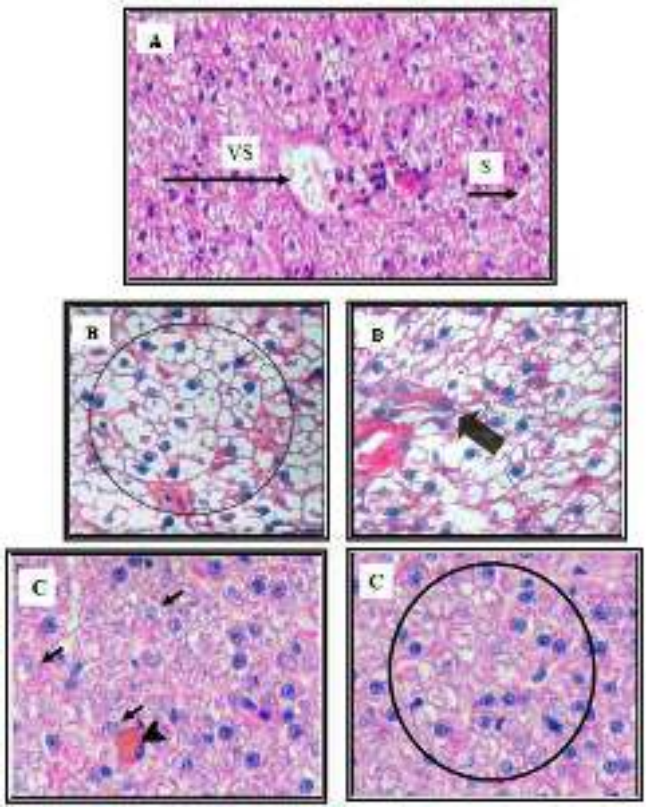

Figure 2. Histological alteration of liver of *C. carpio* after leachate treatment. **[A]** Histologic of liver with no leachate exposure (control), showed vena centralis (VS) and sinusoid (S) (scale: 1,56 cm = 5,524 μm); **[B]** Histologic of liver after leachate exposure of 80 ppm, showed hydropic degeneration (circle) and karyorrhexis (arrow) (scale: 0,66 cm = 1,385 μm); **[C]** Histologic of liver after leachate exposure of 100 ppm, showed karyorrhexis (arrow), melanomacrophage centre (big arrow), and hydropic degeneration (circle) (Scale: 0,84 cm = 1,385) μm).

exposed to leachate with concentrations of 0 ppm showed normal liver histology with characteristics as described previously and it can be seen in the Figure 2.A.

The observation of liver exposed to leachate concentration of 80 ppm showed that there was a change in the form of karyorrhexis cells and hydropic degeneration, while at a concentration of 100 ppm, there was a change in the form of karyorrhexis cells, melanomacrophage center (MMC), and hydropic degeneration. Histology liver with leachate exposure to 80 ppm can be seen in Figure 2.B, whereas changes in fish liver cells with exposure to 100 ppm leachate can be seen in Figure 2.C.

In fish liver cells exposed to leachate concentration of 80 ppm and 100 ppm, there was karyorrhexis found, which is considered as a marker of cell death. Cell death can be defined as a permanent loss of plasma membrane integrity. One type of cell death is apoptosis, which can be determined by the changes in the morphological characteristics of nuclei, one of which is fragmentation (karyorrhexis). These changes occur prior to the disappear of plasma membrane integrity (Golstein & Kroemer, 2006). Karyorrhexis is one of the characteristics of cell death characterised by condensation and rupture of the nucleus into particles or nucleus fragmentation (Lescher, 2011). Karyorrhexis can easily be recognised when the nucleus fragmentation process has been completed along with all parts of the destroyed cell nucleus, as fragments will usually appear with irregular shapes (Obe, 1994).

Karyorrhexis is expected to happen to fish due to low oxygen level in the water, called hypoxia. Hypoxia or lack of oxygen is a phenomenon that occurs in aquatic environments where dissolved oxygen (DO; molecular oxygen dissolved in the water) level reduces, affecting aquatic organisms (Mallya, 2007). This is supported by Geng's statement (2003), which stated that decreasing oxygen level in tissue can cause lesions in the form of necrosis and apoptosis in the organ.

Fish liver cells exposed to leachate 80 ppm and 100 ppm were also shown hydropic degene-

ration. Hydropic degeneration occured due to a disturbance of active transport that has caused inability of the cell to pump out Na^+ ion, resulting the Na^+ ion concentration in the cell to increase. It affects the process of osmosis that causes an influx of water into the cells, causing the cell to swell as the vacuole and nucleus were getting enlarged, granulars in the nucleus can also be seen quite clearly (Robbins et al., 2007).

Hydropic degeneration was also found in liver cells due to the content of ammonia in the leachate. This is supported by the research conducted by Benli et al. (2008), which indicated that the Nile Tilapia (*Oreochromis niloticus* L.) exposed to ammonia has a form of cell changes as hydropic degeneration happens in liver cells. The liver becomes the main organ of various metabolic pathways. Toxic influence of chemicals in general occurs primarily in the liver. Ammonia can be carried away by bloodstream into the liver as nutrient and follow the metabolic pathways (Kucuk, 1999, in Benli et al., 2008). Ammonia and alkali are a strong indicator of toxicity in aquatic environments. Although ion ammonia (NH_4^+) cannot penetrate the cell wall of organism, it can be potentially toxic to fish with the form of molecules (NH_3) which can easily penetrate the tissue, especially when the concentration is quite high (Svobodova et al., 1993 in Emenike et al., 2012).

Melano-Macrophage Centre (MMC) was only found in fish liver cells exposed to 100-ppm leachate concentration. Melano-Macrophage is a type of immune cell that is typical Teleostei and generally found in the spleen. This is phagocytes containing a number of pigments, including melanin (dark brown), hemosiderin, ceroid or lipofuscin (pink yellow to golden brown) located in the vacuole. MM and MMC were also found in the kidney and liver. The form of MMC is considered as a cleaner structure but its role in the immune system is still ambiguous (Genten et al., 2009).

C. carpio exposed to leachate concentration of 100 ppm indicated the presence of MMC in liver cells, which was related to stress and unhealthy condition of the fish due to the leachate exposure. This was in line with Gentens statement et al. (2009) that fish with chronic stress and in unsanitary conditions tend to have a lot more MMC. Leachate contains a number of organic contaminants, so it surely affects water quality and the organisms around. Goyer & Clarkson (2001) also stated that the low oxygen level combined with toxic substances can cause stress response in aquatic ecosystems.

The results of this study are expected to be used as an overview of the impact of an environmental pollution by leachate as indicated from histological damage to the gills and liver of *C. carpio*. So it can give information about the health risk of fish consumption for consideration in the future in better waste management, especially in terms of leachate treatment before being discharged into the water body. It also remains that carp supposed to regional ecological and economic importance in Indonesian.

CONCLUSIONS

The result of this study showed that the greatest damages happened in the leachate exposured to a greater concentration, i.e., 100 ppm. Leachate with concentrations of 80 ppm causes damage in the form of lamella secondary fusion and hyperplasia of epithelial cell in the gills, along with karyorrhexis and hydropic degeneration on the liver. Leachate concentration of 100 ppm causes damage in the form of lamella secondary fusion, hyperplasia of epithelial cell, congestion, and edema in the gills, along with karyorrhexis, hydropic degeneration, and melanomacrophage centre on the liver.

REFERENCES

Authman, M. M. N., Ibrahim, S. A., El-Kasheif, M. A. & Gaber, H. S. (2013). Heavy Metals Pollution and Their Effects on Gills and Liver of the Nile Catfish *Clarias gariepinus* Inhabiting El-Rahawy Drain, Egypt. *Global Veterinaria*, 10(2), 103-115.

Badan Pusat Statistik. (2015). *Penduduk Indonesia menurut Provinsi 2000, 2001, 2002, 2003, 2004, dan 2015*. [Online]. Available at: http://www.bps.go.id/linkTabelStatis/view/id/1267, (Diakses Maret 2016).

Benli, A. C. K., Koksal, G. & Ozkul, A. (2008). Sublethal Ammonia Exposure of Nile Tilapia (Oreochromis niloticus L.): Effects on Gill, Liver and Kidney Histology. *Chemosphere*, 72(9), 1355-1358.

Bhatnagar, A. & Devi, P. (2013). Water Quality Guidelines for the Management of Pond Fish Culture. *International Journal of Environmental Sciences*, 3(6), 1980-2009.

Boran, H., Capkin, E., Altinok, I. & Terzi, E. (2012). Assessment of acute toxicity and histopathology of the fungicide captan in rainbow trout. *Experimental and Toxicologic Pathology*, 64(3), 175-179.

Camargo, M. M. P. & Martinez, C. B. R. (2007). Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. *Neotropical Ichthyology*, 5(3), 327-336.

Campbell. (2004). *Biologi, Edisi Kelima-Jilid 3*. Jakarta: Erlangga.

Chezhian, A., Senthamilselvan, D. & Kabilan, N. (2012). Histological Changes Induced by Ammonia and pH on The Gills of Fresh Water Fish Cyprinus carpio var. communis (Linnaeus). *Asian Journal of Animal and Veterinary Advances*, 7(7), 588-596.

Costa-Pierce, B. A., Soemarwoto, O., Roem, C. M. & Herawati, T. (1990). *Water Quality Suitability of Saguling and Cirata Reservoirs for Development of Floating Net Cage Aquaculture.* Reservoir Fisheries and Aquaculture Development for Resettlement in Indonesia. ICLARM Tech. Rep. 23

Devaraj, S., Arulprakasam, C., Kandhan, A. P., Neelamegam, K. & Kalaiselvan, R. (2014). Toxicological effects of ammonia on gills of *Cyprinus carpio* var. *communis* (Linn.). *Journal of Coastal Life Medicine*, 2(2), 92-98.

Emenike, C. U., Fauziah, S. H. & Agamuthu, P. (2012). Characterization and Toxicological Evaluation of Leachate from Closed Sanitary Landfill. *Waste Management & Research*, 30(9), 88-897.

Evans, D. H., Piermarini, P. M. & Choe K. P. (2005). The Multifunctional Fish Gill: Dominant Site of Gas Exchange, Osmoregulation, Acid-Base Regulation and Excretion of Nitrogenous Waste. *Physiological Review*, 85(1), 97-177.

Fardiaz, S. (1992). *Polusi Air dan Udara*. Yogyakarta: Kanisius.

Ferguson, H. W. (1989). *Gills and pseudobranchs. In: Ferguson, H.W., (ed.), Text book of systemic pathology of fish, 1st ed*. Canada: Lowa state University Press.

Fitriawan, F. (2010). Analisis Perubahan Mikroanatomi dan Variasi Pola Pita Isozim Pada Insang dan Ginjal Kerang Air Tawar *Anodonta woodiana* Terhadap Paparan Logam Berat Kadmium. *Tesis*. Surakarta: Program Studi Biosain. Universitas Sebelas Maret.

Genten, F., Terwinghe, E. & Danguy, A. (2009). *Atlas of Fish Histology*. United States of America: Science Publishers.

Golstein, P. & Kroemer, G. (2006). Cell Death by Necrosis: Towards a Molecular Definition. *TRENDS in Biochemical Sciences*, 32(1), 38-43.

Hadi, A. A. & Alwan, S. F. (2012). Histopathological Changes in Gills, Liver and Kidney of Fresh Water Fish, *Tilapia zillii*, Exposed to Aluminum. *International Journal of Pharmacy & Life Sciences*, 3(11), 2071-2081.

Hartati, E. (2007). Studi pengolahan kandungan ion logam (Fe, Mn, Cu, Zn) lindi sampah oleh zeolit. *Jurnal Sains MIPA edisi khusus,* 13(1), 29-34.

Humason, G. L. (1967). *Animal Tissue Techniques*. United States of America: W. H. Freeman and Company.

Karthigayani, T., Denis, M., Remy, A. R. A. & Shettu, N. (2014). Histological Study of the Intestine and Liver Tissues in the Fish *Oreochromis mossambicus* Exposed to Cypermethrin. *Journal of Modern Biotechnology*, 3(4), 48-54.

Kavidha, K. & Muthulingam, M. (2014). Lead Acetate Induced Glycogen Level Alterations in Gill and Kidney Tissues of Freshwater Fish *Cyprinus Carpio* (Linn.). *International Journal of Modern Research and Reviews*, 2(11), 517-521.

Lescher, P. J. (2011). *Pathology for the Physical Terapist Assistant*. Philadelphia: F.A. Davis Company.

Mallya, Y. J. (2007). *The Effects of Dissolved Oxygen on Fish Growth in Aquaculture*. Tanzania: Kingolwira National Fish Farming Centre.

Mamangkey, J. J. (2011). Konservasi Spesies Ikan Endemik Butini (*Glossogobius matanensis*) di Danau Towuti, Sulawesi Selatan. *Prosiding Forum Nasional Pemacuan Sumber Daya Ikan* III, 18 Oktober 2011.

Natalia, M. (2007). Pengaruh Plumbum (Pb) Terhadap Struktur Insang Ikan Mas (*Cyprinus carpio* L.). *Jurnal Perikanan dan Kelautan*, 12(1), 42-47.

Neelima, P., Kumar, C. L. A., Rao, J. C. S. & Rao, N. G. (2015). Histopathological Alterations in Gill, Liver and Kidney of *Cyprinus carpio* (Linn.) Exposed to Cypermethrin (25%EC). *International Journal of Advanced Research in Biological Sciences*, 2(2), 34-40.

Obe, G. (1994). *Advances in Mutagenesis Research 5*. Berlin: Springer.

Palar, H. (2004). *Pencemaran dan Toksikologi Logam Berat*. Jakarta: Rineka Cipta.

Poleksic, V., Lenhardt, M., Jaric, I., Djordjevic, D., Gacic, Z., Cvijanovic, G., & Raskovic, B. (2010). Liver, Gill, and Skin Histopathology and Heavy Metal Content of the Danube Sterlet (*Acipenser ruthenus* L. 1758). *Enviromental Toxicology and Chemistry*, 29(3), 515-521.

Price, D. R. H. (1979). *Fish as Indicators of Water Quality.* Toronto: John Wiley and Sons. Chicester.

Renou, S., Givaudan, J. G., Poulain, S., Dirassouyan, F. & Moulin, P. (2008). Landfill Leachate Treatment: Review and Opportunity. *Journal of Hazardous Materials*, 150(3), 468-493.

Riani, E. (2012). *Perubahan Iklim dan Kehidupan Biota Akuatik (Dampak Pada Bioakumulasi Bahan Berbahaya dan Beracun & Reproduksi).* Bogor: IPB Press.

Robert, R. J. (2001). *Fish Pathology 3rd Edition*. London: W.B. Saunders.

Salim, F. (2014). Survey on Histopathological Changes in Different Organs of Local Freshwater Fishes in Basra Province. *International Journal Academic Research Multidisciplinary*, 2(10), 236-256.

Saputra, H. M., Marusin, N. & Santoso, P. (2013). Struktur Histologis Insang dan Kadar Hemoglobin Ikan Asang (*Osteochilus hasseltii* C.V) di Danau Singkarak dan Maninjau Sumatera Barat. *Jurnal Biologi Universitas Andalas*, 2(2), 138-144.

Setyowati, A., Hidayati, D., Awik, P. D. N & Abdulgani, N. (2010). *Studi Histopatologi Hati Ikan Belanak (Mugil cephalus) di Muara Sungai Aloo Sidoarjo*. Surabaya: Program Studi Biologi Fakultas Matematika Ilmu Pengetahuan Alam. ITS.

Siregar, Y. I., Zamri, A. & Putra, H. (2012). Penyerapan Timbal (Pb) Pada Sistim Organ Ikan Mas

(*Cyprinus carpio* L). *Jurnal Ilmu Lingkungan*, 6(1), 43-51.

Stentiford, G. D., Longshaw, M., Lyons, B. P., Jones, G., Green, M. & Feist, S. W. (2002). Histopathological biomarkers in estuarine fish species for the assessment of biological effects of contaminant. *Marine Environmental Research*, 55(2003), 137-159.

Suganda, B., Iskandarsyah, M., & Sapari, M. (2012). *Prediksi Arah Pencemaran Air tanah Akibat Tempat Pembungan Sampah Akhir di Daerah Sarimukti dan Sekitarnya*. Sumedang: Fakultas Teknik Geologi. Univeritas Padjadjaran.

Susanah, U., Santosa, K., & Utami, N. (2013). Struktur Mikroanatomi Insang Ikan Bandeng di Tambak Wilayah Tapak Kelurahan Tugurejo Kecamatan Tugu Semarang. *Biosaintifika: Journal of Biology & Biology Education*, 5(1). 65-73.

Susanto, J. P., Ganefati, S. P., Muryani, S., & Istiqomah, S. H. (2004). Pengolahan lindi (leachate) dari TPA dengan sistem koagulasi-biofilter anaerobik. *Jurnal Teknik. Lingkungan P3TL-BPPT*, 5(3), 167-173.

Swingle, H. S. (1969). *Methods of Analysis for waters, organic matter and Pond Bottom Soils Used in Fisheries Research*. Auburn: University Auburn.

Wood, C. M. (2001). *Toxic Responses of the Gill. In: Target Organ Toxicity in Marine and Fresh Water Teleosts, Sclenk, D. and W.H. Benson (Eds.)*. New York: Taylor and Francis.

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Effect of Temperature Shock on the Triploidization Success of Seurukan Fish (*Osteochilus vittatus*)

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Abstract

Seurukan fish (*Osteochilus vittatus*) has many advantages, besides the fish also has disadvantages which are the slow growth, so the temperature shock of triploidization technique was expected to solve the problem. The objective of the present study was to obtain an effective temperature to increase of triploidization success of *seurukan* fish (*Osteochilus vittatus*). The experimental method and completely randomized design model were used in this study. Five levels of temperature shocks at three replicates were tasted: 4°C (cold), 6°C (cold), 28°C (normal), 35°C (heat) and 37°C (heat). The sperms and eggs were fertilized in the plastic jar then a total 100 of fertilized eggs (zygotes) were taken randomly 3 minutes after fertilization and soaked in respective temperature for 90 seconds, and then incubated in incubation jars at the water temperature of 28-29°C. The results showed that the temperature shock gave the significant effect on the hatching and the success of triploidization success ($P<0.05$), but did not give the significant effect the fertility and survival rates ($P>0.05$). The triploid fish can be achieved using cold and heat shock, but the higher triploid fish was recorded at 37°C was the best temperature recommended for triploidization of *Seurukan* fish.

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INTRODUCTION

Bonylip barb, *Osteochilus vittatus* or locally known as seurukan fish is originated from Southeast Asia including Indonesia. This is one of the popular freshwater fish in Indonesia, because of its taste and reasonable price. Several basic studies on bonylip barb have been reported by researchers, for example fecundity of bonylip barb (*Osteochilus vittatus Cyprinidae*) in different waters habitats (Syandri et al., 2015), *Osteochilus vittatus* and *Puntius javanicus* as an agen of biological in Maninjau Lake (Syandri H, 2004), reproduction aspects of sasau fish (Hampala sp.) and lelan fish (*Osteochilus vittatus*) in Singkarak lake (Uslichah and Syandri, 2003). However, the genetic improvement has not been conducted on this fish species. Also, fish farmers claim that the growth performance of the fish was low in captivity. Therefore, it is crucial to develop a practical biotechnology through genetic modification, especially chromosome-set manipulation to overcome this problem.

According to Muchlisin et al. (2014) seurukan fish has potency as a species target for aquaculture, and therefore the cultivation of this species has been initiated in Indonesia especially in Aceh Province. Several studies have been conducted on *O. vittatus*, for example genetic diversity (Mulyasari et al., 2010), breeding (Muchlisin et al., 2014; Adami et al., 2016), feeding ration (Asma & Muchlisin, 2016), application of union (*Allium cepa*) as a prebiotic source (Mayana et al., 2016). The principles of genetic modification method are used to increase production and quality of seed (Nurasni, 2012). Induced triploidy is the only effective method currently available for mass production of reproductively sterile *seurukan* fish (*Osteochilus vittatus*) for aquaculture.

Triploidization is a simple process of genetic technique to establish an individual with three sets of chromosomes (Risnandar, 2001). As the name implies, triploids have three sets of chromosomes in their somatic cells rather than the normal two sets (diploids). Although there are a few naturally occurring triploid species of fish that exist as all-female populations with unique reproductive strategies (Purdom, 1984), for most species triploidy is not a natural condition. Tetraploidy has played a role in the evolution of many widespread and economically important groups of fish, including salmonids (Allendorf and Thorgaard, 1984). The individuals of the triploids are sterile, and therefore the energy requirement for gonadal development are decreased and switched for growing (Lawson and Ishola, 2010). The triploid individual can be generated by preventing the release of polar body II (PB II) on the eggs; this process can be achieved by temperature shock (Hartono et al., 2013).

Study of triploidization using temperature shock technique has been performed on some fish species such as glass catfish (Alawi et al., 2009), nile tilapia (Mukti et al., 2009), goldfish (Mukti, 2005), African catfish (Nurasni, 2012; Olele & Tighiri, 2013), iridescent shark (Puji et al., 2012), yellow catfish (Emilda, 2003), basa catfish (Risnandar, 2001), grass crap (Cassani and Caton, 1985), and Atlantic salmon (Leclercq et al., 2011; Benfey, 2001; Galbreath, et al., 1994). These previous studies showed that growth performance of triploidy fish was faster than diploidy fish. Therefore, a triploidization technique is very promising to boost the growth performance of bonylip barb. This paper was the first reported in combining of the effect of heat and cool shocks on the fertilization, hatching and triploidy successful rates of bonylip barb *O. vittatus*.

This study aims to determine an effective temperature to increase triploidization success and survival rate of *seurukan* fish (*Osteochilus vittatus*). Then the results are expected to provide information of *seurukan* fish triploidization.

METHODS

The study was conducted in hatchery of Batee Iliek, Bireuen District and Research Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences of Syiah Kuala University, Banda Aceh, Indonesia. The completely randomized design was used in this study. Five levels of temperature i.e. 4°C, 6°C, 28°C, 35°C, 37°C were tested with three replications.

The brood fish was injected with Ovaprim at the dosage of 0.1 ml kg^{-1} body weight for male and 0.2 ml kg^{-1} body weight for female. Then the abdomen was stripped gently 9 hours after hormone injection and the eggs and sperm were placed into the beaker (100 ml) and kept in an ice box (4°C) separately. The sperms were diluted with physiological solution at ratio 1: 20 of sperm to the extender (v/v).

Approximately 4 ml of eggs and 1 ml diluted sperm were mixed (1: 4 v/v of sperm: eggs), then two drops of tap water were added and mixed homogenously using a feather, and the sperm and eggs were left in contact for two minutes (Muchlisin et al., 2010). The fertilized eggs were taken randomly and put into the glass slides (every slide has 200 eggs); the slide was immersed into water batch at different temperature for 90

seconds. The slides were removed from the batch then incubated in the plastic jars at temperature 28 °C for 48 h.

The hatching eggs were monitored for 48 hours by interval of two hours, and the number of larvae was recorded 48 hours after incubation. The larvae of seurukan fish were reared in a basin with a water volume of 800 ml for 60 days. The larvae fed on tubifex three times a day until they reach point of satiation (ad libitum).

The successfull of triploid fish was examined based on the size of red blood cell nucleus by the fish age of 60 days. Then the fish was anesthetized in cold waters for 5 min, the tail fin was cut and the blood sample was dripped on object glass for subsequent evaluation. Then the object glass was dripped with 95% alcohol and then re-dripped with 10% Giemsa solution. Then the sample was rinsed with distilled water and dried at room temperature for 30 minutes before observing under the stereo microscope. According to Nurasni (2012), the diameter of the nucleus of red blood cell of diploid is between 9 µm - 10 µm, while 11 µm – 13 µm for triploid. In addition, the fertilization, hatching and survival rates were also analyzed. All data were subjected to analysis of variance (ANOVA), followed by the comparison of means using Duncan's multiple range test at a 95% confidence level (P = 0.05).

RESULTS AND DISCUSSION

Table 1 showed the average percentage of fertility, hatching rate, survival rate, and the success of triploidy at the shock temperature of 4 C, 6 °C, 28 °C (control), 35 °C and 37 °C. The average percentages of fertility were 84.67%, 86.67%, 84%, 80.33 % and 83%; while the hatching rate were 80.67%, 85.33%, 79.67%, 69.33%, and 67%. The survival rate respectively were 83%, 82.67%, 84%, 79.33% and 81.33%; whereas the triploidization success rate were 73.33%, 73.33%, 0%, 53.33% and 86.57%. Generally, it can be concluded that the highest result of fertility and hatching was found at 6 °C, while the triploidy success and body weight are best shown at 37 °C.

The results of ANOVA test showed that the temperature shock gave a significant effect on hatching rate and success of triploidization on seurukan fish, *O. vittatus* (P<0.05). However, it did not give the significant effect on the fertility and survival rates (P>0.05). The study showed that the highest fertility rate was found at the temperature of 6°C, but this value was not significantly different with other treatments. The hatching rate was also highest in the treatment of 6°C; this value was only significantly different with 35°C. Then the highest survival rate was recorded at 28°C, but this value was not significantly different with other treatments. In addition, the highest success of triploidization was found in the treatment of 37°C. However, this treatment was not significantly different with 4°C and 6°C. At least, the highest fish weight gain after 15 days was found at 37°C, but this treatment was not significantly different with 4°C (Table 1). The average value on the same column with different superscript is significantly different (P <0.05).

The results revealed that the highest fertility and hatching rates were obtained at heat shock of 6°C, and the value was decreased slightly when the temperatures were increased or decreased. It was alleged due to the temperature rising that affected the embryos metabolism, thus caused the embryos dead due to lack of oxygen supply. This speculation was supported by Fujaya (2000) who stated that the temperature increase for heat shock treatment caused an increase in metabolic rate 3 - 5 times higher. Thus, it would be increased the oxygen consumption and resulted in embryos death when oxygen availability is less.

The decline in fertility and hatching rates

Table 1. Average percentage and standard deviation (± SD) of fertility, hatching rate, survival rate, success of triploidization and weight gain of *seurukan* fish *(Osteochilus vittatus).*

Temp.	Treatment	Fertility (%)	Hatching (%)	Survival (%)	Triploidization success (%)	Weight of fish after 15 days
4°C	Cold	84.67±2.51^{a}	80.67±7.23^{b}	83.00±2.64^{a}	73.33±2.22^{bc}	1.88±0.58^{bc}
6°C	Cold	86.67±3.51^{a}	85.33±3.51^{b}	82.67±2.08^{a}	73.33±2.28^{bc}	1.70±0.42^{b}
28°C	Normal (control)	84.00±3.60^{a}	79.67±6.65^{b}	84.00±2.64^{a}	0.00±0.61^{a}	1.43±0.30^{a}
35°C	Heat	80.33±2.51^{a}	69.33±1.15^{a}	79.33±0.57^{a}	53.33±1.97^{b}	1.65±0.28^{ab}
37°C	Heat	83.00±3.00^{a}	67.00±2.00^{a}	81.33±1.52^{a}	86.67±1.90^{c}	2.09±0.62^{c}

in thermal shock probably is due to the inhibition of eggs development that caused the death of the embryo, thus reducing the average percentage of fertility and hatching. This assumption was supported by Arsianingtyas (2009) who stated that heat temperatures shock could damage the spindle thread that was formed during the process of cell division of the zygote. Also, Nurasni (2012) reported that the decrease in the hatching rate of African catfish (*Clarias gariepinus*) probably caused by a heat shock treatments that affected on enzyme activities due to the enzyme defection at high temperatures; thus the egg's cytoplasm will be broken that leads to eggs mortality.

Febrianto (2012) reported that the optimum ranges of temperature shock for better hatching of nilem fish (the local synonym name for *O. vittatus*) eggs in the triploidization process are between 23°C to 30°C (heat thermal). However, in this study we found that the best water temperature shock for seurukan fish (*O. vittatus*) eggs was 37°C (heat), but this value was not different significantly with 4°C and 6°C. Therefore, the study showed that the triploid of seurukan fish could be produced by both cold and heat shock treatments; but the fertilization, hatching and survival rates slightly higher in cold shock than in heat shock treatments. According to Risnandar (2001), the treatment of heat shock gave detrimental effect on the survival because the heat shock at the phase of meiosis II may cause damage to the membrane of the embryo. This provision can produce to an abnormal individual that led to a decrease in survival in the early life of larvae.

The observation of the triploidization success was done; by observing the size of red blood cells of the fish. The size of red blood cell of the triploid fish is larger than the common fish (diploid). Alawi et al. (2009) stated that the measurement of red blood cells had been widely used in studies determining the ploidy level of fish, because the increase of chromosomes triploid fish affect the size of red blood cells, including the cell nucleus that will be enlarged.

The study showed that the diameter of the red blood cells of seurukan diploid fish ranges from 7 µm – 9 µm (Figure 1 B), while the size of red blood cells of triploid fish ranges from 10 µm – 13 µm (Figure 1 A). According to Nurasni (2012), the diameter of the red blood cell of hybrid diploid African catfish was between 9 – 10 µm. These results indicated that the effect of temperature shock could be used in the triploid fish production of seurukan fish (*O. vittatus*).

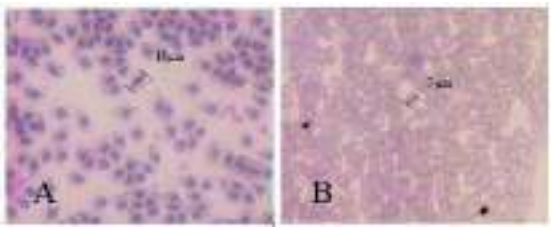

Figure 1. The red blood cells of triploid fish (A) and diploid fish (B) fish with magnification of 400X

Based on the observation of the weight gain, the averages weight of the fish in the treatment of 4°C, 6°C, 28°C, 35°C and 37°C were 1.88 g, 1.70 g, 1.43 g, 1.65 g and 2.09 g, respectively (Table 1). The results showed that the weight gain of triploid fish was higher than normal fish. According to Fujaya (2000), triploid fish is infertile, and therefore the metabolism energy can be saved and used for growing resulted in higher weight gain compared fertile fish as recorded in this study.

However, in general, the success of triploidization in the heat shock was higher than in the cold shock. This is presumably due to the cold shock temperature that gives slow propagation and resulted in many zygotes failure to form triploids. This is supported by Nuraini and Alawi (2008) who stated that low propagation of temperature would cause polar body (PB) II of being apart from the duplication of chromosomes into 3N. Thus, many zygotes formed as normal individuals.

The study indicated that the application of triploidization for seurukan fish in the heat shock at 37°C could be used to increase the growth which is slow become faster with the higher survival rate is still above 80%. It means that the triploidization applies to produce seurukan fish on fish farming can be implemented for fisherman.

This study tried to use both heat and cold shock to determine the viability, triploidy success and best temperature used on the triploidization of seurukan fish (*Osteochilus vittatus*). The results showed the best fertility and hatching rate were found at the temperature of 6°C, while the temperature of 37°C is recommended for triploidy success.

CONCLUSIONS

Induced triploidy is the only effective method currently available for mass production of seurukan fish (*Osteochilus vittatus*) sterile reproductively for aquaculture. The temperature shock

gave a significant effect on hatching rate and success of triploidization on seurukan fish, but did not give the significant effect on the fertility and survival rates. The triploid seurukan fish can be produced by heat and cold shocks, while the best temperature for triploidization of seurukan fish was at 37°C. Further research is recommended to determine the best age of zygote before shocking and the period of soaking.

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REFERENCES

Adami, Y., Fadli, N., Nurfadillah, N., Eriani, K., Jalil, Z., & Muchlisin, Z. A. (2016). A preliminary observation on the effect of sperm extenders on the fertilization and hatching rates of seurukan fish (*Osteochilus vittatus*) eggs. *AACL Bioflux*, 9(2), 300-304.

Alawi, H., Nuraini, & Sapriana. (2009). Induksi triploid ikan selais (*Kryptopterus lympok*) menggunakan kejutan panas. Jurnal *Perikanan dan Kelautan*. 14(1), 37-47.

Allendorf, F. W., & Thorgaard, G. H. (1984). *Tetraploidy and the evolution of Salmonid fishes. p. 1-53. In B.J. Turner (ed.) Evolutionary genetics of fishes.* New York: Plenum Publishing Corporation.

Arsianingtyas, H. (2009). Pengaruh kejutan suhu panas dan lama waktu setelah pembuahan terhadap daya tetas dan abnormalitas larva ikan nila (*Oreochromisniloticus*). *Skripsi*. Surabaya: Fakultas Perikanan dan Kelautan Universitas Airlangga.

Arsianingtyas. H. (2009). *Pengaruh Suhu Panas dan Lama Waktu Setelah Pembuahan Terhadap Daya Tetas dan Abnormlitas Larva Ikan Nila (Oreochromis niloticus).* Surabaya: Fakultas Perikanan dan Kelautan. Universitas Airlangga.

Asma, N., & Muchlisin, Z. A. (2016). Pertumbuhan dan kelangsungan hidup benih ikan peres (*Osteochilus vittatus*) pada ransum harian yang berbeda. *Jurnal Ilmiah Mahasiswa Kelautan dan Perikanan Unsyiah*, 1(1), 1-11

Benfey, T. J. (2001). Use of Sterile Triploid Atlantic Salmon (*Salmo salar* L.) for Aquaculture in New Brunswick Canada. *ICES Journal of Marine Science,* 58(2), 525-529.

Cassani, J. R., & Caton, W. E. (1985). Induced Triploidy in Grass Crap, *Ctenopharyngodon idella* val. *Aquaculture*, 46(1), 37-44.

Emilda. (2003). Tingkat keberhasilan triploidisasi pada ikan baung dengan pemberian kejutan suhu yang berbeda. *Skripsi*. Bogor: Fakultas Perikanan dan Ilmu Kelautan IPB.

Febrianto. (2011). Triploidisasi ikan nilem (*Osteochilus hasselti*) dengan menggunakan tingkat kejut panas dan lama perendaman berbeda. *Skripsi*. Purwokerto: Fakultas Sains dan Teknik Universitas Jenderal Soedirman.

Fujaya, Y. (2000). *Fisiologi ikan*. Makassar: Universitas Hasanuddin.

Galbreath, P. F., Jean, W. S., Anderson, V., Gary, H. & Thorgaard. (1994). Freshwater Performance of All-Female Diploid and Triploid Atlantic Salmon. *Aquaculture*, 128(1-2), 41-49

Hartono, D. P. & Febriani, D. (2013). Pengarauh Lama Waktu Pemberian Kejutan Dingin Pada Pembentukan Individu Triploid Ikan Patin (*Pangasius* sp.). *Aquasains*, 2(1), 61-68.

Lawson, E. O. & Ishola, H. A. (2010). Effect of Cold Shock Treatment in the Survival of Fertilized Eggs and Growth Performance of the Larvae of African Mud Catfish, *Clarias gariepinus* (Burchell, 1822). *Research Journal of Fisheries and Hydrobiology*, 5(2), 85-91.

Leclercq, E., Taylor, J. F., Fison, D., Fjelldal, P. G., Diez-Padrisa, M., Hansen, M. & Migaud, H. (2011). Comparative Seawater Performance and Deformity Prevalence in Out of Season Diploid and Triploid Atlantic Salmon (Salmo Salar) Post-Smolts. *Aquaculture*, 158(1), 116-125.

Mayana, M., Muchlisin, Z. A., & Dewiyanti, I. (2016). Pemanfaatan ekstrak bawang merah (*Allium cepa*) dalam pakan sebagai sumber prebiotik untuk benih ikan seurukan (*Osteochilus vittatus*). *Jurnal Ilmiah Mahasiswa Kelautan dan Perikanan Unsyiah*, 1(1), 25-34

Muchlisin, Z. A., Arfandi, G., Adlim, M., Fadli, N., & Sugianto, S. (2014) Induced spawning of seurukan fish, *Osteochilus vittatus* (Pisces: Cyprinidae) using ovaprim, oxytocin and chicken pituitary gland extracts. *AACL Bioflux*, 7(5), 412-418

Muchlisin, Z. A., Nadiya, N., Nadiah, W. N., Musman, M,. & Siti-Azizah, M. N. (2010) Preliminary study on the natural extenders for artificial breeding of African catfish *Clarias gariepinus* (Burchell, 1822). *AACL Bioflux,* 3(2), 119-124.

Mukti, A. T. (2005). Perbedaan keberhasilan tingkat poliploidisasi ikan mas (*Cyprinuscarpiolinn*.) melalui kejutan panas. *Berkala Penelitian Hayati*, 10, 133-138.

Mukti, A. T., Arsianingtyas, H., & Subekti. S. (2009). Pengaruh kejutan suhu panas dan lama waktu setelah pembuahan terhadap daya tetas dan abnormalitas larva ikan nila (*Oreochromisniloticus). Jurnal Ilmiah Perikanan dan Kelautan*. 1(2), 163-167.

Mulyasari, D. (2010). Karaktristik fenotipe morfomeristik dan keragaman genotype RAPD (Randomly Amplified Polymorphism DNA) ikan Nilem (*Osteochilushasselti*) di Jawa Barat. *Tesis*. Bogor: Fakultas Perikanan dan Ilmu Kelautan IPB.

Mulyasari, D., Soelistyowati, T., Kristanto, A. H., & Kusmini, I. (2010). Karakteristik genetic enam

populasi ikan Nilem (*Oreochromisniloticus)* di Jawa Barat. *Jurnal Riset Aquakultur*, 5(2), 175-182.

Nuraini. H., &. Alawi. (2008). *Produksi benih ikan selais (Ompokhypopthalmus) dengan kejutan suhu berbeda.* Universitas Riau. Pekanbaru.

Nurasni, A. (2012). Pengaruh suhu dan lama kejutan panas terhadap ikan lele sangkuriang (*Clariasgariepinus*). *Indonesian Journal of Applied Sciences,* 2(1), 19-26.

Olele, N. F., & Tighiri, O. H. (2013). Optimization of Triploidy Induction and Growth Performance of Clarias Anguillarias (African Catfish) Using Cold Shock. *Academic Journal of Interdisciplinary Studies*, 2(7), 189-196.

Puji, D., Pindo, H., & Witoko. (2012). Pengaruh jarak waktu pemberian kejutan dingin pada pembentukan individu triploid ikan patin (*PangasiusSp*). *Jurnal Penelitian Pertanian Terapan*, 12(3), 156-162.

Purdom, C. E. (1984). *Atypical modes of reproduction in Fish*. In Oxford Reviews of Reproductive Biology, pp. 303–340. Ed. by J. R. Clarke. Oxford University Press, Oxford.

Risnandar, D. (2001). Pengaruh umur zigot pada saat kejutan panas terhadap keberhasilan triploidisasi serta kelangsungan hidup embrio dan larva ikan jambal siam (*Pangasiushypopthalmus*). *Skripsi*. Bogor: Fakultas Perikanan dan Ilmu Kelautan IPB.

Syandri H. (2004). The use of *Osteochilus vittatus* and *Puntius javanicus* as an agen of biological in Maninjau Lake. *Journal of Natur Indonesia*, 6(2), 87-91.

Syandri, H., Azrita, & Junaidi. (2015). Fecindity of Bonylip Barb (*Osteochilus vittatus Cyprinidae*) in Difeerent Waters Habitats. *International Journal of Fisheries and Aquatic Studies,* 2(4), 157-163.

Uslichah U., & Syandri H. (2003). Reproduction aspects of sasau fish (*Hampala sp*.) and lelan fish (*Osteochilus vittatus*) in Singkarak lake. *Journal of Ichtiology Indonesia*, 1(3), 41-48.

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Study of Mistletoe in Joben Resort Forest Mount Rinjani Lombok

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Abstract

Mistletoes are one group of hemiparasite plants, including the Lorantaceae family that have potential as medicinal. These hemiparasite plants can attack flowering plant (Magnoliophyta) and non-floweing plant (Pinophyta), especially on the main stems, branches and twigs. The objective of this research is to identify the species of mistletoe and its hosts, make identification key, descriptions, and to make a distribution map of mistletoe in Joben Resort forest south of Mount Rinjani Lombok. This study is descriptive explorative research with three kinds of collecting sample methods i.e exploration, continous strip sampling, and delenation method. The research found five species of mistletoes are included in three genera i.e *Amyema cuernosensis*, *Amyema enneantha, Amyema tristis, Macrosolen retusus* and *Scurrula artropurpurea*. These five kinds of mistletoe are associated with 23 hosts species of plants, 18 genera from 13 families. The most favorite host of these mistletoes is *Ficus septica*, and the most agresive mistletoe is *Scurrula artropurpurea.* The important finding of the research is finding new species or new record of mistletoes. The benefit of these new record or new species is providing new material of new medicinal for treating some diseases such as various cancers.

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INTRODUCTION

Mistletoe is one of hemyparasite in Loranthaceae family. It usually attacks shrubsor trees especially on the trunks and branches. These mistletoes will creep the host plants by inserting their haustoria into the host branches or with internal or external epicortical runner (Vidal-Russell & Nickrent, 2008a). Since the growth is ruined, some trees will probably wither event die. Loranthaceae family consist of 73 genera and 950 species. Most of its live in tropical, subtropical or temperate climate. Malesia is reported to have 23 *genera* and 193 species of Loranthaceae family (Barlow, 1997, Vidal-Russell & Nickrent, 2008b).

Indonesia reportedly has 174 species of mistletoes that consists of 26 genera. There are 38species of mistletoe (Loranthaceae family) in Java. The number of species found in West Java, namely 29 species (Sunaryo, 2008), in East Java and Central Java respectively represent 19 and 15 species of mistletoes (Sunaryo, et al., 2010 and Yunita, 2014). In Bali, there are four species of Loranthaceae found in Eka Karya Botanical Garden i.e. *Dendrophthoe pentandra, Helixanthera cylindrica, Scurrula atropurpurea*, and *Scurrula parastisica*. Viscaceae family consists of two species i.e. of *Viscum articulatum* and *Viscum ovalifolium* which are found in Purwodadi (Sunaryo, et al., 2007).

Mistletoe indicated threating to the citrus industry in Ghana. A drastic drop in yield of the citrus plants when attacked by the mistletoe (95%), poor growth of the citrus plants (65%) and mortality when severely infected (55%). Mistletoe also give infestation results in yield loss averaging between 5% and 85.5%. It this agrees with the findings of studies on the prevalence of mistletoe on the citrus orchards in the Eastern Region of Ghana and in Sudan. It was reported that mistletoe infestation causes drastic growth retardation, yield loss and subsequent killing of the citrus plants (Asare-Bediako, et al., 2013).

Mistletoe is not only known as parasit which disturb other plants but also as a potential medicine. Some of research has been done on mistletoe of mango *(Dendrophthoe pentandra)* as the fisrt as a first step towards phytopharmaca among other phytochemical studies to identify the content of the active compound. Based on the test known that the mango mistletoe contains flavonoids quercetin, meso-inositol, rutin, and tannins. The compounds are active as anticancer possibilities (Pramudanti et al., 2013).

Joben Resort forest area is located in the southern slope of Mount Rinjani Lombok. It is rich of water resources. Its terrain and vegetation is a potential place to find mistletoes. Since each area is geographically different, the climate and the environment will also be different. It is very common to find many different kinds of mistletoes. Joben Resort forest area was divided into three areas, namely: 1. the edges of woods and trails tracking composed of secondary forests; 2. the middle section is composed of primary forest; 3. The top section (> 1750 m above sea level (asl.)) is composed of savanna.

The objective of this research is to determine the species of mistletoe, and their hosts, to make identification key, descriptions, and to make a mistletoe species distribution map in Joben Resort forest area which is located in the southern slope of Mount Rinjani Lombok.

METHODS

Mistletoe exploration in Joben Resort forest area which is located in the southern part of Mount Rinjani Lombok. The research was held on July-November 2016. The environmental data were recorded such as the habitat, air temperature, air moisture, altitudes, slopes, and coordinates position of mistletoes finding. Mistletoes specimens and their hosts were identified morphological features by using both keys and descriptions from various taxonomic literatures previously reported such as Backer & Bakh. f. (1965), Barlow (1929, 1991 and 1997), Denser (1935), Radford, et al. (1974) and URL: http://theplantlist.org.

The research was conducted using three kinds of collecting samples methods: exploration method (Rugayah, et al., 2004), continuous strip sampling method (Simon, 2007), and delineation method (Mulyaningsih, et al., 2014). The samples were taken by making five vertical lines in Joben Resort forest area in the southern slope of Mount Rinjani Lombok.

RESULTS AND DISCUSSION

The research found five species of mistletoes which were included in three genera i.e *A. cuernosensis*, *A. enneantha, A. tristis, M. retusus* and *S. artropurpurea*. Four species of mistletoes i.e *Amyema enneantha, Amyema tristis, Macrosolen retusus* and *Scurrula artropurpurea* included new record of mistletoes that were found in Lombok island. These five species of mistletoes are associated to 23 plants species, 18 genera from 13 families.

Key identification of parasite plant

1a.Margin lamina sinuate, leaf and petiole tomentose ..……*Scurrula artropurpurea*

b.Margin lamina entire, leaf and petiole glabrous ……..…2

2a.Haustoria gall external host stem tissues, stem flattened and widened at the end of the internodes……...*Macrosolen retusus*

b.Haustoria gall internal host stem tissues, stem doesn't flattened and widened at the end of the internodes.……3

3a.Trees tall <50 cm, lamina length <10 cm, lamina width <5 cm, petioles subsessile, ……..….*Amyema tristis*

b.Trees tall >50 cm, lamina length >10 cm, lamina width >5 cm, petioles sessile.……4

4a.Nodus swell forming stem knee, the amount of branching <5, leaves ternate, ……...*Amyema enneantha*

b.Nodus swell forming stem tumor, the amount of branching >5, leaves opposite,

……..……..*Amyema cuernosensis*

Description Species of Mistletoe

1. *Scurrula artropurpurea* (Blume) Danser. Bull. Jard. Bot. Buitenzorg 111, 10 (1929) 349, 1 1 (1935) 429. New record based on: Danser (1929 and 1935), Barlow (1991 and 1997), Backer & Bakh. f. (1965), Pelser (2015) (Figure 1).

Aerial stem-parasitic shrubs, slender, drops, 50-150 cm tall, tomentose cream, with external gall and external runners epicortical. Adult stems cylindrical, tomentose cream, fissure, lenticell, young stems flattened at the end of internodes. Leaves alternate, opposite, subopposite; lamina papyraceous, dull, tomentose cream, polymorphic: rounded, obovate, elliptical: 5-13.2 cm length, 3-7.5 cm width; apex: rounded obtuse rarely acute; base: cuneate or oblique or obtuse; margin sinuate; venation reticulate, midrib and the laterals veinsvisible on both sides, 5-6 lateral veins per lamina; petiole 1-2 cm by 0.1 cm, green, dense tomentose cream. Inflorens-censes raceme axillary and at the nodes, 2-8 racemes per node, peduncle 0.2-0.3 cm by 0.05 cm, tomentose cream, 6-7 flowers per raceme; pedicel 0.5-0.8 cm by 0.1 cm, tomentose cream; Bracteole deltoid, tomentose cream, 1 at the tip of pedicel. Flowers tubulate, 1.5-2 cm length, 0.5 cm width, perianth gamosepalous, 4 merous; calyx lobe rounded, tomentose cream; corolla green: 1.2-1.5 cm by 0.5 cm, lobe oblanceolate, 0.5-0.8 cm by 0.5 cm, reflected, curve, green inside and tomentose cream outside. Stamens 4, epipetalous, baxifixed, 0.4 cm length; filament brownish red, 0.2 cm length; anther purplish red, 0.1 cm length. Stigma capitate, red; stylus 1-1.45 cm length, red-brown; ovary clavate, 0.5 cm by 0.1 cm, tomentose cream. Fruit berry, green, clavata, 0.8-1 cm by 0.2 cm, tomentose cream. Seeds 1, light green, covered with yellow sticky layer that lies between endocarpium and testa.

Figure 1. *S. artropurpurea*: a. habitus, b. haustoria, c. the cross section haustoria, d. leaf, e. flower, f. Fruit

Vernacular name: mengandi (Sasak Joben)

Habitat and ecology: secondary forests, altitude: 757-1000 masl., humidity: 79-91.5%, temperature: 23-27^{0}C. Host plants: *Baccaurea racemosa, Citrus hystrix, Calliandra haemotecephala, Dalbergia latifolia, Euchesta horsfieldii, Ficus fistulosa, F. septica, Ficus* sp., *Glochidion* sp., *Laportea stimulan, Leocoyke capitellata, Macaranga tanarius, Mallotus moluccanus, Melastoma mabathrichum, Phylanthus* sp., *Persea americana, Syzigium* sp., *Saurauria pendula, Pterospermum javanicum,* and ***A. enneantha***. This mistletoe sticks to the trunk and branched of secondary, tertiary at a height of 3-15 m above the ground. This species can attach to other mistletoe such *A. enneantha.* Benefits: traditional utilization namely water decoction of the leaves can cure smallpox. Specimens examined: East Lombok, Joben Resort forest area is located in the southern part of Mount Rinjani:WDF: 1, 2, 16, 17,18, 21 are stored in the herbarium of the Faculty of Mathematics and Science, Mataram University Lombok Indonesia (MUL).

2. *Macrosolen retusus* (Jack) Miq. Fl. Ind. Bat. 1, 1 (1856) 828. New record based on: Barlow (1997) (Figure 2).

Aerial stem-parasitic shrubs, thick, erect, 45-150 cm tall, glabrous. Haustoria were formed external gall and external runners epicortical Adult stem cylindrical, fissure, lenticell, young stems flattened and widened at the end of node, levies, glabrous, green. Leaves opposite-decussate, subopposite, coriaceous, glabrous, dull,

polymorphic: obovate to elliptical, 4.5-9.8 cm length, 2-6.3 cm width; apex: rounded, obcordate, obtuse rarely acute; base: cuneate, oblique or obtuse; margin entire; venation pinnate, midrib and lateral veins distinct above, 7-8 veins per leaf; petiole 0.3-0.5 cm by 0.3 cm, glabrous. Inflorenscenses raceme axillary and at the nodes, 1-2 racemes per peduncle, peduncles 0.5-0.8 cm by 0.1 cm; 6-7 flowers per raceme; bracteole deltoid, glabrous, light green, imbricate, 3 at the tip of pedicle. Young flowers tubulate, 0.5-0.7 cm length, 0.2 cm width, perianth 6 merous, green, glabrous. Ovary botuliform, glabrous. Fruit berry, green, elipsoid, 0.7cm by 0.3 cm, glabrous. Seeds 1, light green, covered with white sticky layer that lies between endocarpium and testa.

Figure 2. *M. retusus*: a. habitus, b. haustoria, c. the cross section haustoria, d. leaf, e. flower, f. fruit.

Vernacular name: Mengandi (Sasak Joben)

Habitat and ecology: secondary forests and in the open area, altitude: 627-683 als., air-humidity: 71-97%, air temperature: 24-27^0 C. Host plants: *D. latifolia, F. superba, F. fistulosa,* and *Mangifera indica*, attached to the trunk and the secondary branched, 3-15 m above the ground. Specimens examined: East Lombok, Joben Resort forest area is located in the southern part of Mount Rinjani: WDF: 10, 12, 13 and 14 (MUL).

3. *Amyema tristis* (Zoll.) Tiegh., Bull. Soc. Bot. France 41 (1894) 507; Danser, Bull. Jard. Bot. Buitenzorg III, 11 (1931) 351; Backer & Bakh. f., Fl. Java 2 (1965)71; Barlow, Blumea 36 (1992) 371. New record based on: Barlow (1992 and 1997), Backer & Bakh. f. (1965) and Danser (1931) (Figure 3).

Aerial stem-parasitic shrubs, thick, erect, 30-45 cm tall, glabrous. Haustoria were formed internal gall and external runners epicortical. Adult stem cylindrical, fissure, lenticel, brown, young stems cylindrical, levies, green; nodes swell forming gall, 0.5-1 cm by 0.3-1 cm.

Figure 3. *A. tristis*: a. habitus, b. haustoria, c. the cross section haustoria, d. stem *A. tristis* attack himself, e. nodus swell forming stem tumor, f. leaf

Leaves opposite, coriaceous, glabrous, polymorphic: ovate-elliptical, 4.6-7.1 cm length, 2-4 cm width; apex: acuminate, acute; base: truncate, oblique, obtuse; margin entire; venation pinnate, midrib and lateral veins visible, 5-6 veins per leaf; petiole sub-sessile, glabrous.

Vernacular name: mengandi (Sasak Joben)

Habitat and ecology: secondary forests, altitute 695 m als., humidity: 62%, temperature: 27^0 C. Host plants: *F. septica* and *A. tristis*. Mistletoes attached to the trunk and secondary branched at 2 m above the ground. Specimens examined: East Lombok, Joben Resort forest area is located in the southern part of Mount Rinjani, WDF: 11 (MUL).

4. *Amyemaenneantha* Barlow, Blumea 36 (1992) 329. New record based on: Barlow (1992 and 1997) (Figure 4)

Aerial stem-parasitic shrubs, thick, erect, 50-150 cm tall, glabrous. Haustoria were formed internal gall and external runners epicortical. Adult stem cylindrical, fissure, lentisel, brown, 1-1.5 cm in diameter; internodes 3.5-10 by 0.3-1 cm; nodes swell forming knee gall, 0.5-1.2 by 0.6-1.5 cm; young stems cylindric, levies, green. Leaves ternate; lamina coriaceous, glabrous, ovate to lanceolate: 5-14 cm length, 2.5-6.5 cm width; apex: acuminate or acute; base: attentuae; margin entire; venation pinnate, obscure, midrib

prominent on both side, 5-6 veins per leaf; petiole sessile- sub-sessile 0-0.2cm by 0.1 cm, glabrous. Inflorenscenses umbel simple, axillary and at the nodes, 6-9 umbels per peduncle, peduncles cylindrical, sessile-sub-sessile 0-0.1cm by 0.1 cm, green, glabrous; 6-8 flowers per umbel; pedicels 0.1 cm by 0.1 cm, glabrous. Barcteole triangular, 0.1 cm by 0.1 cm, glabrous, green, 1 at the end of pedicel. Flowers campanulate, 2-2.7cm length,1 cm width, perianth 5 merous; calyx gamosepalous, lobes: 0.1 cm by 0.1 cm, glabrous, yellowish green; corolla, glabrous, red, glabrous, choriopetalous 2-2.7 cm by 1 cm, reflected, curve. Stamens 5, epipetalous, baxifixed, 1 cm length; filament yellow: 0.6 cm length; anther brown: 0.4 cm length. Stigma capitate, red; stylus 1.5-2 cm length, red; ovary botuliform, 0.3 cm by 0.2 cm, glabrous. Fruit berry, green, eliptical, 0.5-1 cm by 0.3 cm, glabrous. Seeds 1, brown, covered with sticky layer that lies between endocarpium and testa

Figure 4. *A. enneantha*: a. habitus, b. haustoria, c. the cross section haustoria, d. leaf, e. flower, f. Fruit

Vernacular name: Mengandi (Sasak Joben)

Habitat and ecology: secondary forests, altitude: 759-1500 mals., air humidity: 75-85%, air temperature: 24-27^0 C. Host plants: *F. septica, Glochidion* sp., *L. stimulan, M. mabathrichum*, *S. pendula, M. tanarius*. Mistletoes attached to trunk and secondary branched, at 3-5 m above the ground.

Benefits: utilization traditionally namely water decoction of the leaves can cure hemorrhoid. Specimens examined: East Lombok, Joben Resort forest area is located in the southern part of Mount Rinjani, WDF: 4, 5, 6, 19 and 20 (MUL).

5. *Amyema cuernosensis* (Elmer) Barlow, Blumea 36 (1992) 323, Pelser (2015) (Figure 5).

Aerial stem-parasitic shrubs, thick, erect, 50-150 cm tall, glabrous. Haustoria were formed internal gall and external runners epicortical. Adult stem cylindrical, fissure, lenticell, brown, 1-12 cm in diameter; internodes 5-9.5 cm by 0.5-1 cm; nodes swell forming gall, 0.7-1 by 1-1.7 cm; young stems cylindrical, levies, green. Leaves opposite; lamina coriaceous, glabrous: ovate to eliptical, 7-17 cm length, 5-10 cm width; apex: acuminate-acute; base: attentuae-rounded; margin entire; shining above and dull bellow; venaton pinnate, midrib and lateral vein prominent visible on both side, 5-6 veins per leaf; petiole sessile-subsessile: 0-0.3 cm by 0.2 cm, green, glabrous.

Figure 5. *A. cuernosensis*: a. habitus, b. haustoria, c. the cross section haustoria, d. leaf, e. flower, f. fruit

Inflorenscenses umbel simple, axillary and at the node, 6-8 umbels per node, peduncles: 1-1.3 cm by 0.1 cm, glabrous; 6-8 flowers per umbel; pedicels: 0.2 cm by 0.1 cm, glabrous. Bracteole triangular, 0.1 by 0.1 cm, glabrous, green, 1 at the end of pedicel. Flowers campanulate, 3-3.5 cm length, 1-1.5 cm width, perianth 5 merous; calyx gamosepalous, glabrous, yellowish green, lobe, 0.1 by 0.1 cm; corolla red, choriopetalous: 2-2.7 cm by 1 cm, reflected, curve, puberulent. Stamens 5, epipetalous, baxifixed, 1 cm length; filament yellow, 0.6 cm length; anther brown, 0.4 cm length. Stigma capitate, yellow to red; stylus 1.5-2 cm length, red; ovary botuliform, 0.3 cm by 0.2 cm, glabrous. Fruit berry, green, eliptical, 0.5-1cm by 0.3 cm, glabrous. Seeds 1, brown, coatted with white milky sticky layer that lies between endocarpium and testa.

Vernacular name: Mengandi (Sasak Joben)

Habitat and ecology: secondary forests altitude 679-747 masl., air humidity 93%, air temperature 24^0 C. Host plants: *F. septica* and *L. Stimulan*. Mistletoes attached to trunk and secondary branched 5-10 m above the ground.

Specimens examined: East Lombok, Joben Resort forest area is located in the southern part of Mount Rinjani, WDF: 9 (MUL).

Mistletoe Diversity and Host Range

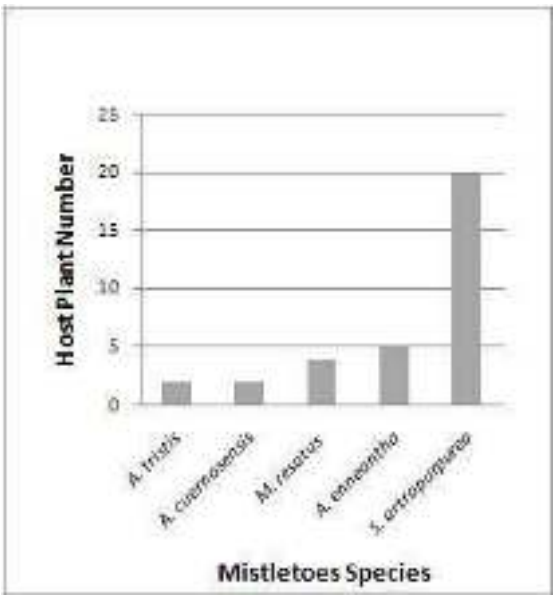

Figure 6. Graph host range of mistletoe in Joben Resort forest in the southern slope of Mount Rinjani Lombok.

A total of five mistletoes species belonging to three genera including Loranthaceae family (*Amyema, Macrosolen*, and *Scurrula*, were new recorded in the study area. These five mistletoes species were parasitizing 24 host plants belonging (Figure 6).

The fifth species of mistletoes distribution pattern can be shown (Figure 7) that each species has been found at specific altitude, on range of 627-1500 masl. Each species of mistletoe was found on different altitute.

For example: mistletoes were found at an altitude below 700 masl., such as: *A. tristis* (695 masl.), *A. cuernosensis* (679-747 masl.) and *M. retusus* (627-623 masl.), while the mistletoes were found above an altitude of 750 masl., e.g.: *A. enneantha* (759-1500 masl) and *S. artropurpurea* (757-1000 masl). The species of mistletoe that most impact host is *S. Artropurpurea*: attaching 192 individuals from 20 host species, *A. Enneantha*: attaching 71 individuals from six host species, *M. retusus*: attaching five individuals from the four host species, *A. Cuernosensis*: attaching two individuals from two species host, and *A. tristis* only attaching one individual (Figure 6 and 7). Mistletoes do not like the conditions thick canopy vegetation that the sun light doesn't up to the forest floor. This is due to mistletoes life as hemyparasite. They live under open areas, because they need sunlight to perform photosynthesis. The map of the distribution can be observed also the host of mistletoes:*A. enneantha* and *S. artropurpurea* have many of the populations most othersspecies of mistletoes. However in Nigeria *lbazia lebbeck* was the most vulnerable to mistletoe attack. (Dlama et al., 2016).

Figure 7. Map of distribution pattern of mistletoes in Joben Resort forest in the southern slope of Mount Rinjani Lombok: A. cuernosensis, A. enneantha, A. tristis, M. resutus, S. Arropurpursa

The important finding of the research are finding new species or new record of mistletoes, their hosts. The benefit of these new record or new species are providing new material of new medicinal for treating some diseases such as various cancers.

CONCLUSIONS

The research found five species of mistletoes were included in three genera i.e. *A. cuernosensis*, *A. enneantha, A. tristis, M. retusus* and *S. artropurpurea*. These five kinds of mistletoes were associated to 23 different species of plants from 19 genera and included in 13 families. The number of parasitized host every species of mistletoe is *A. cuernosensis* infect as much as two plants species; *A. enneantha* infect six plants species; *A. tris-*

tis infact one plant species; *M. retusus* infact four plants species; and *S. artropurpurea* infect most that 19 species of host plants. The most favourite host of these mistletoes was *Ficus septica* from Moraceae family. The most aggressive mistletoe was *Scurrula artropurpurea.*

REFERENCES

Asare-Bediako, E., Addo-Quaye, A. A., Tetteh, J. P., Buah, J. N., Van Der Puije, G. C., & Acheampong, R. A. (2013). Prevalence of Mistletoe On Citrus Trees In The Abura-Asebu-Kwamankese District of The Central Region of Ghana. *International Journal of Scientific & Technology Research*, 2(7), 122-127.

Backer, C. A. & Bakhuizen Van Den Brink, R. C.. (1965). *Flora of Java (Spermato- phytes Only).* Vol. II, Angiosperm Families 111-160. N.V. P. Noordhoff-Groni-ngen, The Hague, Netherlands. Loranthaceae, pp. 67-74.

Barlow, B. A. (1991). Conspectus of the genera *Scurrula* L. and *Taxillus* Tieghem (Loranthaceae) in the Malesian region. *Blumea-Biodiversity, Evolution and Biogeography of Plants,* 36(1), 63-85

Barlow, B. A. (1992). Conspectus of the genus *Amyema* Tieghem (Loranthaceae). *Blumea-Biodiversity, Evolution and Biogeography of Plants,* 36(2), 293-381

Barlow, B. A. (1997). Loranthaceae. In: C Kalkman, DW Kirkup, HP Nootebom, PF Stevens, WJJO de Wilde (eds.) *Flora Malesiana Series.* 13 (1): 209-401. Noordhoff. Groningen.

Danser, B. H. (1929). *On the taxonomy and the nomenclature of the Loranthaceae of Asia and Australia*. Archipel Drukkerij.

Danser, B. H. (1931). *The Loranthaceae of the Netherlands Indies*. Archipel Drukkerij.

Danser, B. H. (1935). *A revision of the Philippine Loranthaceae*. Bureau of Printing.

Devkota, M. P., Joshi, G. P., & Parajuli, P. (2010). Diversity, distribution and host range of mistletoe in protected and unprotected areas of Central Nepal Himalayas. *Banko Janakari*, 20(2), 14-20.

Dlama, T. T., Oluwagbemileke, A. S., & Enehezeyi, A. R. (2016). Mistletoe presence on five tree species of Samaru area, Nigeria. *African Journal of Plant Science*, 10(1), 16-22.

Mulyaningsih, T., Marsono, D., Sumardi, & Yamada, I. (2014). Selection of superior breeding infraspecies gaharu of *Gyrinops versteegii* (Gilg.) Domke. *Agricurtural Science and Technology,* B4(2014), 485-492.

Pelser, P. B. (edt.). (2015). Loranthaceae in Philippine. URL.: http://www.philippine plants.org/Families/Loranthaceae.html.

Pramudanti, D. R., Padaga, M. C., & Winarso, D. (2013). *Pengaruh terapi ekstrak air benalu mangga (Dendrophthoe pentandra) terhadap kadar albumin dan gambaran histopatologi ginjal hewan model tikus (Rattus norvegicus) hiperkolesterolemia*. Program Studi Pendidikan Dokter Hewan, Program Kedokteran Hewan, Universitas Brawijaya

Radford, A.E., Dickison, W. C., Massey, J. R., & Bell, C. R. (1974). *Vascular plant systematic*. New York: Harper & Row, Publishers, Inc.

Rugayah, E., Widjaja, A. & Pratiwi. (2004). *Pedoman pengumpulan data keaneka-ragaman flora*. Bogor: Pusat Penelitian Biologi LIPI.

Simon, H. (2007). *Metode inventore hutan*. Yogyakarta: Pustaka Pelajar.

Sunaryo. (2008). Pemarasitan benalu *Dendrophthoe pentandra* (L.) Miq. Pada tanaman koleksi Kebun Raya Cibodas, Jawa Barat. *Natur Indonesia,* 11(1), 48-58.

Sunaryo, R. E., Rachman, E. & Uji, T. (2007). Keanekaragaman Jenis Benalu Parasit Pada Tanaman Koleksi Di Kebun Raya Eka Karya. Bali. *Berkala Penelitian Hayati*, 13, 1-5.

Sunaryo, S., Rachman, E., & Uji, T. (2010). Kerusakan morfologi tumbuhan koleksi Kebun Raya Purwodadi oleh benalu (Loranthaceae dan Viscaceae). *Berita Biologi,* 8(2), 129-139.

Vidal-Russell, R., & Nickrent, D. L. (2008a). The first mistletoes: Origins of aerial parasitism in Santalales. *Molecular Phylogenetics and Evolution*, 47(2), 523-537.

Vidal-Russell, R., & Nickrent, D. L. (2008b). Evolutionary Relationships In The Showy Mistletoe Family (Loranthaceae). *American Journal of Botany*, 95(8), 1015-1029.

Yunita, E. (2014). Morfologi dan sebaran benalu pada tumbuhan inang di desa Kedondong Sidoarjo Jawa Timur. *Skripsi*. Malang: Fakultas Matematika dan Ilmu Pengetahuan Alam. Universitas Negeri Malang.

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Improving Post-graduate Students Learning Activities through Lesson Study in Learning Forest-Prototype

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Abstract

Improving learning quality in 21st can not be separated from contextual learning and student-centered learning paradigm. The contextual lesson study program conducted in learning-forest prototype to build a learning community. The objectives of this research were to improve learning activities of postgraduate students in Biology Education department and to build a learning community. The implementation of lesson study was conducted in the Biology Learning Innovation subject for postgraduate students while practicing to observe open lesson in the undergraduate students which used learning forest-prototype. The postgraduate students took roles as planner, observer, and reflector in the plan, do (open lesson), and see (reflection) activities. The implementation was done in three cycles in even semester of academic year 2015/2016. Students learned collaboratively and contextually. The postgraduate students' learning activities were observed by six observers from lecturer colleagues. The research results showed that the students were able to implement planning, open lesson, and reflection properly. The average of student's learning activity grade was 91.11% from all of students, with the grade averages for planning, open lesson, and reflection activities were 88.89%, 93.33%, and 91.11% respectively. The implementation of this lesson study in the learning forest-prototype can be done in other relevant subjects to strengthen learning activities.

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INTRODUCTION

Lesson study is an approach to improve learning quality and teacher professionalism development which is conducted by teachers in Japan. In conducting lesson study, teachers work collaboratively, learn curriculum, formulate learning objective, observe lesson research, and do reflection to perfect learning (Susilo et. al., 2009). This lesson study concept is in accordance with studies in Depdiknas (2009). According to Hendayana (2007) and Slamet et. al., (2010), lesson study is a model of educator professional development model through studies of sustainable and collaborative learning, based on mutually assisting collegiality principles in learning to form a learning community. This is an activity to study learning by teachers and their colleagues. The learning aspects to study include planning, conducting, evaluation, and learning reflection. In implementing lesson study, teachers can use varying methods or learning strategies according to situations, conditions, and problems the educators deal with. In this learning activity, collaborations occur between students; mutually learning, scrutinizing, and assisting (Masaaki, 2012; Manabu, 2012; Saito et. al., 2015).

Along with the importance of the learning community, contextual learning also emphasizes the importance of students' activities in the learning community, to develop the students' curiosities, to learn in groups, to build critical thinking ability, to transfer multidisciplinary knowledge, where information is collected, analyzed, and synthesized from varying sources and point of views. Contextual learning involves meaningful components, conducts meaningful works, cooperates, thinks critically and creatively, mutually assists students, and uses standard and authentic assessments (Johnson, 2009).

In accordance to learning community formation in the implementation of contextual learning, according to social constructivism theory, during learning process students experience conceptual changes as results of social and academic interactions. These conceptual changes, experienced by students, are enlightenment into more complex students' understanding and improvement of their critical thinking abilities (Depdiknas, 2002; Muhfahroyin, 2012). According to Ridlo & Alimah (2013) the student-centered learning of environmental exploration may be conducted using active- and cooperative-based strategies. Examples of these strategies are contextual learning, participatory learning, and inquiry learning.

Relationship patterns of classroom with outside environments are very much emphasized to synchronize knowledge had been built inside students through constructivism. Subsequently, this knowledge already possessed by students is reflected again for further developments through collaborative learning, self-finding, and mutual learning (Saito et. al., 2015). Similar with statement above, BNSP (2010) and Frydenberg & Andone (2011) state that learning in 21st need to be held with collaborative, creative and innovative learning. In other word, the teachers must prepare and plan overall to facilitate the students contextual, participatory, active, and creative in their learning.

There are many learning sources, which are able to empower students to understand science cognitively and psychomotorically, have been developed. However, real learning sources in nature (contextual), which are able to prepare students in cognitive, affective, and psychomotoric domains, and which are able to support environmental care character, are rare to develop. By a fundamental research, a learning source with initiation of a project based on critical land, which had no educational and economic values before, was developed (Muhfahroyin, 2013). This critical land was empowered for learning into a *forest prototype* with educative benefits and it was able to build environmental care characters for students. By having forest prototype learning activity, students were trained to think critically and creatively, to build environmental care attitude, work skilfully which represented cognitive, affective, and psychomotoric domains (Muhfahroyin, 2016). This forest prototype learning development supports natural environment issues such as global warming, climate change, and *save our earth* movement. Related to learning, this research is also in synergy with philosophies of constructivism, student centered learning, collaborative learning, and contextual teaching and learning. By this forest prototype learning, learning can be used as media and vehicle to implement lesson study.

The objective of this research was to improve postgraduate students' learning activities in implementing lesson study to build learning community. The benefits of this research were to increase lecturers and students insight in lesson study, as well as utilization of learning forest-prototype as a contextual learning resource for students.

METHODS

The research was conducted at Depart-

ment of Biology Education Muhammadiyah University of Metro in even semester academic years 2015/2016, start in March to July 2016.

The research population deals with 48 postgraduate students of biology education. Sampling was taken by purposive sampling to determine the class which is used as research locations consist of 18 students.

Learning implementation in this research was conducted in the biology learning innovation subject for postgraduate students of biology education. Postgraduate students took roles as planners in PLAN, observers in DO (*open lesson*), and reflector in SEE (reflection). Open lesson was conducted in plant morphology subject for undergraduate students. Lesson study activity was started with planning with an objective to produce a learning plan. Planning was conducted collaboratively between postgraduate students and undergraduate lecturers. Planning activity discussed lesson plan, student's activity sheet, student's observation sheet, and other learning instruments.

In conducting open lesson, postgraduate students took roles as observers for undergraduate students in learning of plant morphology. In the reflection activity, postgraduate students provide suggestions on findings when they observe undergraduate students' learning.

The instruments exerted to measure dependent variables in this research is observation, applied to have students learning activities in three cycles of lesson study (PLAN-DO-SEE). The data in plan session were activities of observing, questioning, answering, responding, and providing suggestion activity. The data in do session were observing, recording, finding unique matters, adjusting position at learning groups. The data in see session were observing, suggesting findings, responding, providing suggestions, and reflecting. The data collection steps in this research consist of as long as learning.

Research data were analyzed descriptively to describe postgraduate students' activities in implementing lesson study which included activities of planning, open lesson, and reflection.

RESULT AND DISCUSSION

The implementation of lesson study was conducted by postgraduate students in three activities of lesson study cycles, namely PLAN-DO-SEE. Explanation of the result and discussion is as follows.

Students' Learning Activities in Plan

The learning was started with planning to produce lesson plan. Planning was conducted collaboratively by lecturers who delivered the subject, other colleague lecturers, and 18 postgraduate students in biology education department. Planning discussed lesson plan, student's activity sheet, student's observation sheet, and other learning instruments. In this planning activity, postgraduate students did activities and it was recorded to determine percentage of activities had been done. Research data of learning activities in PLAN is show in Table 1.

Table 1. Percentage of Students Learning Activities in PLAN of Lesson Study

Activities	Amount	
	Participants	Percentage (%)
Observing	18	100.00
Questioning	15	83.33
Answering	14	77.78
Responding	16	88.89
Providing suggestion	17	94.44
Average		88.89

Planning was conducted to obtain better learning preparation. All participants in planning activity conducted positive activities. High percentage of observing, questioning, answering question, responding, and providing suggestion activities were indicators of active participations and enthusiasm of participants in planning activity. Planning discussed all related matters to do in learning. It was in accordance with Kemdiknas (2012) that lesson plan, student's activity sheet, learning instruments, lay out of learning students, observation sheet, list of participants in learning groups were prepared in the planning. Here participants were mutually listening opinions and suggestions for conducting contextual learning. It was not only simulation, but a real learning (Johnson, 2009; Muhfahroyin, 2007).

Based on previous understanding, all observers actively observe planning activity properly and contextually. In addition, observers also understood forest prototype learning which was conducted by the model lecturer (Muhfaroyin, 2013). As beginner teachers, the postgraduate students needed training about lesson study implementation. Similarly, Chichibu (2016) stated that beginner teachers needed to be trained to develop their instructional and critical thinking skills. Further, Monnier (2016) explained that to

implement lesson study, training for the prospective teachers was required, so that as a training approach lesson study could facilitate practices and theory articulations and allowed teachers to improve students' learning qualities.

Students' Learning Activities in DO (Open Lesson)

Open lesson is the implementation of planning stage in the real and contextual learning. Eighteen postgraduate students took roles as observers in the undergraduate students' learnings on plant morphology subject. Postgraduate students' activities were observing, recording, finding unique matters, adjusting position at learning groups. Data of the learning activities in DO (open lesson) is shown in Table 2.

Table 2. Percentage of Students Learning Activities in DO (open lesson) of Lesson Study

Activities	Amount	
	Participants	Percentage (%)
Observing	18	100.00
Notting	18	100.00
Recording	14	77.78
Finding unique matters	18	100.00
Adjusting position	16	88.89
Average		93.33

The average percentage of postgraduate students' activities as observers during learning was 93.33% (very good) from total of postgraduate students. As observers, postgraduate students had been prepared with methods of being good observers. Learning conducted by the model lecturer was not only simulation of lesson study, but it was a real learning. Observers should be able to do their roles properly, carefully, and precisely (Kemdiknas, 2012). Observers should be able to observe all students' activities in learning and record unique findings during learning. In order to observe properly, the observers adjusted their standing positions to avoid disturbing students in their learning and to facilitate observations.

With a good planning at lesson study, learning outside classroom would be able to improve students' learning activities. Good learning planning would create good learning process. Similarly, Mellvig & Nilsson (2015) stated that there was no difference between traditional classroom teaching and outdoor teaching. According to researchers, no matter where the learning location was, when learning was planned and conducted properly and seriously by maintaining its quality, it would produce good results. Produce result in the learning indicated by students-centered learning which can empower students intelelectual ability. In knowledge age, 21st century, students-centered learning is required to have high quality human resources with high intellectual ability. It requires solution which can critical thinking and problem solving, tools for working, in the real living in the world (BNSP, 2010; Frydenberg and Andone, 2011).

Students' Learning Activities in Reflection

Reflection activity was conducted after observation during learning. Postgraduate student observers provided their findings and suggestions during their observations. Reflection activities included observing, suggesting findings, responding, providing suggestions, and reflecting. Data of the learning activities in SEE (reflection) can is in Table 3.

Table 3. Percentage of Students Learning Activities in SEE (reflection) of Lesson Study

Activities	Amount	
	Participants	Percentage (%)
Observing	18	100
Reporting findings	17	94.44
Responding	14	77.78
Providing suggestions	18	100.00
Reflecting	15	83.33
Average		91.11

The average of reflection activity was 91.11% (very high) from total student participants. The activities of observers referred to understand the learning reflection. When the lecturer model delivered the results of reflection, all participants in reflection activity followed carefully. After that, participants were provided opportunities to present their observation results, then a floor for responses to the learning results was held. The participants then gave suggestions to the model lecturer for the next learning. Through this reflection, collaboration occurred in the lesson study team to solve problems during open lesson. Similarly, Yamaji (2016) stated that with the collaborative reflection, the learning could support participating structure in which students learned collaboratively.

In this reflection, participants did not criticize model lecturer, but focused to present results of learning during open lesson. Observers focused on students with learning difficulties and other students' learning behaviors (Parmin, 2007; Kemdiknas, 2012). The results of reflection would become considerations to conduct next learning.

In conducting lesson study, teachers collaboratively: 1) learn curriculum and formulate learning lesson and the development objectives of their learners (to develop their life skill), 2) plan learning to obtain the objectives, 3) conduct and observe a research lesson, and 4) do reflection to discuss the next learning (Lewis et. al., 2006 in Susilo et. al., 2009). A learning, conducted in a lesson study for learning community (LSLC), emphasizes a collaborative learning where students are together mutually assisting and scrutinizing to listen ideas and concepts between them in a collaborative group (Saito et. al., 2015). The collaborative learning is the core of learning community. Similarly, Inprasitha & Inprashita (2014) state that there are 5 steps of the lesson study process: 1) the teachers in the lesson study group, collaborate in developing the knowledge management plan, 2) the usage of knowledge management plan and classroom observation, 3) the classroom reflection, 4) the conclusions of teachers' learning, 5) the modification of knowledge management plan. To implement lesson study the teacher can conducting contextual learning in outside classroom would be able to improve students' learning activities, as well as learning in learning forest prototype.

The contextual learning has seven main components: (1) *constructivism* – students are able to construct understanding along with learning experiences has been done and to give a meaning through real experiences; (2) *inquiry* – students are able to self-finding concepts, facts, and principles in daily life; (3) *questioning* – students question to encourage understanding to explore and to master; (4) *learning community* – to obtain perfect understanding, students learn in groups to build learning society; (5) *modelling* – to ease understanding assimilation, students make reproducible and developable modelling in the learning; (6) *reflection* – students reflect what has been done for contemplation and take the meaning and to use it to construct understanding in the future; and (7) *authentic assessment* – assessment of all of the learning processes have been done and this is conducted by assessing the true process sequences from the beginning to the end (Muhfahroyin, 2007; Johnson, 2009).

This research focuses on how the lesson study can be done by postgraduate students as a mean for lesson study implementation training. The postgraduate students are commonly teachers coming from some regions, and they will disseminate the practice of lesson study in their respective region to build learning community and improve learning quality.

CONCLUSIONS

Based on the research results, it is concluded that students were able to implement planning, open lesson, and reflection properly. The average of total of learning activity was 91.11% from all postgraduate students with averages of activities for planning, open lesson, and reflection were 88.89%, 93.33%, and 91.11% respectively. This lesson study implementation was planned properly and implemented to biology learning innovation subject. The lesson study implementation is recommended to be applied to other relevant subjects to increase students learning activities in the forest-prototype based learning.

REFERENCES

BNSP. (2010). *Paradigm of National Education in 21st*. Jakarta: BSNP.

Chichibu, T. (2016). Impact on lesson study for initial teacher training in Japan: focus on mentor roles and kyouzai-kenkyuu. *International Journal for Lesson and Learning Studies*, 5(2), 155-168.

Depdiknas. (2009). *Lesson Study Dissemination Program for Strengthening Teacher Education in Indonesia-LEDIPSTI. Program Perluasan Lesson Study untuk Penguatan LPTK*. Jakarta: Direktorat Ketenagaan Ditjen Dikti.

Frydenberg, M. & Andone, D. (2011). Learning for 21stCentury Skills. *IEEE's International Conference on Information Society.* London 27-29 June 2011, 314-318.

Hendayana. (2007). *Lesson Study a Strategy to Improve Professionality of Teachers. Lesson Study Suatu Strategi untuk Meningkatkan Keprofesionalan Pendidik (Pengalaman IMSTEP-JICA).* Bandung: UPI Press.

Inprasitha, N. & Inprasitha, M. (2014). *Lesson Study: An Implemented Innovation in the Thai Context*. Presented in the WALS Conference, Indonesia, November 25-28 2014. Makalah Disajikan pada Seminar International WALS di Indonesia pada Tanggal 25-28 Nopember 2014.

Johnson, E. B. (2009). *Contextual Teaching and Learning* (*Pembelajaran Kontekstual*). *Menjadikan Kegiatan Belajar Mengajar Mengasyikkan dan Bermakna*. Bandung: MLC

Kemdiknas. (2012). *Guideline of School-based Lesson Study Panduan untuk Lesson Study Berbasis*

MGMP dan Lesson Study Berbasis Sekolah. Jakarta: International Development Center of Japan.

Masaaki, S. (2012). *Dialogue and Collaboration in Junior High School Dialog dan Kolaborasi di Sekolah Menengah Pertama, Praktik Learning Community*. Jakarta: JICA.

Manabu, S. (2012). *School Reform, Learning Community Concept and Practice Mereformasi sekolah, Konsep dan Praktik Komunitas Belajar*. Jakarta: JICA.

Mellvig, S., & Nilsson, A. (2015). Outdoor learning contra indoor learning. *International Journal for Lesson and Learning Studies*, 4(2), 174-177.

Muhfahroyin. (2007). Pembelajaran Contextual Teaching and Learning untuk Mengintegrasikan Nilai-nilai IMTAQ dalam Pembelajaran Biologi. *Jurnal Pendidikan dan Pembelajaran*, 5(1).

Muhfahroyin. (2012). Meningkatkan Kemampuan Berpikir Kritis dan Pengembangan Karakter melalui TPSS dalam Lesson Study. *Jurnal Bioedukasi*, 3(1).

Muhfahroyin. (2013). Inisiasi Prototype Hutan Pembelajaran sebagai Wahana Implementasi Scientific Approach bagi Mahasiswa Pendidikan Biologi. *Makalah Disajikan pada Seminar Nasional Pendidikan Sains di Universitas Sebelas Maret* pada Tanggal 9 Nopember 2013.

Muhfahroyin. (2016). The Development of Forest-Prototype-Based Learning Model to Activate Science Process Skill. *A Paper Presented at International Conference of Multidiciplinary Education in Malaysia* May 19, 2016.

Monnier, M. D. (2016). Developing the skills of future teachers to fine-tune pupils' learning through lesson studies. *International Journal for Lesson and Learning Studies*, 5(3), 239-254.

Parmin. (2007). Strategi Meningkatkan Kualitas Pembelajaran Melalui Lesson Study. *Lembaran Ilmu Kependidikan*, 36(2).

Ridlo, S. & Alimah, S. (2013). Competency and Conservation-Based Strategies in Biology Learning. *Biosaintifika Journal of Biology & Biology Education*. 5(2), 121-129.

Saito, E., Murase, M., Tsukui, A., & Yeo, J. (2015). *Lesson Study for Learning Community*. New York: Routledge.

Slamet, Subad, T., Sutama, & Khotimah, R. P. (2010). Peningkatan Kompetensi Guru Sekolah Dasar melalui Lesson Study. *Warta*, 13(1), 55-64.

Susilo, H, Chotimah, H, Joharmawan, R, Jumiati, Dwita sari, Y, & Sunarjo. (2009). *Lesson Study Berbasis Sekolah.* Malang: Bayumedia Publising.

Yamaji, A. (2016). Teacher discourse supporting peer collaboration in mathematics. *International Journal for Lesson and Learning Studies*, 5(3), 255-270.

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Resistance of Advanced Soybean Lines to Pod Borrer (*Etiella zinckenella*)

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E. zinckenella; soybean lines; infected pod; infected seed

Abstract

The increasing and stabilizing of soybean product in Indonesia face many limitations. One of the limiting factors is pod borrer (*Etiella zinckenella* Treitschke) infestation that is able to cause yield loss up to 80%. Objective of the research was to find out some advanced soybean lines that resistant to pod borrer. Design was randomized complete block with three replications. Soybean lines were grown gradualy to ensure the simultanously flowering. The plants were caged at 35 days after planting (DAT) and infested with the imago of *E. zinckenella* at 56 DAT. Results showed that different soybean lines affected imago population, eggs population, larvae population, infected pods and infected seeds. Some genotypes were consistantly resistant to *E. zinckenella*. The resistance of those genotypes were non preference resistance based on eggs population, larvae population, infected pod and infected seeds. This study discovered nine soybean lines that is resistant to *E. zinckenella*, so that it can be beneficial for improving soybean resistance to this pest through releasing as a new resistant pod borer variety after tested further in potential yield and genetic x environment interaction trials. In addition, there were three varieties and two germplasm accessions that can be used as gene sources for improving the resistance of the varieties. The three varieties are able to be cultivated directly in field to decrease the *E. zinckenella* occurrence.

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INTRODUCTION

One of the limiting factors in increasing and stabilization of soybean product is pest attack (Tengkano et al., 2006). Pod borer, *Etiella zinckenella* Treitschke (Lepidoptera: Pyralidae) is the one of major pests in soybean (Baliadi et al., 2008a). Fluctuation and peak of eggs and larvae population vary according to the season, rainfall, variety, growing pattern, other host availability, natural enemy, and pest control activity using insectiside (Wagiman et al., 1987). The highest population of pod borer in soybean occurs in dry season, although there is *Crotalaria* sp. as the host along the year. Host plant can be served as source of pest population and as direct or indirect pest controller (Baliadi et al., 2008b).

Usually, pod borer controlling in Indonesia is conducted by using chemical insecticide causing negative effect to human health and ecosystem stability (Baliadi et al., 2008b). Beside, insecticide controlling is also inefficient because requires high cost (Tabata & Yasuda, 2011). Some of alternative environment friendly for controlling strategy to *E. zinckenella* are conducted by aplying resistant variety, trap crop, release of *Trichogramma* sp. paracitoid, sex-pheromon, and resistant gene transfer through biotechnology. The usage of resistant variety is able to decrease pesticide residue in environment and ecoomicaly benefit (Oki et al., 2012).

There are variability responses of soybean genotypes to pod borer *Etiella zinckenella* Treitschke (Amro et al., 2009; Taghizadeh et al., 2012). Variety of Grobogan is less preferred by imago pod borer as site for laying eggs with eggs population about 0.6–2 eggs per hill (Tengkano et al., 2010). Soybean genetic variability to pod borer are need to be identified to provide gene sources in development of soybean that resistant to pod borer. Soybean lines derived from crossing activity are also need to be identified to find out the combination of resistance trait and agronomical traits in a soybean line. Resistant cultivars is one of integrated control components (Inayati & Yusnawan, 2016).

The objective of the research was to study non preference resistance level of soybean genotypes to pod borer *E. zinckenella* Treitschke, and to find out the genotypes that resistance to this pod borer. The finding of the resistance level can be studied further to understand the resistance mechanism and the role of plant resistance gene. Besides, the resistant genotypes will benefit to be used in improving soybean resistance to pod borer or as promising lines that can be tested further for their yield potential, and then can be released as a new variety for pod borer resistance.

METHODS

The experiment was in laboratory and greenhouse of Indonesian Legume and Tuber Crops Research Institute (ILETRI), Malang. The design was a randomized complete block design (RCBD) with three replications. The materials consisted of 50 genotypes from crosses, five resistant genotypes (IAC 100, IAC 80, G 100 H, Detam 1, and No. 29) and two susceptible genotypes (Ichyou and Wilis).

Soybean variety "Wilis" was grown sequentially in ILETRI's greenhouse with one week interval for seven times planting date in the area of 7 m x 5 m every planting date. Plants were fertilized using Urea 0.4 g/hill and NPK 1.2 g/hill. Bean fly pests were controlled with insecticides of cypermethrin on 8 DAP, and leaf pests were controlled with insecticides of cyhalothrin at 14, 21, and 28 days after planting. After the plant was 28 DAP, controls were carried out manually.

Pod borer insects were obtain from collecting of instar 5 larva in Ngale Research Station, Ngawi Regency, East Java Province, Indonesia. The collected larva were taken to ILETRI's pest laboratory. Larva were reared in the plastic container, where in the container was filled with sawdust. Larvae were reared until transforming to be pupae and then the pupae moved to copulation cage. At the top of the inside cage, there were hanging cotton layer filled with 10% honey solution for feeding the imago that out from the pupa. At three days after the imago appeared, imago was identified for the sex, and the damages of foot and antene by using binocullar microscop. At four days age, the selected imagos were ready to be applied in treatment plants.

Planting was conducted in RCBD with three replications, where every replication consisted of 6 polybags/genotype with four seeds/polybag. Planting was conducted based on flowering age of each genotype in order pod setting start simultaneously. Fertilizers were applied by using Urea 0.4 g and NPK 1.2 g per polybag at sowing date. Watering were carried out by monitoring the water availability in the soil and water will be added to maintain field capacity. Thinning was applied at 14 DAP with remaining 2 plants/polybag. Similar to the feed preparation, bean fly pests were controlled with insecticides of sipermetrin on 8 DAP, and leaf pests were controlled with insecticides of sihalothrin at 14, 21, and 28 days after planting.

The plants were caged by using screen textile at 35 DAP. When the plant age was 21 days after flowering (DAF), pod borer infestation in

treatment plants was carried out at 14.00 Western Indonesian Time with two couples of imago for every genotype in one replication. Hence, there were 144 couples or 228 imago in one replication. Number of eggs was observed 2 days after infestation (DAI) at the pods by using binocular microscope. Each observation of larva population and percentage of pod borer attacking were recorded at two polybags consisted of four plants at 14 DAI, before larva got down to the soil for transforming to be pupa. Data were analyzed by using analysis of variance and continued with least significant difference at 5% significance level.

Attacking percentage was calculated as follows:

$$Pod\ damage = \frac{Number\ of\ damage\ pods}{Number\ of\ total\ pods} \times 100\%$$

$$Seed\ damage = \frac{Number\ of\ damage\ seeds}{Number\ of\ total\ seeds} \times 100\%$$

Determination of resistance criteria based on the formula bellow (Chiang & Talekar, 1980):

< X – 2 SD	= HR (Highly Resistant)
X – 2 SD to X – SD	= R (Resistant)
X – SD to X	= MR(Moderately Resistant)
X to X + SD	= S (Susceptible)
>X + SD	= HS (Highly Susceptible)

Where:

X	= Mean of pod damage or seed damage
SD	= Standard deviation

RESULTS AND DISCUSSION

Imago population

Analysis of variance showed that the differences of soybean genotype affected significantly on population of imago, eggs, and larva, and number of attacked pod and seed damage. Imago population of *E. zinckenella* on 50 evaluated genotypes, six check resistant genotypes, and one check susceptible genotypes revealed that imago population ranged 2.33–8.33 imago/genotype (Fig. 1). The highest imago population were on genotype of Tgm/Anj-599 and Tgm/Anj-764 (8.33 imago/genotype), while the lowest imago population were on genotype of Tgm/Anj-833 (2.33 imago/genotype). Imago population on check resistant genotype ranged between 5-5.67 imago/genotype, while imago population on check susceptible genotype were 5.67 imago/genotype. Based on imago population, there were two genotype indicated highly resistant of non-preference manner or were not chosen as an alighting site, i.e. Tgm/Anj-833 and Anjasmoro. Beside, there were seven genotype indicated as resistant genotypes, i.e. Tgm/Anj-530, Tgm/Anj-847, Tgm/Anj-889, Tgm/Anj-910, Tgm/Anj-912, Tgm/Anj-918, and Tgm/Anj-959. Probably, these genotypes had short trichome, because the genotypes with many short trichomes are prefered by *E. zinckenella* in depositing eggs (Permana et al., 2012).

Population of eggs, and larvae of *E. zinckenella*

E. zinckenella eggs population on 57 tested genotypes were between 3.67-43.67 eggs/hill. The highest eggs population was on genotypes Tgm/Anj-571 (43.67 eggs/hill), while the lowest population was on genotype IAC 100 (3.67 eggs/hill) less lower than genotype G100H (4.67 eggs/hill). Those two genotypes were the check of resistant genotypes. Based on eggs population, there were three genotypes indicated as resistant genotypes in non-preference manner or were not chosen by imago as the site to put the eggs, i.e. Tgm/Anj-778, Tgm/Anj-847, and Tgm/Anj-909 with eggs population 5.00, 9.33 and 8.67 eggs/hill respectively.

In this study, varieties of Tanggamus, Anjasmoro, and Detam 1 showed criteria of moderately resistant or less chosen by *E. zinckenella* imago as site for putting the eggs with eggs population 12, 10 and 15.67 eggs/hill. This study was supported by Tengkano et al. (2011) that varieties of Tanggamus and Anjasmoro were not chosen by pod borer imago as site for putting the eggs with eggs population 0 egg/hill, while Detam 1 was less chosen by imago for putting the eggs. Taghizadeh et al. (2012) also reported different mean number of eggs laid among the cultivars.

Genotypes of Ichyou and Wilis, as the check susceptible genotypes, also showed low eggs population namely 4.67 and 11 eggs/hill respectively. Hence, Ichyou and Wilis were indicated as resistant and moderately resistant respectively. This result is different to Santi et al. (2014) that stated Ichyou was chosen by *E. zinckenella* imago as site for putting the eggs. Presumably, this difference caused by the number of genotypes, where in this study we used 57 genotypes that was more than Santi et al. (2014) leading lower possibility to be chosen as eggs place. Ichyou is a genotype with hairless pod. Genotype with hairless pods is not preferred by the imago to lay eggs because they do not have hair that can protect the eggs (Susanto & Adie, 2008). However, based on the observations of Ichiyou pods in laboratory, pod borer eggs were laid under the petals at the base of the pod (Santi et al., 2014). Perhaps, Ichyou has secondary chemical compounds that can attract imago pod borer to lay their eggs on the

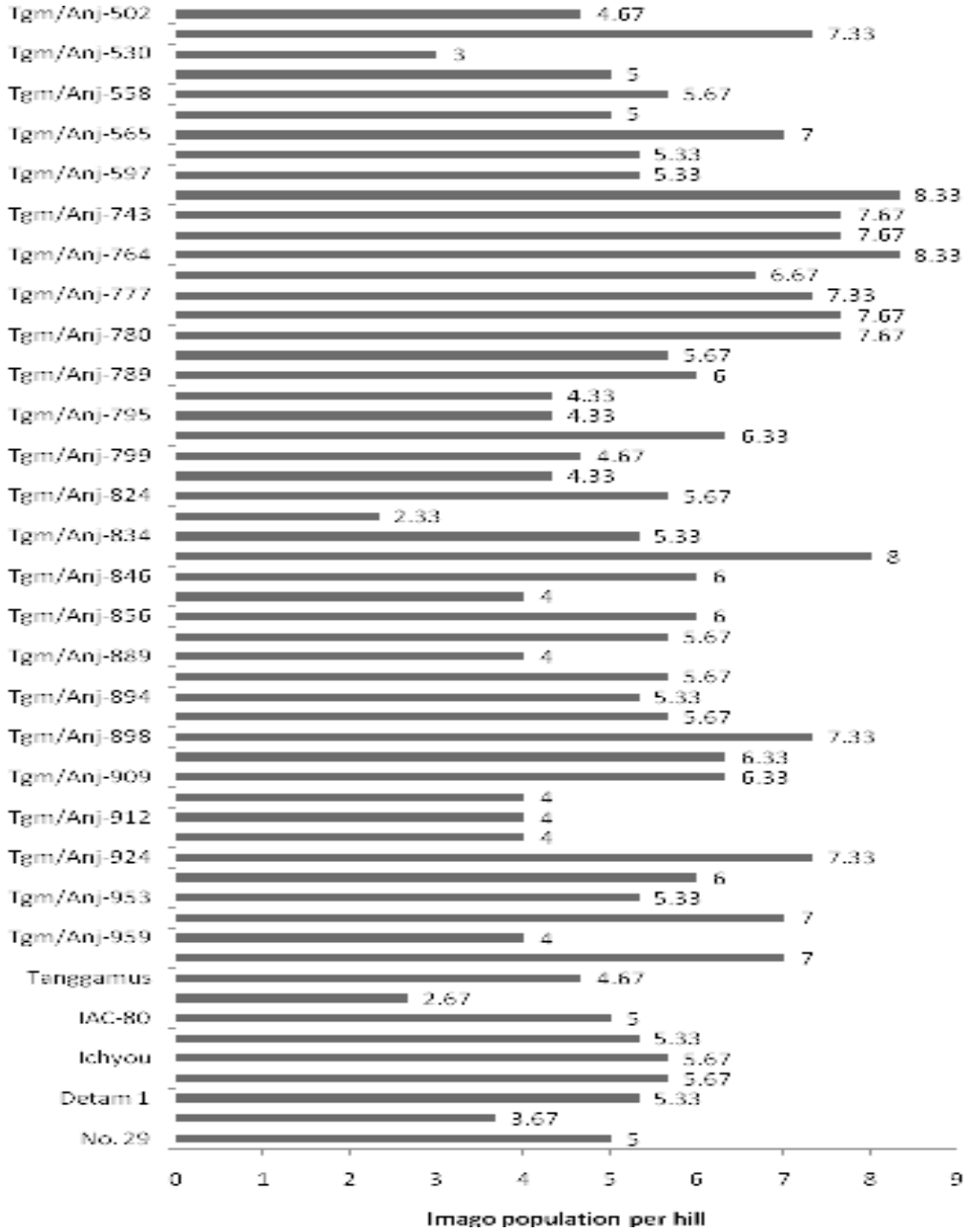

Figure 1. Population of imago of *E. zinckenella*

pods by hiding them under the petals pods. On hairy pods, eggs were laid by pod borer imago on the pod skin among the hairs. This may be due to the pod borer imago want the eggs laid among the hair can not be taken by predators such as imago of *Paederus* sp. Observation showed that when the pod borer eggs removed from the pod, it would soon be devoured by imago *Paederus* sp.

Differences soybean genotypes also significantly affected populations of *E. zinckenella* larvae (Table 1). The population of pod borer larvae on these genotypes ranged between 37.67-255 larvae/hill. The highest larval population was found on the Tgm/Anj-784, while the lowest population was found on Anjasmoro variety. Based on the larval population and the analysis of variance it can be argued that Anjasmoro was indicated as a highly resistant variety, where the resistance was non-preferences or not selected by the larvae as their food. Beside Anjasmoro, there were three genotypes that indicated as resistant genotypes based on larval population i.e. Tgm/Anj-744 (92.33 larvae/hill), Tgm/Anj-833 (93.67 larvae/hill), and Tgm/Anj-897 (86 larvae/hill). In addition, there were 20 genotypes that indicated as medium resistant with larval population between 113.33-143.67 larvae/hill.

In this study, genotype IAC 100 and

Table 1. Population of egg and larva of *E. zinckenella*

Genotype	Egg population per hill	Criteria	Larva population per hill	Criteria
Tgm/Anj-502	34.00 a-g	HS	158.33 b-i	S
Tgm/Anj-522	42.33 a-b	HS	140.33 b-i	MR
Tgm/Anj-530	17.33 a-j	MR	161.00 b-i	S
Tgm/Anj-537	32.33 a-f	HS	156.33 b-i	S
Tgm/Anj-558	14.00 b-j	MR	156.67 b-i	S
Tgm/Anj-561	29.00 a-h	S	127.00 b-i	MR
Tgm/Anj-565	18.33 c-j	MR	197.67 a-e	HS
Tgm/Anj-571	43.67 a-b	HS	188.67 a-e	HS
Tgm/Anj-597	14.67 c-j	MR	156.33 b-i	S
Tgm/Anj-599	16.67 a-j	MR	142.33 b-i	MR
Tgm/Anj-743	11.00 e-j	MR	121.67 d-j	MR
Tgm/Anj-744	20.00 a-j	S	92.33 f-j	R
Tgm/Anj-764	24.00 a-h	S	127.00 b-i	MR
Tgm/Anj-773	15.67 a-j	MR	207.67 a-d	HS
Tgm/Anj-777	21.33 a-h	S	122.67 c-j	MR
Tgm/Anj-778	5.00 i-j	R	140.67 b-i	MR
Tgm/Anj-780	16.33 a-j	MR	120.00 d-j	MR
Tgm/Anj-784	10.00 f-j	MR	255.00 a	HS
Tgm/Anj-789	13.00 c-j	MR	125.33 b-j	MR
Tgm/Anj-790	19.33 a-i	MR	135.33 b-i	MR
Tgm/Anj-795	15.33 c-j	MR	143.67 b-i	MR
Tgm/Anj-796	26.00 a-h	S	156.00 b-i	S
Tgm/Anj-799	16.00 a-j	MR	157.67 b-i	S
Tgm/Anj-803	12.00 e-j	MR	169.00 a-g	S
Tgm/Anj-824	13.00 c-j	MR	133.00 b-i	MR
Tgm/Anj-833	15.00 c-j	MR	93.67 f-j	R
Tgm/Anj-834	11.67 d-j	MR	177.33 a-f	S
Tgm/Anj-844	27.00 a-h	S	210.67 a-c	HS
Tgm/Anj-846	12.67 f-j	MR	194.67 a-e	HS
Tgm/Anj-847	9.33 g-j	R	190.00 a-e	HS
Tgm/Anj-856	34.33 a-f	HS	113.33 e-j	MR
Tgm/Anj-871	17.67 c-j	MR	121.00 d-j	MR
Tgm/Anj-889	23.67 a-j	S	165.33 b-h	S
Tgm/Anj-890	32.33 a-e	HS	169.67 a-g	S
Tgm/Anj-894	38.33 a-e	HS	152.67 b-i	S
Tgm/Anj-897	17.33 a-j	MR	86.00 g-j	R
Tgm/Anj-898	19.33 a-i	MR	132.67 b-i	MR
Tgm/Anj-908	18.00 a-j	MR	138.67 b-i	MR
Tgm/Anj-909	8.67 g-j	R	153.33 b-i	S
Tgm/Anj-910	18.67 a-j	MR	197.33 a-e	HS
Tgm/Anj-912	27.33 a-g	S	136.33 b-i	MR
Tgm/Anj-918	39.33 a-c	HS	153.33 b-i	S
Tgm/Anj-924	42.00 a	HS	149.33 b-i	S
Tgm/Anj-933	23.33 a-h	S	213.00 a-b	HS
Tgm/Anj-953	13.67 c-j	MR	139.33 b-i	MR
Tgm/Anj-957	34.67 a-d	HS	137.00 b-i	MR
Tgm/Anj-959	24.33 a-h	S	142.67 b-i	MR
Tgm/Anj-982	27.67 a-h	S	182.33 a-e	S
Tanggamus	12.00 e-j	MR	154.67 b-i	S
Anjasmoro	10.00 e-j	MR	37.67 j	HR
IAC-80	17.00 a-j	MR	165.33 b-h	S
IAC-100	3.67 j	R	72.67 i-j	R
Ichyou	4.67 i-j	R	161.33 b-h	S
G 100 H	4.67 i-j	R	80.33 h-j	R
Detam 1	15.67 a-j	MR	111.00 e-j	MR
Wilis	11.00 e-j	MR	140.00 b-i	MR
No. 29	7.67 h-j	R	143.33 b-i	MR

The number followed by the same letter is not different based on least significant different at 5% level (LSD 5%), HR = highly resistant, R = resistant, MR = moderately resistant, S = susceptible, HS = highly susceptible

G100H as check resistant genotypes were consistanly resistant with larval population 72.67 and 80.33 larva/hill respectively. However, genotype IAC 80 as resistant check genotype showed as susceptible criteria with larval population 165.33 larvae/hill, while Wilis as susceptible check variety showed medium resistant cirteria with larvae population of 140 larva/hill. There may be other factors that caused genotype IAC 80 selected by larvae as their food. The resistance of the tested genotypes were non preference, it meant that the genotype is not chosen or not favored by pod borer larvae as their food. The genotypes were not chosen by the larvae as food materials alleged to contain chemical compounds that can be detected and disliked by pod borer larvae.

The percentage of pods and seeds that attacked by *E. zinckenella*

Analysis of variance showed that the differences in soybean genotypes significantly affected the percentage of pod damage and seeds by pod borer (Table 2). The percentage of infected pods on 57 tested genotypes ranged from 37.29% to 81.58%. The highest percentage of pod damage by *E. zinckenella* larvae was found on No. 29 variety with 37.29%, while the lowest percentage was found on Tgm/Anj-790 with 81.58%. Based on

the percentage of infected pods, there are 8 resistant genotypes i.e. Tgm/Anj-744, Tgm/Anj-824, Tgm/Anj-796, Tgm/Anj-856, Tgm/Anj-871, Tgm/Anj-897, Tgm/Anj-908, Tanggamus, and Anjasmoro. Beside, there were 12 genotypes that indicated as moderately resistant (Table 1).

Figure 2. Outside view of soybean pods attacked by *Etiella zinckenella*

Figure 3. Inside view of soybean pods attacked by *Etiella zinckenella*

Table 2 also shows that the differences in soybean genotype significantly affected percentage of attacked seed by *E. zinckenella*. The percentage of seed damage of the 57 tested genotypes ranged from 30.17% to 70.74% per hill. The highest percentage of seed damage by *E. zinckenella* larvae was found on genotype Tgm/Anj-790, while the lowest percentage of pod damage found on genotype Tgm/Anj-871. Based on the percentage of seed damage, there were five resistant genotype i.e. Tgm/Anj-846, Tgm/Anj-871, Tgm/Anj-897, Tgm/Anj-908, and Anjasmoro. Beside, there were 17 genotypes that indicated as moderately resistant (Table 2).

Table 2. Percentage of pod damage and seeds by *E. zinckenella*

Genotype	Pod damage (%)	Criteria	Seed damage (%)	Criteria
Tgm/Anj-502	68.69 a-j	S	54.97 a-n	S
Tgm/Anj-522	72.40 a-g	HS	55.27 a-n	S
Tgm/Anj-530	75.96 a-d	HS	59.77 a-j	S
Tgm/Anj-537	78.81 a-b	HS	67.26 a-e	HS
Tgm/Anj-558	69.86 a-i	S	60.81 a-i	HS
Tgm/Anj-561	67.72 a-k	S	53.28 a-n	S
Tgm/Anj-565	66.70 a-l	S	54.24 a-n	S
Tgm/Anj-571	75.73 a-e	HS	69.31 a-c	HS
Tgm/Anj-597	69.21 a-i	S	60.02 a-j	S
Tgm/Anj-599	55.76 d-o	MR	41.48 h-q	MR
Tgm/Anj-743	52.41 f-o	MR	40.50 j-q	MR
Tgm/Anj-744	49.13 i-o	R	40.71 j-q	MR
Tgm/Anj-764	51.59 g-o	MR	43.09 g-q	MR
Tgm/Anj-773	73.54 a-f	HS	68.45 a-d	HS
Tgm/Anj-777	67.97 a-j	S	58.56 a-k	S
Tgm/Anj-778	50.55 h-o	MR	42.12 h-q	MR
Tgm/Anj-780	61.50 a-n	S	53.68 a-n	S
Tgm/Anj-784	78.44 a-c	HS	62.78 a-g	HS
Tgm/Anj-789	53.28 f-o	MR	49.30 d-q	MR
Tgm/Anj-790	81.58 a	HS	70.74 a-b	HS
Tgm/Anj-795	60.55 a-n	MR	49.39 c-q	MR
Tgm/Anj-796	45.74 l-o	R	40.67 j-q	MR
Tgm/Anj-799	54.80 d-o	MR	40.65 j-q	MR
Tgm/Anj-803	54.65 e-o	MR	39.58 k-q	MR
Tgm/Anj-824	46.67 k-o	R	43.20 g-q	MR
Tgm/Anj-833	62.49 a-n	S	53.85 a-n	S
Tgm/Anj-834	60.60 a-n	S	46.22 f-q	MR
Tgm/Anj-844	81.11 a	HS	61.15 a-h	HS
Tgm/Anj-846	53.13 f-o	MR	37.85 m-q	R
Tgm/Anj-847	72.45 a-g	HS	52.48 a-o	S
Tgm/Anj-856	49.84 i-o	R	47.78 e-q	MR
Tgm/Anj-871	42.48 m-o	R	30.17 q	R
Tgm/Anj-889	68.72 a-j	S	51.55 b-o	S
Tgm/Anj-890	67.82 a-k	S	54.21 a-n	S
Tgm/Anj-894	69.99 a-i	S	56.88 a-m	S
Tgm/Anj-897	47.60 j-o	R	37.43 m-q	R
Tgm/Anj-898	71.34 a-h	S	72.36 a	HS
Tgm/Anj-908	49.71 i-o	R	36.40 n-q	R
Tgm/Anj-909	65.83 a-l	S	59.44 a-k	S
Tgm/Anj-910	61.04 a-n	MR	44.72 g-q	MR

Tgm/Anj-912	54.07 f-o	MR	47.89 e-q	MR
Tgm/Anj-918	63.92 a-l	S	52.81 a-o	S
Tgm/Anj-924	61.30 a-n	S	51.67 b-o	S
Tgm/Anj-933	77.15 a-c	HS	64.81 a-f	HS
Tgm/Anj-953	62.33 a-n	S	51.27 b-o	S
Tgm/Anj-957	69.37 a-i	S	58.17 a-l	S
Tgm/Anj-959	53.12 f-o	MR	46.00 f-q	MR
Tgm/Anj-982	66.81 a-l	S	50.01 c-q	S
Tanggamus	49.60 i-o	R	42.41 h-q	MR
Anjasmoro	41.64 n-o	R	33.24 o-q	R
IAC-80	63.80 a-l	S	50.54 c-p	S
IAC-100	50.30 h-o	MR	38.38 l-q	R
Ichyou	57.56 c-o	MR	48.81 d-q	MR
G 100 H	41.44 n-o	R	37.46 m-q	R
Detam 1	57.67 b-o	MR	40.95 i-q	MR
Wilis	63.19 a-m	S	35.46 n-q	R
No. 29	37.29 0	HR	31.17 p-q	R

The number followed by the same letter is not different based on least significant different at 5% level (LSD 5%), HR = highly resistant, R = resistant, MR = moderately resistant, S = susceptible, HS = highly susceptible

Based on Table 1 and 2, it can be seen that the resistance of soybean genotypes against *E. zinckenella* determined based on eggs population, larval population, the percentage of pod damage, and the percentage of seed damage. Imago population was not used as a resistance determinant because imago was mobile, especially when they were observed. Based on the four determinants, there were nine soybean lines that consistently resistant to *E. zinckenella*, i.e. Tgm/Anj-599, Tgm/Anj-743, Tgm/Anj-778, Tgm/Anj-789, Tgm/Anj-795, Tgm/Anj-824, Tgm/Anj-871, Tgm/Anj-897, and Tgm/Anj-908. Of the seven resistant check genotypes, there were three varieties (Anjasmoro, Detam 1, and No. 29) and two accessions (IAC 100 and G100H) that were indicated as resistant based on eggs population, larval population, percentage of pod damage and percentage of seed damage; whereas genotypes of IAC 80, Tanggamus and Wilis were susceptible. IAC 80 was suscaptible on three determinant variables, while Tanggamus and Willis were susceptible on one determinant variable. Probably, the location of IAC 80 based on randomization in the greenhouse was positioned close to the sun at noon and light at night, affecting the imago in choosing a place to lay their eggs and larvae to eat IAC 80 than other genotypes.

The nine soybean line can be assessed further for the potential yield if they be released as the new high yielding varieties that resistant to *E. zinckenella*. The three varieties and two accessions identified as *E. zinckenella* resistant can be studied further to ensure the gene resistance role and its mechanism in physiological aspect. These varieties are able to be used as gene sources for development a new resistant superior variety. Also, they can be cultivated in farmer field to reduce the impact of *E. zinckenella* incident. By using these varieties, the yield loss due to the *E. zinckenella* infestation can be decreased.

CONCLUSION

There were 10 genotypes that consistently resistant to *E. zinckenella*, i.e. Tgm/Anj-599, Tgm/Anj-743, Tgm/Anj-778, Tgm/Anj-789, Tgm/Anj-795, Tgm/Anj-824, Tgm/Anj-871, Tgm/Anj-897, Tgm/Anj-908, dan Anjasmoro. The resistance of the resistant genotypes were antixenosis resistance (non-preference resistance) or the rejection of plants because of the morphological characters on the plant causing the insects did not like the plants as food and roost and shelter.

REFERENCES

Amro, M. A., Abdel-Moniem, A. S. H., & Omar, M. S. (2009). Determination of the resistance status of experimental soybeans to the lima bean pod borer, *Etiella zinckenella* Treitschke and the Whitefly, *Bemisia tabaci* Gennadius at El-Dakhla Oases, New Valley, Egypt. *Archives of Phytopathology and Plant Protection*, 42(6), 552-558.

Baliadi, Y., Tengkano, W., & Marwoto. (2008a). Soybean pod borer, *Etiella zinckenella* Treitschke (Lepidoptera: Pyralidae), and the controlling strategy in Indonesia. *Jurnal Litbang Pertanian*, 27(4), 113-123.

Baliadi, Y., Tengkano, W., Bedjo, & Purwantoro. (2008b). Validation of recommendation of soybean integrated pest management in lowland field with planting patern of rice-soybean-soybean. *Agritek*, 16(3), 492-500.

Chiang, H. S., & Talekar, N. S. (1980). Identification of source of resistance and to the bean fly and two other agromyzid flies in soybean and mungbean. *Journal of Economic Entomology,* 73(2), 197-199.

Inayati, A., & Yusnawan, E. (2016). Characteristics of Superior Soybean Breeding Lines Tolerance to Rust (*Phakopsora pachyrhizi*). *Biosaintifika: Journal of Biology & Biology Education*, 8(1), 47-55.

Oki, N., Komatsu, K., Sayama, T., Ishimoto, M., Takahashi, M., & Takahashi, M. (2012). Genetic analysis of antixenosis resistance to the com-

mon cutworm (*Spodoptera litura* Fabricius) and its relationship with pubescence characteristics in soybean (*Glycine max* (L.) Merr.). *Breeding Science,* 61(5), 608-617.

Permana, A. D., Johari, A., Putra, R. E., Sastrodihadjo, S., & Ahmadi, I. (2012). The influence of trichome characters of soybean (*Glycine max* Merrill) on oviposition preference of soybean pod borer *Etiella zinckenella* Treitschke (Lepidoptera: Pyralidae) in Indonesia. *Journal of Entomology and Nematology*, 4(3), 15-21.

Santi M. Y. I. B., Baliadi, Y., Suhartina, & Tengkano, W. (2014). Response of soybean promising lines for acid soil and drought tolerances to pod borer. Proceeding *Proceeding of National Seminar of Legume and Tuber Crops Research Results 2013*. Indonesian Center for Food Crops Research and Development. pp. 245-255.

Susanto, G. W. A., & Adie, M. M. (2008). Characteristic of morphological resistance on soybean genotype pod borer insect (*Etiella zinckenella* Tr.). *Jurnal Penelitian Pertanian Tanaman Pangan* 27(2), 95-100.

Tabata, J., & Yasuda, K. (2011). The sex pheromone of the lima bean pod borer *Etiella zinckenella* (Treitschke). *JARQ,* 45(1), 47-50.

Taghizadeh, R., Talebi, A. A,, Fathipour , Y., & Khalghani, J. (2012). Effect of ten soybean cultivars on development and reproduction of lima bean pod borer, *Etiella zinckenella* (Lepidoptera: Pyralidae) under laboratory conditions. *Appl. Ent. Phytopath*. 79, 15-28.

Tengkano, W., Purwantoro, & Baliadi, Y. (2010). Identification of early maturing soybean genotypes that resistant to pod borer pest *Etiella Zinckenella* Tr. (Lepiodoptera: Pyralidae). *Proceeding of National Seminar of Legume and Tuber Crops Research Results.* Indonesian Center for Food Crops Research and Development, p. 217-232.

Tengkano, W., Supriyatin, Suharsono, Bedjo, Prayogo, Y., & Purwantoro. (2006). Status of soybean pests and natural enemies in acidic dryland Lampung. p: 511-526. *In* Suharsono et al. (eds). *Proceeding of National Seminarof Increasing Legume and Tuber Crops Production for Supporting Food Self Sufficiency*. Indonesian Center for Food Crops Research and Development.

Wagiman, F. X., Turnipseed, S., & Linser, W. (1987). An evaluation of soybean pests, factors affecting their abundance, and recommendation for integrated pest management in Java. *Survey Report*. Departement of Entomology and Phytopathology, Faculty of Agriculture, Gajah Mada University, Yogyakarta. 21 pp.

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Antioxidant Activity of Dominant Plants Species in *Obat Pahit* from Lingga Malay Ethnic in Riau Archipelago

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Abstract

Obat Pahit is a potion that has been long commonly consumed by Lingga Malay society for generations as stamina keeper. The most dominant plants found in the packaging of the Obat Pahit were namely *Bauhunia semibifida, Cnestis palala* and Penawa Root (3 species). This research aimed to investigate and determine activity of antioxidant contents in *Obat Pahit* from five Traditional Medicine Practitioners (TMPs) in the district of Lingga. The tested samples were mashed then being soaked into 2 types of solvent: distilled water and methanol, containing HCl 1%. DPPH method was also used in this research. Quantitatively antioxidant activity test of *Obat Pahit* from the five TMPs by using methanol solvent had extremely highest activity compared to the distilled water solvent. The test, using TLC plate by spraying the extract from three dominant plants with 0.1 mM of DPPH solution, produced a pale-yellow spots at a wavelength of 366 nm. On the other hand, the test using HPLC at wavelengths of 230 nm and 280 nm showed the presence of two dominant secondary metabolites contents: flavonoid and phenolic. IC50 (ppm) of *Bauhinia semibifida* (6.6247), Penawa Root (5.0124) and *Cnestis palala* (5.9968) were much lower than IC50 of mangosteen's rind (41.7675), vitamin C (6.6612) and Stimuno drug (8.333). This antioxidant analysis has not been reported previously. This proof contributed greatly to uncovering potentially native natural resources as an indigenous Indonesian drug which is expected to decrease dependence on imported drugs especially imunomodulator, antihypertensive, antidiabet etc. This research would be beneficial and excellent manifestation for the development of natural antioxidant-based medicines from traditional knowledge of Indonesia's local ethnicities.

How to Cite

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INTRODUCTION

Antioxidants are compounds that can prevent from the oxidation of other compounds which occurs either in the body or interaction of other compounds which are easily oxidized. It also can block the oxidation of free radicals occurring inside our body cells by neutralizing or destroying (scavenging) them (Lautan, 1997). Free radicals are atoms or molecules that have one or more unpaired electrons. This causes them are chemically reactive, which can cause chemical changes and damage on various components of living cells. In the human body, free radicals are considered to play a role in the occurrence of some diseases, such as aging. Currently, exposured by them is quite widespread in society, ranging from pollution to unhealthy foods (Winarsi, 2007). Therefore, various studies to obtain a safe antioxidant from natural sources were mostly done. Alkaloids, Flavonoids, tannins, polyphenols, vitamin C, vitamin E, and carotenoids are classes of compounds group from natural materials that possess the potency as antioxidants (Miller, 1996; Prior, 2003; Pokorny et al. 2001; Teruna et al. 2007, Zamri et al. 2016)

One of potions that derived from natural ingredients is *Obat Pahit*, which is commonly consumed by Malay ethnicity in Lingga, Riau Archipelago. This potion is efficacious to maintain body's power and fitness. Each village in Lingga has a Traditional Medicine Practitioner (TMP), who still uses and mixes ingredients of *Obat Pahit* with different types of medicinal herbs based on the knowledge inherited by generations. Some TMPs who are famous in concocting of *Obat Pahit* are originally from Kalan Village, SP4 Village, Linau Village, Resun Village dan Musai Village. This knowledge will different from one village to another because village difference of residence, which is affected by customs and procedures and behavior of local society (Irawan *et al.* 2013). In packaging of the potion from TMPs, there are some dominant plants species mentioned Kangkang Valves Root "*Akar Kangkang Katup*" (*Bauhinia semibifida),* Seven Layers Root "*Akar Tujuh Lapis*" (*Cnestis palala*), and *Akar Penawa*, as well as other complementary plants.

With varying TMPs and composition of *Obat Pahit*'s ingredients, it was necessary to examine the activity of antioxidant contents in *Obat Pahit* obtained from five TMPs, who are originally from Lingga, Riau Archipelago, as well as the investigation of antioxidant contents over the most three dominant plants used in the packaging of *Obat Pahit*. Overall, it was expected that this research would be beneficial and excellent manifestation for the development of natural antioxidant-based medicines from traditional knowledge of Indonesia's local ethnicities.

METHODS

Materials used in this study were *Obat Pahit* potion from the five TMPs, plants sample of *Akar Kangkang Katup* (*Bauhinia semibifida*), *Akar Tujuh Lapis* (*Cnestis palala*), *Penawar* root, mangosteen's rinds (*Garcinia mangostana*), vitamin C, Drug Stimuno (adjusted for human dose and converted according to mouse dose by serving in infusa form), distilled water, methanol, silica gel GF254, ethyl-acetate, and DPPH powder (1,1-Diphenyl-2-picrylhydrazyl).

Sample Extraction

Obat Pahit potion with aquades solvent

Each samples of *Obat Pahit* ingredients, at weight of 250 g, was crushed to powder using a grinder. This powder was then used for the extraction of secondary metabolite constituents by adding 1 liter of distilled water into a jar containing 250 g of *Obat Pahit* powder until the powder was submerged thoroughly. After that, it was then soaked for 1 hour. The extraction was filtered with filter paper. The filtrate was then evaporated to form a solid-liquid extract which will be used for the future test of antioxidant activity.

Obat Pahit potion with methanol solvent

Potions of *Obat Pahit* potion from five TMPs was grinded by using a blender. Then, it was obtained a powder which was used for extraction. A 100 g of Simplicia powder was macerated with methanol until it was submerged, and being soaked for a day. All extract was collected and evaporated with a rotary vacuum evaporator at 50 ° C to obtain a solid-liquid extract. The resulted extract was then measured.

The dominant plants of Obat Pahit (Akar Penawar, Akar Tujuh Lapis, Akar Kangkang Katup/Sebaju)

Potions of three dominant plants from the packaging of *Obat Pahit* potion was then crushed to powder using a blender. A total of 5 grams of powder samples were soaked in methanol for 24 hours and then ultrasonized for 30 minutes, followed by the filtration to obtain a liquid extract. The liquid extract was then evaporated by rotary evaporator till getting a thick layer.

Antioxidant Activity in Quantitatve and Qualitative Obat Pahit potion

Quantitatively antioxidant activity test of Obat Pahit ingredients from five TMPs and examination of three dominant plants with DPPH Method

The test of antioxidant activity used a two-fold-delution microplate reader with DPPH method (1,1- diphenyl-2-picryl hydrazyl) (Zhang et al., 2006; Wahdaningsih et al. 2013) at a wavelength of 520 nm. A sample of 2 mg was dissolved in 2 mL of MeOH until the concentration of the sample became 1000 mg / mL. Line A was inserted a sample of 100 mL (plate consisting of rows A-H, respectively amounted to 12 wells). A total of 50 mL MeOH was inserted into each well on line B-F. 50 mL from row A was then put into row B, 50 mL from row B was subsequently inserted into the line C, the same preparation was continually done to the line F. However, 50 mL from line F was discarded in order to obtain a concentration of 1000, 500, 250, 125, 62.5, and 31.25 g / mL. On the other hand, line G to H was filled with only 50 mL of MeOH, specifically only wells on the line 1-6 of row H filled. Line A-G for DPPH method was added by 80 mL of MeOH with a concentration of 80 mg / mL, and then incubated for 30 minutes. The activity of radical bounding was measured as a decrease of DPPH absorbance by the presence of microplate reader and data processing. Positive control was used as a comparison for ascorbic acid gradient at a concentration of 50 ug / mL. The percentage of inhibition value was calculated by the following formula (Andayani et al. 2008):

% Inhibition = (A Control-A Sample)/(A Control) x 100 %

Note: A control = Absorbansi uncontained sample

A sample = Absorbansi sample

Qualitatively antioxidant activity test of three dominant plants with Thin Layer Chromatography Methods (TLC)

0.5 ml of liquid extract (from point A) was then inserted into the vial. 5 samples were prepared for a TLC plate with a length of 5 cm elution. The respective samples were applied at the start layer in the elution from the chamber to the finish lines. The pattern of spots was appeared on the lamp of Uv ray at separation λ = 254 nm and 336 nm.

Qualitatively antioxidant activity test of three dominant plants with High Performance Liquid Chromatography (HPLC)

HPLC analysis was performed using elution method. Samples were dissolved in methanol (HPLC grade) (Susanti et al., 2017) (1 mg in 1mL methanol), then filtered with a 0.45 µ of PTFE 13mm. Filtrat as much as 20 mL were injected into the column, and then the samples were analyzed for 25 minutes using water:asettonitril (HPLC grade at 1 mg in 1mL methanol). ODS column was detailed with a length and a diameter of 150 x 4.6 mm.

RESULTS AND DISCUSSION

Quantitatively antioxidant activity test of Obat Pahit from five TMPs with two types of solvent: Distilled water and methanol

Based on the results of quantitatively antioxidant activity test with the solvent of distilled water, only *Obat Pahit* from TMP's Linau village had the lowest value of IC50 compared to the IC50 value of *Obat Pahit* from TMP's other villages, at 73.6347. This indicated that the antioxidant constituents of *Obat Pahit* potion from Linau village were belonged to the category of powerful antioxidants. On the other hand, by using methanol solvent on quantitatively antioxidant activity, *Obat Pahit* potion from all TMPs had a very low value of IC50 as following: TMP from Kalan (4.9285), TMP from SP4 (13.1546), TMP from Linau (11.1490), TMP from Resun (27.9204) and TMP from Musai (9.2948); therefore this *Obat Pahit* potions, in other words, were classified as a very powerful antioxidant. IC50 values of each *Obat Pahit* potions from the five TMPs on the result test of quantitatively antioxidant activity by using DPPH method stated in Table 1.

Antioxidant Activity Test of Three Dominant Medicinal Plants in the packaging of Obat Pahit potion

Qualitatively Antioxidant Activity test of three dominant medicinal plants using TLC Method

The qualitatively antioxidants activity experiment was done by using TLC method. The extraction of *Obat Pahit* was spotted on TLC plate and then eluted by using eluen n-Heksan : ethyl acetate (5:5), eluen ethyl acetate : methanol (8:2), eluen ethyl acetate (100%), eluen ethyl acetate : methanol (6:4). The reason of using these eluen n-Heksan : ethyl acetate (5:5) was in order to be able to eluting by the clear appearence of colours and spot distance when spotted on TLC plate. This was according to its polarity, eluen n-Heksan : ethyl acetate n-Heksan : ethyl acetate (5:5), eluen ethyl acetate : methanol (8:2), eluen ethyl acetate (100%), eluen ethyl acetate : methanol (6:4). The reason of using eluen n-Heksan : et-

hyl acatete (5:5) was because of their attributes which are more non-polar so that compounds which were separated were also non-polar, this association based on the TLC principle, "like dissolve like". After the TLC plate eluted, it was then sprayed by DPPH and next observed under UV light with different wave lengths. The spot appearing with yellow colour showed the occurence of free anti-radicals activity. Antioxidant compounds will react to DPPH radicals through the mechanism of hydrogen atoms donation and also cause the occurence of decaying colour from purple to yellow (Molyneux, 2004).

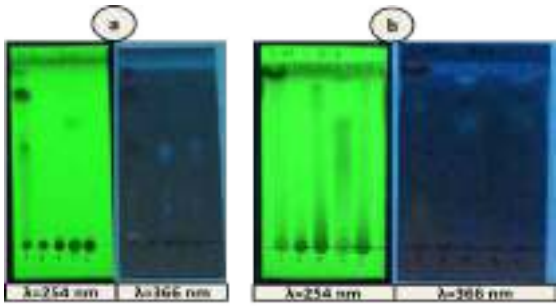


Figure 1. KLT Profile of plant was sprayed DPPH with Eluen N-Heksana : Etil asetat = 5:5(a) and Eluen Etil asetat : Methanol = 8:2(b)

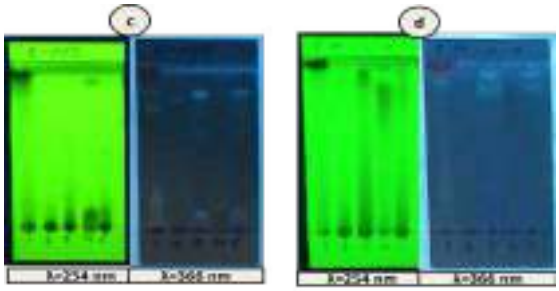
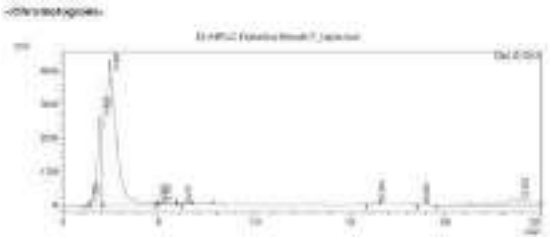


Figure 2. KLT Profile of plant was sprayed DPPH with Eluen Etil asetat = 100 % (c) dan Eluen Etil asetat : Metanol = 6:4 (d)
Note:1 = Mangosteen peel; 4 = *Penawar* Roots; 2 = *Cnestis palala;* 5 = Mixture; 3 = *Bauhinia semibifida*

Based on the TLC results, it can be compared between patches or spots from the five plant extracts with two different wavelengths, namely λ = 254 nm and λ = 366 nm. On the samples, more than one dots indicated that the samples had an organic compound or antioxidant compound. At a wavelength λ = 254 nm, antioxidant compounds such as terpenoids and flavonoids were not visible. However, at the wavelength λ = 366 nm, they are visible. One of them that can be seen from the TLC plate by using eluen ethylacetate:methanol (6:4) at a wavelength λ = 366 nm was more vivid than other eluens.

Qualitatively Antioxidant Activity test of three dominant medicinal plants using HLPC Method

Based on the HPLC assay with wavelengths of 230 nm and 280 nm, it was obtained some compounds which were dominant on the three plant samples and mixture sample. Wavelengths of 230 nm and 280 nm are the wavelengths to identify flavonoids and phenolic contents. It was also proved by the test of three dominant plants on antioxidant activity that showing a high antioxidant activity, because of phenolic and flavonoids contents are categorized as antioxidants (Figure 3; Figure 4; Figure 5; Figure 6).

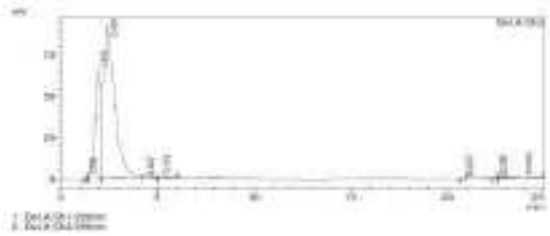


(a)

Table 1. Results of Quantitatively Antioxidant Activity Test of *Obat Pahit* potion from each TMPs with Distilled Water and Methanol Solvent

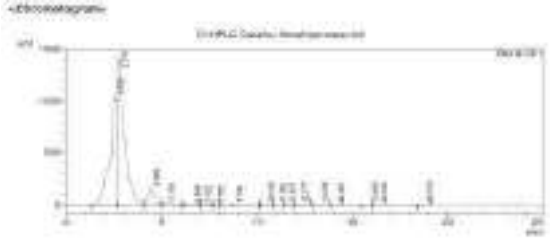
TMP	IC50 Value (ppm) Distilled Water	Antioxidant Activity Criteria*	IC50 Value ppm) Methanol	Antioxidant Activity Criteria*
Kalan	150.2199	Moderate	4.9285	Very Strong
SP4	122.3022	Moderate	13.1546	Very Strong
Linau	73.6347	Strong	11.1490	Very Strong
Resun	202.9095	Very Weak	27.9204	Very Strong
Musai	161.1920	Weak	9.2948	Very Strong

*Antioxidant activity criteria base on (Zuhra, 2008)

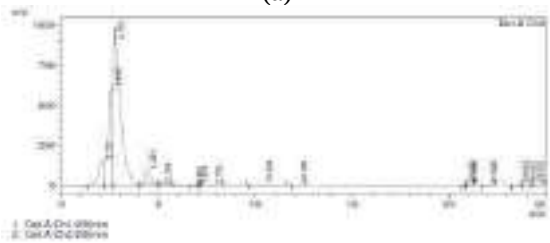


(b)

Figure 3. Result of HPLC from *Akar Tujuh Lapis* with Wavelength Detector (A) 230 nm and (B) 280 nm

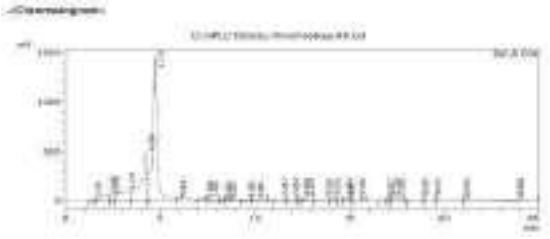


(a)



(b)

Figure 4. Result of HPLC from *Akar Penawar* with Wavelength Detector (A) 230 nm and (B) 280 nm



(a)

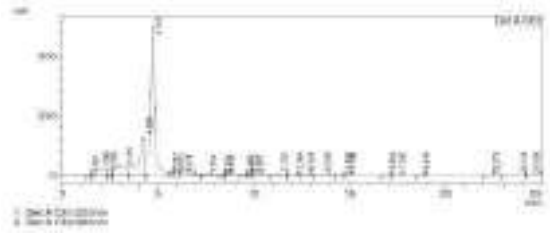


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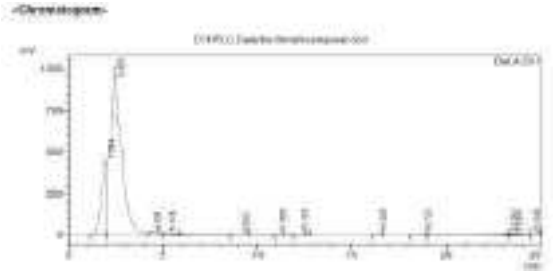
Figure 5. Result of HPLC from *Akar Kangkang Katup* with Wavelength Detector (A) 230 nm and (B) 280 nm



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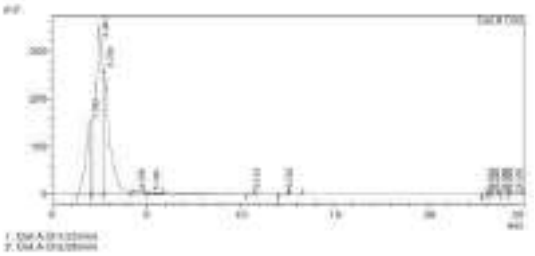

(b)

Figure 6. Result of HPLC from Mixture of *Akar Kangkang Katup*, *Akar Tujuh Lapis* dan *Akar Penawa* with Wavelength Detector (A) 230 nm and (B) 280 nm

Quantitatively Antioxidant Activity test of three dominant medicinal plants using DPPH Method

Quantitatively antioxidant activity test was also conducted on the single-most dominant herbs. This was carried out to observe the antioxidants activity on the herbal plants within *Obat Pahit* potion stated in Table 2 below:

Table 2. Results of Quantitatively Antioxidant Activity Test of Three Dominant Medicinal Plants used in *Obat Pahit* potion Packaging with Methanol Solvent

Samples' name	IC50 Value (ppm)	Antioxidant Activity Criteria*
Bauhinia semibifida	6.6247	Very strong
Penawar root	5.0124	Very strong
Cnestis palala	5.9968	Very strong
Mixture	29.6644	Very strong
Garcinia mangostana	41.7675	Very strong
Asam Askorbat	6.6612	Very strong
(*Phyllanthus niruri*)	8.333	Very strong

*Antioxidant activity criteria base on (Zuhra, 2008)

Positive controls used in this study were vitamin C (Ascorbic Acid), Mangosteen's rinds and immunomodulatory drugs (Stimuno). Vitamin C is an antioxidant that is soluble in water. The use

of positive control of this test is to find out how strong antioxidant potential exist in three types of methanol extract obtained from the plants, when compared to vitamin C. When the IC50 value of the samples is equal or close to the IC50 value of the positive control, it can be considered that the sample could potentially become as a powerful alternative antioxidant.

Based on the results of this test, the IC50 values of Penawar Roots, Seven-Layer Roots and Kangkang-Valve Root are 5.0124; 5.9968; 6.6247 (ppm), respectively. The three plants can be considered to have a very strong antioxidant activity, because it has a lower IC50 values than positive controls, i.e mangosteen peel (41.7675 ppm), IC50 in pure ascorbic acid (6.6612) and IC50 Stimuno (8.333 ppm). This indicates that the Penawar Roots, Seven-Layer Roots and Kangkang-Valve Root have a great potential as an antioxidant.

Since substantial evidences indicate that numerous human disorders and diseases present the involvement of oxidative stress (Teixeira et al*,* 2015). The attention of scientists has turned on the prospection and evaluation of antioxidant agents for the prevention and treatment of several diseases such as diabetes, atherosclerosis, aging, immune suppression and neurodegeneration (Saeed et al, 2012). The results from this study demonstrate high potential of these plant species also as sources of natural antioxidants. Natural antioxidants derived from plants, either in the form of raw extracts or their chemical constituents, are accepted to be very effective preventing the deleterious processes caused by oxidative stress (Cruz et all*,* 2014). This antioxidant analysis was proof contributed greatly to uncovering potentially native natural resources as an indigenous Indonesian drug which is expected to decrease dependence on imported drugs especially imunomodulator, antihypertensive, antidiabet etc.

CONCLUSION

The antioxidant activity of *Obat Pahit* potion from the five TMPs using methanol was significantly very strong compared to the solvent of distilled water. The qualitatively antioxidants test of *Bauhinia semibifida*, *Cnestis palala* and Penawar Roots by TLC and HPLC methods indicated the presence of flavonoid and terpenoids contents. Qualitatively antioxidant activity of IC50 values obtained from Penawar Roots, *Cnestis palala* and *Bauhinia semibifida*, respectively 5.0124 mg / mL; 5.9968 mg / mL: 6.6247 mg / mL, is much lower than the IC50 value of ascorbic acid (6.6612 mg / mL) and Stimuno (8.3333), even 4 times the mangosteen peel extract (41.7675 g / mL). Future studies are expected to examine the antioxidant contents of the active compounds of *Obat Pahit* potion and the three dominant plants in packaging.

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REFERENCES

Andayani, R.Y., Lisawati & Maimunah. (2008). Penentuan Antioksidan, Kadar Fenolat Total dan Likopen pada Buah Tomat (*Solanum lycopersicum* L). *Jurnal Sains dan Teknologi Farmasi,* 13(1).

Cruz, L. C., Batista, J. E. S., Zemolin, A. P. P., Nunes, M. E. M., Lippert, D. B., Royes, L. F. F., ... & Franco, J. L. (2014). A study on the quality and identity of Brazilian Pampa biome honey: evidences for its beneficial effects against oxidative stress and hyperglycemia. *International Journal of Food Science*, Article ID 470214.

Irawan, R.I., Fitmawati, Herman. (2013). Pengetahuan Tumbuhan Obat Dukun Sakai Desa Sebangar Duri Tiga Belas dan Desa Kesumbo Ampai Duri Kabupaten Bengkalis. *Biosaintifika: Journal of Biology & Biology Education*, 5(1), 30-35.

Lautan, J. (1997). Radikal Bebas pada Eritrosit dan Leukosit. *Cermin Dunia Kedokteran,* 9(116), 49-52.

Miller, A. L. (1996). Antioxidant flavonoids: structure, function, and clinical usage. *Alternative Medicine Review*, 1(2), 103-111.

Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology*, 26(2), 211-219.

Prior, R. L. (2003). Fruits and Vegetables in The Prevention of Cellular Oxidative Damage. *The American journal of clinical nutrition,* 78(3), 570S-578S.

Pokorny, J., Yanishlieva, N., & Gordon, M. (2001). *Antioxidant in food, Practical Application.* New York: CRC Press.

Saeed, N., Khan, M. R., & Shabbir, M. (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant

extracts *Torilis leptophylla* L. *BMC complementary and alternative medicine*, 12(1), 221.

Susanti, H., Wahyuono, S., Susidarti, R. A., & Sari, I. P. (2017). Diosgenin Determination of Costus speciosus Water Extract Using HPLC Method. *Traditional Medicine Journal*, 22(1), 1-6

Teixeira, M. P., Cruz, L., Franco, J. L., Vieira, R. B., Stefenon, V. M. (2015). Ethnobotany and antioxidant evaluation of commercialized medicinal plants from the Brazilian Pampa. *Acta Botanica Brasilica*, 30(1), 47-59

Teruna, H. Y., & Waterman, P. G. (2007). Alkaloids from the stem bark of *Orophea hexandra* (Annonaceae). *Biochemical systematics and ecology*, 35(7), 454-455

Wahdaningsih, S., Wahyuono., & Setyowati, E. P. (2013). Isolation and Identification of Antioxidant Compounds in Fern Stems (Alsophila glauca J. SM) U sing DPPH Method (2,2-Diphenyl-1-Picrylhydrazyl). *Traditional Meditional Journal*, 18(1), 38-45

Winarsi, H. (2007). *Antioksidan Alami dan Radikal Bebas.* Yogyakarta: Kanisius.

Zamri. A., Teruna H. Y., & Ikhtiarudin, I. (2016). The Influences of Power variations on Selectivity of Synthesis Reaction of 2'-Hydroxychalcone Analogue under Microwave Iradiation. *Molekul Journal*, 11(2), 299-307

Zhang, Q., Zhang, J., Shen, J., Silva, A., Dennis, D. A., & Barrow, C. J. (2006). A simple 96-well microplate method for estimation of total polyphenol content in seaweeds. *Journal of Applied Phycology*, 18(3), 445-450.

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Plant Response to Environmental Gradient Mediated by Trait Through Ontogeny on Common Tree Species at Two Contrasting Habitats in Karst Forest of Southern Taiwan

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Abstract

One of central goal in ecology is to understand how plants respond to environment and what kind of attributes that can be obtained with an easy way to interpret the complexity of nature, especially on vegetation response. Ecologists use functional traits to understand how plants respond to environmental changes. Plant species may have experienced different environmental conditions during their ontogeny. Thus, they may show different patterns of ontogenetic trait variation (OTV) as their response to different environmental condition. In this study, the relationship between trait variation on different habitat and across ontogenetic stages both on community and population levels were investigated. Five selected leaves traits (leaf area, specific leaf area, leaf thickness, leaf dry matter content, and leaf succulence) were examined to look at plant response along soil water content and light gap interception gradient in Kenting forest dynamics plot (KFDP), Southern Taiwan. Leaf area was the most varied trait across habitat and ontogeny. Leaf thickness reveals an opposite pattern compare to leaf area. Leaf dry matter content (LDMC) showed less variation either between or within species and across ontogeny. Shift of community responses on environmental gradient by trait through ontogeny showed that intraspecific variation is important to be considered in ecological study. The other important finding in this study was by only using mean species we can misleading in understanding of plant responses to the environmental gradient in order to their adaptation both across different habitat and ontogenetic stages.

How to Cite

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INTRODUCTION

Over the past several decades, ecologists have been linking environmental condition to structure and species composition of plant communities. However, these studies have not been able to answer the big question about how a species can grow and thrive in a certain habitat while others could not. There is a stage in ecological process that missing when we try to make a link between environmental factors, plant performance (demographics), and plants distribution. This stage is about the process of how plants respond to environment and what kind of attributes that can be obtained with an easy way to interpret the complexity of nature, especially on vegetation response.

Trait-based approach in community ecology has been investigated increasingly in two decades recently. Functional trait is all the character of a plant that includes morphology, physiology, and phenology that give indirect effect on plant performance such as growth rate, reproduction and survivorship (Violle et al., 2007). It often measured at the individual level and usually used for comparisons between species (McGill et al., 2006).

Morphological and physiological adaptation may as the results of plants strategy in responding to different environmental factors. Thus, variation in morphological and physiological plants features maybe visible along environmental gradients (Schöb et al., 2013). Furthermore, based on functional trait possessed as a form of adaptation to the environment, plants can be grouped into several functional groups (Lavorel et al., 1997). For example, plant species are dominant in the resource-rich habitat, can be characterized by a short leaf life span with fast tissue turnover, high resource capture and fast growth abilities. Species are classified as exploitative plant which has a functional trait such as having a high specific leaf area (SLA) associated with photosynthetic capacity or have high leaf nitrogen content (LNC) correlated with rate of plant growth (Reich et al., 1997; Wright et al., 2004). The other group is conservative plant species, plants which have slower tissue turnover and longer leaf life span which is characterized by a low SLA and LNC. This plant group is also characterized by the large investment in high-density tissues, one of which is a high leaf dry matter content (LDMC) that can be used as a good estimator (Ryser & Urbas, 2000).

In most of previous studies, species mean value was wide be used for interpreting mechanisms and processes on ecology. It is based on the inter-specific variability or between species trait variation (BTV) and unconsidered intraspecific trait variability (ITV) properties (Albert et al*.,* 2011). Plant traits are however variable within species, depending on genetic, developmental, and environmental factors (Coleman et al., 1994).

Plant species may have experienced different environmental conditions during their ontogeny. Thus, they may show different patterns of trait values as their response to different environmental conditions at different ontogenetic stages. Ontogenetic trait variation showed differences in resource use strategy on young and adult plants. Trait associated resource take and allocation showed contrasting patterns through ontogeny (Martin & Thomas, 2013). Moreover, ontogenetic trait variation is also strongly suspected role by showing the shift of the relative importance of different assembly mechanisms based on different patterns of functional diversity and community weighted mean trait across ontogenetic stages (Spasojevic, et al., 2014). The small plants tend to grow far from their large plants (Murdjoko et al., 2016).

Trait variation and its pattern in relation to how plants respond to environmental change become increasingly attractive for further investigation. Furthermore, shift of plant community of conservative traits at lower resource availability to opportunistic traits at higher resource availability (Reich et al., 1997, Wright et al., 2004) became one of essential pattern to be considered in study of plant ecology. The important thing is that strength of this shift in trait pattern may depend on ontogenetic. Therefore, assessing patterns of trait variation among ontogenetic stages across contrasting habitats become very important in seeing how plants respond to environmental changes.

Aim of this study was to answer the question about whether there is any difference trait values of communities and populations growth on different habitat and across ontogenetic stages. We predict that there are differences of trait value in plants that grow in different habitats, in this case on ridge and at valley. Trait across ontogenetic stages also will show the differences between groups of small, medium and big trees. Therefore, body size as a parameter of ontogeny can be considered as a function of trait variations.

METHODS

Study area

This study was conducted in the Kenting forest dynamics plot which is located in Kenting Uplifted Coral Reef Nature Reserve of Kenting national park, Hengchun peninsula, Pingtung, Southern Taiwan. The Kenting Karst Forest Dynamics Plot is located in an undisturbed forest in the northeastern part of the Reserve (East Longitude: 120°49'; North Latitude: 21°57'; elevation 250- 300 m). It is a 10-ha rectangle plot which runs 400 m long (east-west axis) and 250 m wide (north-south axis) with 1000 sub plots (Wu et al., 2011).

Figure 1. The location of study site (Kenting forest dynamics plot, Kenting national park, Hengchun peninsula, Pingtung, Southern Taiwan).

According to data from Hengchun Weather Station, Central Weather Bureau (in years 2000 – 2010), Kenting forest area has annual precipitation of 2,000 mm in average. About 87% rainfall occurs between June and November due to *Mei-Yu* season and typhoons, while from October to April are drier with strong northeast monsoon. The mean annual temperature is 25.4°C with a small difference between the average temperature of the coldest and hottest, from 20.9°C on January to 28.4°C on August. On average, 2.3 typhoons landed on the Hengchun Peninsula every year from 1897 to 2007 (Data source: Central Weather Bureau cited in Wu et al., 2011).

Sampling

Dominant tree species in Kenting forest dynamic plot which have abundance rank from 1-10 and it comprises more than 80% in total abundance in Kenting plot area (Wu et al., 2011) were chosen. Samples were taken from two different habitats (valley and ridge) by considering main paths at study area. The numbers of the collected samples were between 40-66 individuals for each species. Traits were measured on 5 leaves for each individual. For quantifying body size as function of trait, diameter on the breast high (DBH) of each tree were measured. Then we classified them into three different size classes: 1-5 cm DBH (small tree), 5.1-15 cm DBH (medium size tree) and bigger than 15 cm DBH (big tree).

There were 46 sub plots (10 x 10 m for each sub plots) selected to assess environmental factors. Soil water content, soil bulk density, soil porosity, soil particles, and leaf area index were collected from these sub plots.

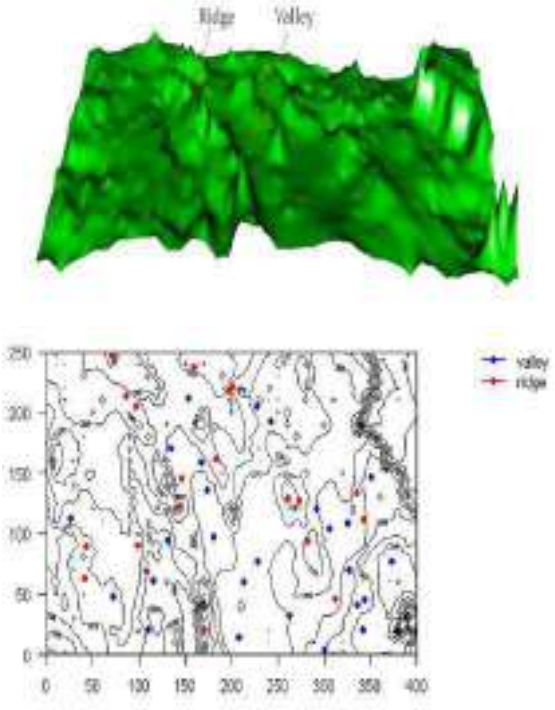

Figure 2. Topography and distribution of 46 sub plots for collecting samples of traits and environmental factor in Kenting plot.

Data collection

Environmental factor data

After careful removal of the dry litter layer from the top, random soil samples of the size (10x10x10 cm) were collected from the (0-10 cm) layer, at least from three different stands of each sub plot. Then samples were put in sealed plastic bag for preventing water loss. Fresh weight of samples soil was measured as soon as possible. After that, samples were put in paper bag and oven-dried for 3 days (72 hours) in 105 degree celcius. Dry mass of soil was measured every day until there was no difference weight. Soil water content was calculated by thermogravimetric method as (soil wet mass – soil dry mass)/ soil

dry mass multiplied by 100 to get value in percentage (ISO 1993 cited in Smith and Mullins 2005). Soil bulk density was calculated as the ratio of sample soil volume to oven-dried weight. Soil porosity was calculated by bulk density divided by particle density. Soil particles analysis was measured by calculating the percentage of sand, silt, and clay in the inorganic fraction using hydrometer method (Bouyoucos, 1962). Leaf area index was measured on each habitat using LAI 2200 plant canopy analyzer manufactured by LI-COR Bioscience based on its protocols.

Trait data

In this study, we used variables of leaf traits that included: leaf area (LA), specific leaf area (SLA), leaf dry matter content (LDMC), leaf thickness (L_{th}), and leaf succulence (LS). These traits were selected because they are widely used in trait-based ecology studies. Also, these traits are easy to collect and measured compared to other traits, such as root traits or whole plant traits.

The area of a leaf (also called leaf area, LA) is the most common metric for leaf size and is defined as the one-sided or projected area of an individual leaf. Leaf size has important consequences of the leaf energy and water balance (Cornelissen et al., 2003). Leaf size is a compromise between functional and resource-use efficiency. Specific leaf area (SLA), the ratio of leaf fresh surface area to dry mass, is a key component of the leaf economics spectrum (Wright et al., 2004), and reflects the tradeoff between rapid resource uptake and resource conservation. Leaf dry matter content (LDMC), the ratio of leaf dry mass to fresh mass, is also related to the leaf economics spectrum and correlates positively with leaf lifespan, water use efficiency, and herbivore resistance (Cornelissen et al., 2003). Leaf thickness (L_{th}) plays a key role in determining the physical strength of leaves. In optimization theory, balancing photosynthetic benefits against C costs of respiration and transpiration, predicts that L_{th} should be higher in sunnier, drier and less fertile habitats, as well as in longer-lived leaves. Leaf succulence is often seen as an anatomical trait common to plants with a high development of a water storage tissue (Kluge & Ting, 2012). Succulence leads to high leaf water content (LWC) and leaf thickness (Cornelissen et al., 2003).

Leaf traits are measured according to handbook for standardised measurement of plant functional traits worldwide by Pérez-Harguindeguy et al. (2013). Fresh leaf weight was measured with an electronic balance to the nearest 0.1 mg. Leaf area (LA; cm^2) was measured with a flatbed scanner directly within 12 hours of collection then was calculated using image-J software based on the scanned images. Leaves were then oven dried for 72–96 hours at 60 °C until a constant weight was reached. Specific leaf area (SLA; $cm^2\ g^{-1}$) was calculated as leaf area per dry leaf mass. Leaf Dry Matter Content was measured from oven-dry mass (mg) of a leaf, divided by its water-saturated fresh mass (g), expressed in mg g^{-1}. Leaf thickness was measured using a dial thickness gauge with accuracy up to 0.01 mm.

Table 1. Ten common trees species and its abundance in Kenting forest dynamic plot (Wu et al., 2011). Relative density was calculated from species density divided by total density in Kenting FDP.

Species name	Family	Tag.Code	Density	Relative density (%)	Rank	Individual sampled
Diospyros maritima	Ebenaceae	DIOSMA	22079	56.48	1	66
Drypetes littoralis	Euphorbiaceae	DRYPLI	3456	8.84	2	61
Aglaia formosana	Meliaceae	AGLAFO	1648	4.22	3	55
Champereia manillana	Opiliaceae	CHAM-MA	970	2.48	4	48
Dendrocnide meyeniana	Urticaceae	DENDME	940	2.4	5	63
Cryptocarya concinna	Lauraceae	CRYPCO	759	1.94	6	41
Melanolepis multiglandulosa	Euphorbiaceae	MELAMU	758	1.94	7	46
Macaranga tanarius	Euphorbiaceae	MACATA	687	1.76	8	45
Palaquium formosanum	Sapotaceae	PALAFO	608	1.56	9	46
Diospyros philippensis	Ebenaceae	DIOSPH	569	1.46	10	55
Total			32474	83.08		

Leaf succulence was calculated as (leaf wet mass – leaf dry mass)/leaf area (gH2O per cm^{-2}) as proposed by Mantovani (1998).

Data analysis

All statistical data were analyzed by using "R" statistical program (R Core Team, 2015). We used coefficient of variation (CV) for quantifying intra-specific trait variability on each species. CV is the ratio of the standard deviation to the mean for calculating data dispersion. Variance decomposition was to investigate the structure of trait variation by using a restricted maximum likelihood (REML) method in the "varcomp" and "lme" function, I fitted a general linear mixed model which consist only random factors to the variance across different scales nested one into another. For community level the scales are: leaf, individual, ontogeny, species, and habitat. For population level (only ITV) the scales are: leaf, individual, ontogeny, and habitat. Habitat here is divided into two, ridge and valley in the study site. The code used in R to calculate the variance partitioning of the traits across ecological scales on community level for the full model on LA was: `varcomp.la<-varcomp(lme(log10(la)~1, random=~1|habitat/spname/size/tag, data=pft, na.action=na.omit),1)`.

Only LA and SLA were log transformed for normalized the data. The "ape" and "nlme" libraries are necessary to use the "varcomp" and "lme" commands. For calculating another trait, it only changes LA with the other trait in model. When calculating on species level, I remove "spname" from random factor. Then the result from variance decomposition was timed by 100 to get percentage values of all.

Two-way analysis of variance (ANOVA) was used to assess differences in trait values between communities, as well as across species ontogenetic stages. The F value and p value were used as the basis of whether there is difference between groups were compared. More specifically to determine trait variation through ontogeny, I used ordinary least squares (OLS) regression. This analysis is to investigate the trait patterns trough ontogeny. All bivariate relationships were log-transformed to meet the assumption of homogeneity of data, and fit with an ordinary least-square (OLS) regression slope, with DBH as the predictor variable. Coefficient of determination (R^2), slope and the p value were used to describe the effect of high breast diameter (DBH) of trees to the trait variations through ontogeny.

RESULT AND DISCUSSION

Measurement of soil water was taken by mass-based. Data distribution of soil moisture (Figure 8) shows that there are significant differences between soil water content in the valley and ridge area (t = -5.6, p-value = <0.001). The average soil water content in the valley is 18% while in the ridge area is 24%.

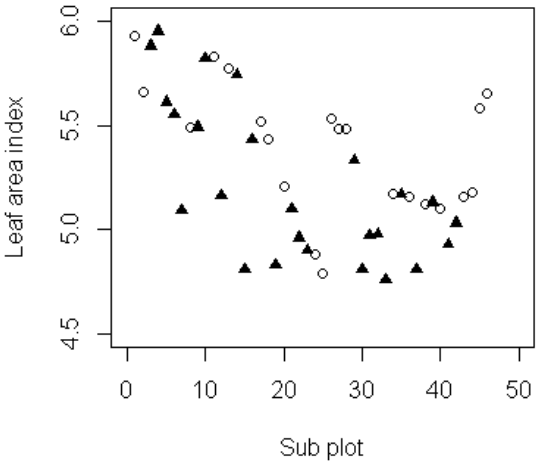

Figure 3. Leaf area index (LAI) values on different habitat (○: valley, ▲: ridge) of 46 sub plot. t = 1.7717, p-value = 0.08337

Based on the proportion of soil particles (clay, silt, and sand), we can classify the soil samples to different soil textures. Composition of soil texture that occurred on the ridge and the valley (Figure 9) shows that both habitats have similar type of soil, that is "loam" soil (55% of total samples on each habitat). What distinguished of these two habitats that approximately 35-40% of soil samples at the valley had "sandy loam" texture, whereas in the ridge area was "clay loam" soil.

Bulk density has a significant negative relationship to soil water content (figure S2). While soil water content increased significantly as increasing of soil porosity figure S2). Therefore, soil samples on ridge habitat that has lower bulk density and higher porosity than soil at valley habitat influence soil at ridge habitat has higher water content than valley. Furthermore, composition of soil texture on ridge habitat which has more clay soil than at valley also supports that soil water content on ridge habitat is higher than at valley habitat (Figure 5).

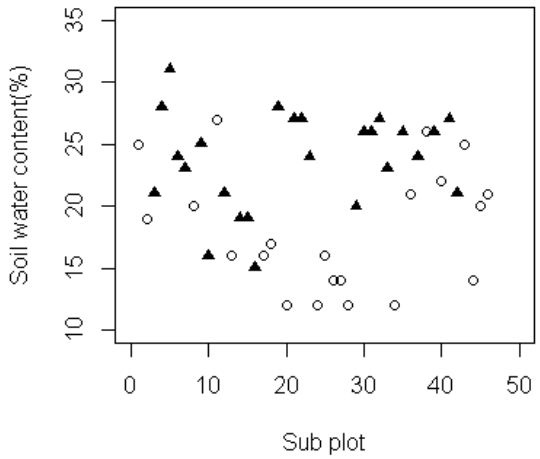

Figure 4. Soil moisture measured as soil water content by mass values on different habitat (○: valley, ▲: ridge) of 46 sub plot. t = -4.1997, p-value = 0.0001556

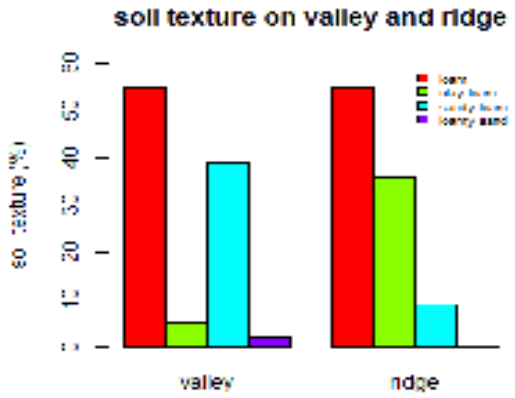

Figure 5. Percentage of each type of soil samples on two different habitat (at the bottom of valley and on the top of ridge). Soil texture by color (red: loam, green: clay loam, cyan: sandy loam, and blue: loamy sand).

Based on the average value for each species, it was found that inter-specific trait variation was very large (Table 2). For example in the leaf area, differences between species can up to 10-fold. For example, leaf area of CHAMMA was only 23.14 cm^2, but in MELAMU it reached to 236.28 cm^2, leaf thickness of MELAMU was only 0.09 mm compared to that of PALAFO which was 0.32 mm. Differences in SLA is also quite large, ranging from 81.69 cm^2/g in DIOSPH to 256.49 cm^2/g in MELAMU. Differences in LDMC and leaf succulence also relatively high. It ranged from 162.39 gH_2O/cm^2 to 391.4 gH_2O/cm^2 and 101.37 mg/g to 251.8 mg/g, respectively.

Based on coefficient of variation (CV), the largest intra-specific trait variation (ITV) was in leaf area of AGLAFO which has the highest CV (0.57). In general, leaf area (LA) showed the largest ITV compared to other traits across species. CV range for LA was 0.39 - 0.57 followed by SLA (0.18-0.38) and leaf thickness (0.13-0.35) then leaf succulence (0.14-0.3) and LDMC which has smallest CV ranges from 0.06 - 0.19.

To determine the structure of variation in each trait measured, variance decomposition analysis was used based on the value of the trait resulting from sampling by hierarchical method. Measurements at the scale of the leaves, and then gradually to the next scales, that are individual (tree), ontogenetic, species, and sampling at two different habitats (on the top of ridge and at the bottom of valley). At the community level (Figure 6f.), trait variation structured mostly from inter-specific variation trait or between species trait variation (BTV). It was explained approximately 60% on a leaf thickness up to 80% for LDMC, while BTV for the other three traits (leaf area, SLA, and succulence) were 70% respectively. Other sources of variation derived from the intra-specific variation of trait (ITV) which is divided into several hierarchies. Ontogeny turns the greatest role in ITV for leaf area and leaf thickness, whereas for the SLA, LDMC, and leaf succulence variations were more explained by individual scale.

Variance partitioning (ITV) at the level of population showed a similar pattern with ITV at the community level. In general, the variation of the difference in size class explained more than 40% ITV for leaf area and leaf thickness respectively. While variation structures for SLA, LDMC and leaf succulence were most explained by individuals with almost has similar percentage (40% - 80% variations). At leaves scale, only a few species which have large variations, such as LDMC on CRYPCO and DIOSPH, or leaf succulence on MELAMU and PALAFO. There was no different trait variation coming from differences habitat (ridge and valley). Only leaf succulence in some species and LDMC on AGLAFO that have slightly different CV between two habitat and it only 5% - 15% variance explained from this scale.

Variance decomposition results from each trait (Figure 6) show that the role of BTV approximately 70%, while ITV 30% in contributions to trait variability. Similar results (comparison for 70% BTV and 30% ITV) were also reported by Albert et al. (2010) for SLA and LDMC on alpine ecosystems and Hulshof & Swenson (2010) for SLA and leaf water content on dry tropical forest. In accordance with the spatial variance partitioning (SVP) hypothesis (Albert et al., 2011)

Table 2. Mean, variance, and coefficient of variation (CV) trait values of ten common species in study area.

Trait	stat. desc	Aglaia formosana	Champereia manillana	Cryptocarya concinna	Dendrocnide meyeniana	Diospyros maritima	Diospyros philippensis	Drypetes littoralis	Macaranga tanarius	Melanolepis multiglandulosa	Palaquium formosanum
Leaf Area	mean	64.22	23.14	25.23	166.81	43.01	130.91	29.09	174.22	236.28	84.86
	var	1320.66	110.50	172.98	5023.81	423.21	2336.79	238.03	9366.10	8428.61	2041.12
	cv	0.57	0.45	0.52	0.42	0.48	0.37	0.53	0.56	0.39	0.53
SLA	mean	113.14	113.35	108.19	241.54	126.96	81.69	79.87	158.70	256.49	86.29
	var	768.53	465.02	445.5	4359	842.96	988.58	284.97	773.21	7583.7	497.92
	cv	0.25	0.19	0.20	0.27	0.23	0.38	0.21	0.18	0.34	0.26
Leaf Thickness	mean	0.24	0.28	0.22	0.17	0.19	0.25	0.30	0.12	0.09	0.32
	var	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
	cv	0.27	0.25	0.24	0.22	0.20	0.23	0.22	0.13	0.35	0.24
LDMC	mean	365.11	281.80	410.1	162.39	385.2	435.63	389.7	391.40	275.51	323.36
	var	2069.5	824.59	2268.	963.13	1591.	1991.7	1720.	561.38	795.51	1652.8
	cv	0.12	0.10	0.12	0.19	0.10	0.10	0.11	0.06	0.10	0.13
Leaf Succulence	mean	161.82	235.70	138.2	227.36	131.5	176.72	203.9	101.37	110.42	251.80
	var	1171.3	2682.8	594.3	774.33	238.1	577.65	971.2	449.88	1115.4	1348.2
	cv	0.21	0.22	0.18	0.12	0.12	0.14	0.15	0.21	0.30	0.15

then the value of inter-specific variation trait or trait variation between species (BTV) is higher than the intra-specific variation of trait (ITV) at the broader scales.

Deeper study for understanding of variation pattern in different ecological scale of different traits will make us easier to take a decision to do ecological studies based on different questions. Messier et al. (2010) on the Figure 8, explain that variations at different scales are affected by different playing ecological and evolutionary processes. For example, water and nutrient status during leaf flushing will affect the variation of the trait on leaf scale. This is what might be able to explain that by partitioning variance in my research, traits related to water status (LDMC and leaf succulence) on a scale of leaves have a greater proportion of the percentage variation in contributing of ITV compared with ontogenetic scale. Even in some species, variations of these two traits are bigger than the individual scale.

Generally, plants on ridge habitat have smaller and thicker leaves with lower SLA and higher leaf succulence compare to plants that grow at valley. In addition, small trees tend to have larger and thinner leaves with higher SLA and lower leaf succulence then medium and big trees. Whereas LDMC on small and medium trees were relatively similar which have lower values than big trees. Summary of analysis of variance (table 3) shows that at level of community, there are significantly different (p-value < 0.05) of trait values between habitats and across ontogenetic stages. However, only LDMC values between ridge and valley community was not significantly different. All leaves trait between group comparisons has very low p-value which means that in average, the variation of leaf trait between habitats and across ontogenetic stages was much larger than the variation of trait values within a habitat or in same stage of ontogeny.

At the population level, LDMC tend to have a stable value in most species either between or across ontogeny population, only on population AGLAFO, DIOSMA and PALAFO which showed a significant difference. Leaf area and leaf thickness is a trait with the greatest value variation across ontogenetic stages. This is shown by the significant differences across different species at all life stages, only on MACATA for leaf thickness were not significantly different.

Comparisons between populations of a certain size in a particular habitat did not show significant differences when compared to the size of the population in different habitats. The difference in value trait each leaf area on CRYPCO, SLA and leaf on PALAFO, leaf succulence on DIOSPH, and LDMC on CHAMMA, CRYPCO, and DENDME indicate a difference between the

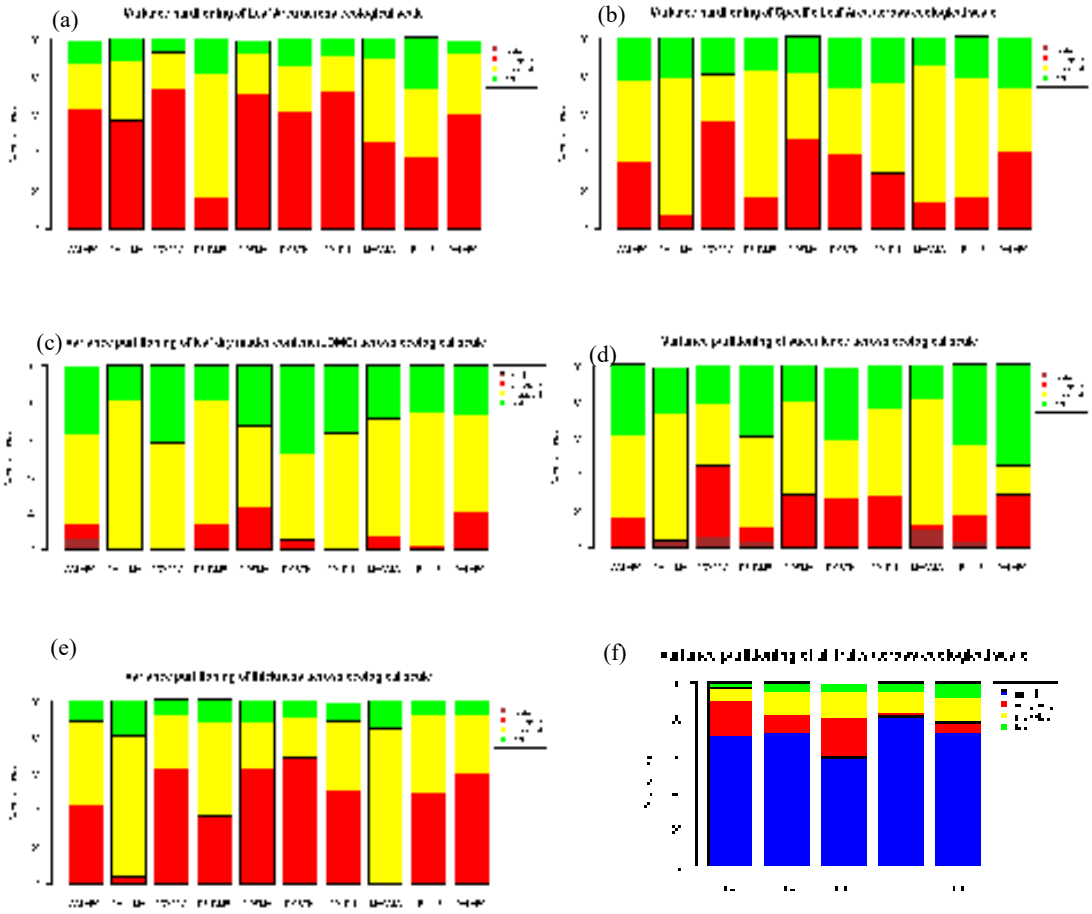

Figure. 6. Composition of variations by variance partitioning on single trait across ecological scales. Relative variance decomposition at the population level for (a) leaf area (LA), (b) specific leaf area (SLA), (c) leaf dry matter content (LDMC), (d) leaf succulence, (e) leaf thickness, and community level for (f) all species. Species codes come from the Table 1.

size class on Certain habitats compare to other size classes or similar class on different habitat.

Since the ontogeny trait variation is matter in variance partitioning of ITV, here I try to explore more about body tree size related to trait variation. Ordinary least-squares (OLS) regression was used to examine the relationship between tree size with a functional trait for each species. Data of diameter breast high (DBH) tree was used as a predictor (x) to determine the value of a functional trait variation (y). All data were taken from the average value of each individual species with different DBH.

Trend lines represent leaf trait-DBH structural relationships based on regression analysis on a log-log transformed data (Figure 7). Overall, five leaf traits (leaf area, SLA, thickness, LDMC and succulence) has a significant relationship (p-value <0.001; table S2) to body size. But there are some species that do not have a significant relationship such as LDMC on CHAMMA, CRYPCO, DIOSPH, and DRYPLI, then SLA and succulence in MACATA.

Leaf area and SLA negatively correlated to body size, while leaf thickness, succulence and LDMC has a positive correlation. The highest relationship was between leaf area and DBH in CRYPCO ($R^2 = 0.82$) and the lowest was thickness-DBH relationship on MACATA ($R^2 = 0.06$). The average values of R^2 for each trait are 0.62, 0.34, 0.45, 0.12, and 0.22 for leaf area, SLA, thickness, LDMC, and succulence respectively. Slope value, which represents the magnitude of the effect of body size on the trait measured, the highest (-0.665) on leaf area for MACATA and the lowest (0.043) on LDMC for MACATA. Therefore, based on R^2 and slope values it was known that body size (DBH) described the greatest to variation of leaf area, while the smallest relationship was with LDMC across ontogeny.

One of ecological scale in the study of Messier et al. (2010) is strata. This scale is important since most of leaf trait sampling in another study only taken on the leaves which get full sun

Table 3. ANOVA summary of trait values on community level between habitat and across size class

Trait	Parameter	F value	p value	signif.codes
Leaf Area	habitat	4.78	0.029	*
	size	13.67	0.0000016	***
	habitat:size	0.27	0.762	
SLA	habitat	20.95	0.0000059	***
	size	6.32	0.0019	**
	habitat:size	2.83	0.06	.
Leaf thickness	habitat	22.51	0.000002691	***
	size	21.54	0.000000001	***
	habitat:size	2.62	0.074	.
LDMC	habitat	2.46	0.1172	
	size	7.78	0.00046	***
	habitat:size	1.9	0.15024	
Leaf succulence	habitat	25.18	0.00000071	***
	size	9.18	0.00012	***
	habitat:size	0.57	0.56659	
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1				

exposure. Yet the fact that most of the foliage there is contained in branching in under the canopy on the same individual at different foliage or individual with different body sizes (ontogenetic stage). The different strata that affect the acquisition of light availability and other resources may influence of ITV and can revive the ecological process that affected the plant's performance.

In this study, ontogenetic stage occupied the similar scale as the strata in term of light resource availability. Small trees would get less light in small gap of light interception (high LAI) area compared to medium and bigger size trees. There are two environmental conditions and ecological process that influence the trait variation in addition to water and nutrient status on this scale, namely humidity and wind; and resource allocation (Figure 8). Environmental conditions associated with humidity and winds are often associated with the response of plants in order to self-defense, while resource allocation strategy is closely related to plants in selecting of which resources are more to be a priority than others. Secondly, it is consistent with the findings that the trait variation on ontogenetic stage has a greater proportion trait that plays a role in self-defense and resource allocation strategy (leaf thickness, leaf size, and SLA).

A very significant influence of the body size to trait variation through ontogeny and a substantial contribution from ontogenetic stages of the trait variability both at the level of populations and communities (Figure 7) strengthen our assumptions for the important to consider ontogenetic trait variation (OTV) in the study of ITV and trait-based community ecology. Further OTV role is increasingly clear to explain species and community response to environmental gradient mediated by trait.

To become dominant species (high abundance and high frequency), plants should adapt to different environmental conditions and respond to environmental changes in such habitat. Therefore, the dominant species should have adaptive traits, responsive traits or both in order to be abundant in large distribution at the study site. When species are only able to adapt to particular environmental conditions, they could have a high abundance in certain habitat types in accordance with its adaptive trait but less common

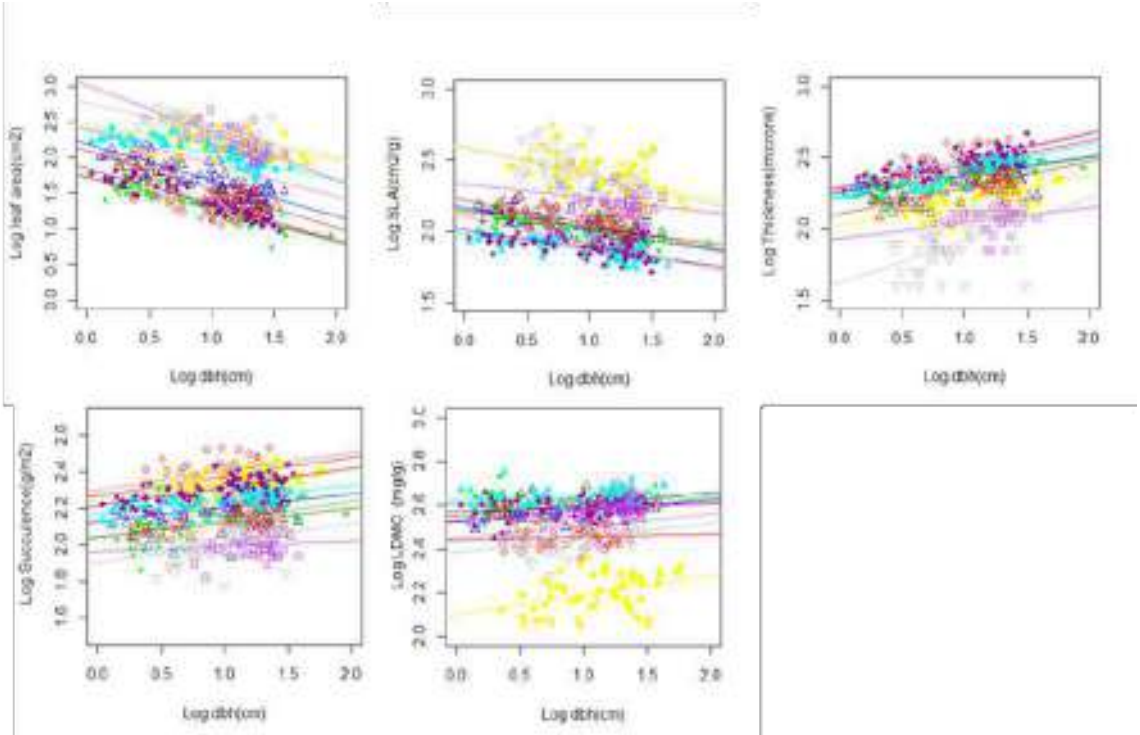

Figure 7. Trait (Leaf area, SLA, Thickness, Succullence, and LDMC) as a function of DBH across common tree species in Kenting karst forest (species code; AGLAFO(blue), CHAMMA(red), CRYPCO(green), DENDME(yellow), DIOSMA(brown), DIOSPH(cyan1), DRYPLI(dark magenta), MACATA(darkorchid1), MELAMU(gray), PALAFO(hotpink)).

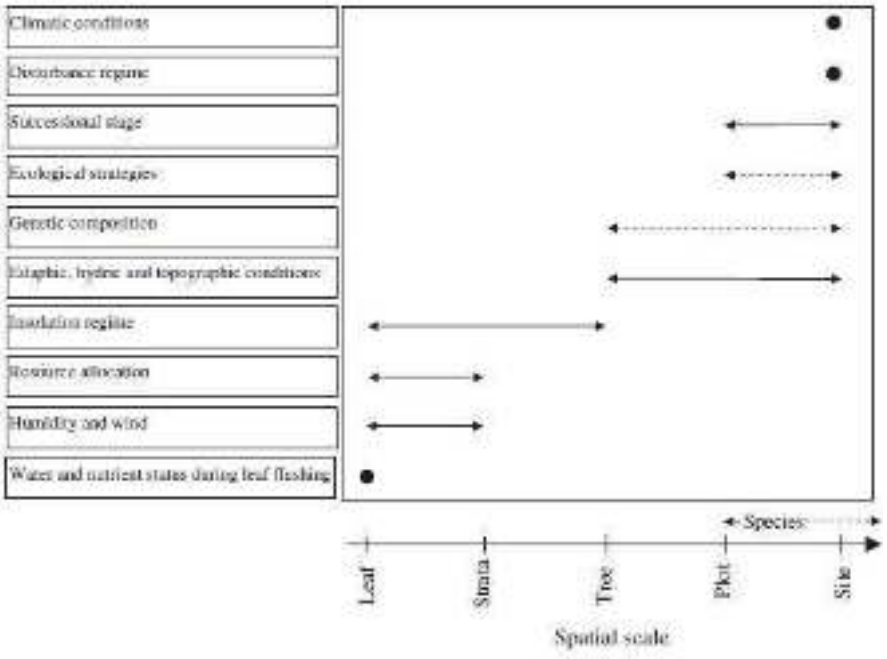

Figure 8. Processes affecting trait variation on different ecological scales. The abscissa represents the spatial scale and the ordinate lists the processes. The scale of species is drawn using a dashed line and overlaps the plot and site levels. The processes affecting a spatial scale via its effects on species are also drawn using a dashed line (Messier et al., 2010).

in other habitat types. In my case, dominant species could be able to grow in both habitats (valley and ridge) or abundant only in one habitat type (valley or ridge).

At valley habitat, plants exposed to conditions of less light availability, especially for small and medium trees. Various types of soil texture are quite diverse (loam, sandy loam, clay loam, and loamy sand), allowing soil water gradient on this habitat. Furthermore, proportion of sand particle in soil sample is relatively higher than ridge soil, allowing soil conditions at valley habitat has lower soil water content than ridge habitat. In contrast, on ridge habitat, LAI values tend to be lower than valley habitat. This allows for more light availability. In addition, ridge area condition that tends to be more exposed and has higher altitude than the valley, allows for getting higher solar radiation and hotter air temperature. This condition can cause evapotransipration process will be faster, so plants that grow on the ridge habitat should be adapt for changing status of soil water content. Unfortunately, in this study I did not measure soil water content at different times so we could not see fluctuations of soil water content status at study site.

Occurrence of dominant species on different environmental conditions of both habitat (valley and ridge) was probably as result of local adaptation process at a study site for long time periods. Whether because of plasticity response, evolutionary changes, or both, there is plenty of evidence for local adaptation in plants (Franks et al., 2014). Local adaptation is defined as a dynamic process that applies in the population to maintain or increase the frequency of traits that increase survival or reproductive success of individuals with the trait and value of an adaptive trait for an individual is measured relative to other individuals with properties (Taylor, 1991).

Among dominant species showed a similar pattern of trait values on contrasting habitats. On the ridge habitat, leaves tend to have lower SLA, higher leaf thickness, and higher leaf succulence compare to valley habitat. These three traits (SLA, leaf thickness and leaf succulence) may be can be used as a candidate as both adaptive and responsive traits. As an adaptive trait, it was known that SLA is one of key traits in plant functional grouping involved conservative versus opportunistic plants (Reich et al., 1997, Wright et al., 2004). While leaf thickness and leaf succulence, both have a strong relationship in strategies for optimizing water use efficiency and allocation of capacity to water storage in leaf organs (Smith, 1978). Lower SLA (due to thicker and/or denser leaves) contribute to long leaf survival, nutrient retention and protecting from desiccation (Mooney & Dunn 1970). Higher SLA (due to larger and thinner leaves) tend to have high leaf nitrogen content (LNC) reflected a faster potential rate of return on investment in leaves (Wright et al., 2004). Also, reviews of these three traits have significant relationships along both environmental gradients. Furthermore, these traits have relatively high CV as a form of adaptation to changes in environmental factors within habitat.

The last trait is leaf dry matter content (LDMC). This trait is proposed as one of the potential adaptive traits. In addition, its value was relatively stable at individuals within and among species, LDMC was less variation trait both between habitat and across ontogenetic stage. LDMC is also known as less sensitive trait to soil resources variation in different types of sand dunes (Yulin et al., 2005). No significant different of LDMC in two different habitats indicate that plant is able to adapt to different environments, probably through certain investment values in leaf tissue density (Chaves et al., 2002) or adjust to adapt from similar physical hazards such as herbivory and the wind (Cornellisen et al., 2003) at the study site.

CONCLUSIONS

This research reveals trait variability across ecological and ontogenetic stages scales between contrasting habitats on community and population levels. There are fundamental finding that has important contribution to trait-based plant ecological study; inter-specific trait variation was higher than intra-specific trait variation on dominant species community in tropical karst forest, body size had remarkable effect to trait variation through ontogeny, and plants that grow on ridge habitat tend to have smaller and thicker leaves, lower SLA and higher leaf succulence compare to plants at valley. The important finding in this study was by only using mean species we can misleading in understanding of plant responses to the environmental gradient in order to their adaptation both across different habitat and ontogenetic stages.

REFERENCES

Ackerly, D., Knight, C., Weiss, S., Barton, K., & Starmer, K. (2002). Leaf size, specific leaf area and microhabitat distribution of chaparral woody plants: contrasting patterns in species level and community level analyses. *Oecologia*,

130(3), 449-457.

Adler, P. B., Salguero-Gómez, R., Compagnoni, A., Hsu, J. S., Ray-Mukherjee, J., Mbeau-Ache, C., & Franco, M. (2014). Functional traits explain variation in plant life history strategies. *Proceedings of the National Academy of Sciences*, *111*(2), 740-745.

Albert, C. H., Thuiller, W., Yoccoz, N. G., Soudant, A., Boucher, F., Saccone, P., & Lavorel, S. (2010). Intraspecific functional variability: extent, structure and sources of variation. *Journal of Ecology*, 98(3), 604-613.

Albert, C. H., Grassein, F., Schurr, F. M., Vieilledent, G., & Violle, C. (2011). When and how should intraspecific variability be considered in trait-based plant ecology?. *Perspectives in Plant Ecology, Evolution and Systematics*, *13*(3), 217-225.

Bouyoucos, G. J. (1962). Hydrometer method improved for making particle size analyses of soils. *Agronomy journal*, 54(5), 464-465.

Chaves, M. M., Pereira, J. S., Maroco, J., Rodrigues, M. L., Ricardo, C. P. P., Osório, M. L., ... & Pinheiro, C. (2002). How plants cope with water stress in the field? Photosynthesis and growth. *Annals of botany*, *89*(7), 907-916.

Coleman, J. S., McConnaughay, K. D., & Ackerly, D. D. (1994). Interpreting phenotypic variation in plants. *Trends in Ecology & Evolution*, 9(5), 187-191.

Coley, P. D. (1983). Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecological monographs*, *53*(2), 209-234.

Cornelissen, J. H. C., Lavorel, S., Garnier, E., Diaz, S., Buchmann, N., Gurvich, D. E., ... & Pausas, J. G. (2003). A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian journal of Botany*, 51(4), 335-380.

Cornwell, W. K., & Ackerly, D. D. (2009). Community assembly and shifts in plant trait distributions across an environmental gradient in coastal California. *Ecological Monographs*, *79*(1), 109-126.

Dent, D. H., DeWalt, S. J., & Denslow, J. S. (2013). Secondary forests of central Panama increase in similarity to old-growth forest over time in shade tolerance but not species composition. *Journal of Vegetation Science*, *24*(3), 530-542.

Franks, S. J., Weber, J. J., & Aitken, S. N. (2014). Evolutionary and plastic responses to climate change in terrestrial plant populations. *Evolutionary Applications*, *7*(1), 123-139.

Givnish, T. J., & Vermeij, G. J. (1976). Sizes and shapes of liane leaves. *The American Naturalist*, 110(975), 743-778.

Hulshof, C. M., & Swenson, N. G. (2010). Variation in leaf functional trait values within and across individuals and species: an example from a Costa Rican dry forest. *Functional Ecology*, 24(1), 217-223.

Jung, V., Violle, C., Mondy, C., Hoffmann, L., & Muller, S. (2010). Intraspecific variability and trait-based community assembly. *Journal of ecology*, 98(5), 1134-1140.

Keddy, P. A. (1992). Assembly and response rules: two goals for predictive community ecology. *Journal of Vegetation Science*, *3*(2), 157-164.

Kleyer, M., Dray, S., Bello, F., Lepš, J., Pakeman, R. J., Strauss, B., ... & Lavorel, S. (2012). Assessing species and community functional responses to environmental gradients: which multivariate methods?. *Journal of Vegetation Science*, 23(5), 805-821.

Kluge, M., & Ting, I. P. (2012). *Crassulacean acid metabolism: analysis of an ecological adaptation* (Vol. 30). Springer Science & Business Media.

Kitajima, K. (1994). Relative importance of photosynthetic traits and allocation patterns as correlates of seedling shade tolerance of 13 tropical trees. *Oecologia*, *98*(3-4), 419-428.

Lavorel, S., McIntyre, S., Landsberg, J., & Forbes, T. D. A. (1997). Plant functional classifications: from general groups to specific groups based on response to disturbance. *Trends in Ecology & Evolution*, 12(12), 474-478.

LI-COR Inc. (2011). LAI 2200 Plant canopy analyzer, instruction manual. LI – COR, Inc. Canada.

MacArthur, R. H. (1972). *Geographical ecology: patterns in the distribution of species*. Princeton University Press.

Malhado, A. C. M., Malhi, Y., Whittaker, R. J., Ladle, R. J., Ter Steege, H., Phillips, O. L., ... & Arroyo, L. (2009). Spatial trends in leaf size of Amazonian rainforest trees. *Biogeosciences*, 6(8), 1563-1576.

Mantovani, A. (1999). A method to improve leaf succulence quantification. *Brazilian Archives of Biology and Technology*, 42(1).

Martin, A. R., & Thomas, S. C. (2013). Size-dependent changes in leaf and wood chemical traits in two Caribbean rainforest trees. *Tree physiology*, 33(12), 1338-1353.

McGill, B. J., Enquist, B. J., Weiher, E., & Westoby, M. (2006). Rebuilding community ecology from functional traits. *Trends in ecology & evolution*, 21(4), 178-185.

Messier, J., McGill, B. J., & Lechowicz, M. J. (2010). How do traits vary across ecological scales? A case for trait-based ecology. *Ecology letters*, 13(7), 838-848.

Mooney, H. A., & Dunn, E. L. (1970). Photosynthetic systems of mediterranean-climate shrubs and trees of California and Chile. *American Naturalist*, 104(939), 447-453.

Murdjoko, A., Marsono, D., Sadono, R., & Hadisusanto, S. (2016). Plant Species Composition and Their Conspecific Association in Natural Tropical Rainforest, South Papua. *Biosaintifika: Journal of Biology & Biology Education*, 8(1), 33-46.

Parkhurst, D. F., & Loucks, O. L. (1972). Optimal leaf size in relation to environment. *The Journal of Ecology*, 505-537.

Perez-Harguindeguy, N., Diaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., ... & Urcelay,

C. (2013). New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of botany*, 61(3), 167-234.

R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/

Raunkiaer, C. (1934). The life forms of plants and statistical plant geography; being the collected papers of C. Raunkiaer. *The life forms of plants and statistical plant geography; being the collected papers of C. Raunkiaer*. Oxford University Press, Oxford.

Reich, P. B., Walters, M. B., & Ellsworth, D. S. (1997). From tropics to tundra: global convergence in plant functioning. *Proceedings of the National Academy of Sciences*, 94(25), 13730-13734.

Ryser, P., & Urbas, P. (2000). Ecological significance of leaf life span among Central European grass species. *Oikos*, 91(1), 41-50.

Schöb, C., Armas, C., Guler, M., Prieto, I., & Pugnaire, F. I. (2013). Variability in functional traits mediates plant interactions along stress gradients. *Journal of Ecology*, 101(3), 753-762.

Smith, K.A. and Mullins, C.E. (2005). *Soil and environmental analysis: physical methods, revised, and expanded*. CRC Press.

Spasojevic, M. J., Yablon, E. A., Oberle, B., & Myers, J. A. (2014). Ontogenetic trait variation influences tree community assembly across environmental gradients. *Ecosphere*, 5(10), 1-20.

Taylor, E. B. (1991). A review of local adaptation in Salmonidac, with particular reference to Pacific and Atlantic salmon. *Aquaculture*, *98*(1), 185-207.

Vendramini, F., Díaz, S., Gurvich, D. E., Wilson, P. J., Thompson, K., & Hodgson, J. G. (2002). Leaf traits as indicators of resource-use strategy in floras with succulent species. *New Phytologist*, *154*(1), 147-157.

Violle, C., Navas, M. L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., & Garnier, E. (2007). Let the concept of trait be functional!. *Oikos*, 116(5), 882-892.

Webb, L. J. (1959). A physiognomic classification of Australian rain forests. *The Journal of Ecology*, 551-570.

Westoby, M., Falster, D. S., Moles, A. T., Vesk, P. A., & Wright, I. J. (2002). Plant ecological strategies: some leading dimensions of variation between species. *Annual review of ecology and systematics*, 33(1), 125-159.

Wright, I. J., Reich, P. B., Westoby, M., & Ackerly, D. D. (2004). The worldwide leaf economics spectrum. *Nature*, 428(6985), 821.

Wright, I. J., Reich, P. B., & Westoby, M. (2001). Strategy shifts in leaf physiology, structure and nutrient content between species of high-and low-rainfall and high-and low-nutrient habitats. *Functional Ecology*, *15*(4), 423-434.

Wu, S. H., Hseu, Z. Y., Shih, Y. T., Sun, I. F., Wang, H. H., & Sen, Y. C. (2011). Kenting karst Forest dynamics plot: tree species characteristics and distribution patterns. *Taiwan Forestry Research Institute, Taipei*, 1-306.

Yulin, L. I., Johnson, D. A., Yongzhong, S. U., Jianyuan, C. U. I., & Zhang, T. (2005). Specific leaf area and leaf dry matter content of plants growing in sand dunes. *Botanical Bulletin of Academia Sinica*, *46*.

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Ethnobotanical Study of Medicinal Plants in Karangwangi, District of Cianjur, West Java

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Abstract

The knowledge and usage of plant as medicinal remedy by current generation are not as extensive as previous; therefore, many rural communities with restricted modern medical access still rely on traditional medicine. This paper provides significant ethnobotanical information on medicinal plants in Karangwangi Village of Cianjur District, West Java Indonesia. This study aimed to identify plants collected for medical purposes by the local people as well as to document the local names, uses, preparation, and location of these plants. Ethno botanical data was recorded by opting people participation and key informant approach involving semi-structured interviews, group discussions and filling of questionnaires. The results showed a total of 114 medicinal plants belonging to 50 families were identified. Zingiberaceae was the most-frequently cited (nine species), followed by Asteraceae, Euphorbiaceae, and Fabaceae (seven species each). The most-used plant parts were leaves (51.8%), followed by stems (22.9%) and the most common preparations were decoction, poultice and squeezed. Most of the plants were obtained from the house-yard and total of 30 medicinal uses were recorded. The ethnobotanical result documented in this study showed that this area is rich in medicinal plants and these plants are still commonly used for medicinal purposes among the people in their daily lives. Ethnobotanical heritage should be preserved, however, there is a gradual loss of traditional knowledge about these plants in new generation. Further, the findings can be used as baseline information for further scientific investigation for analyzing phytochemical, pharmaceutical and other biological activities for future drug discovery.

How to Cite

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INTRODUCTION

Many kinds of medicinal plants have been used worldwide, especially in rural communities of developing countries. The using of plants as medicine has been done for generations and was passed on from one generation to the next (Kumalasari, 2006). The World Health Organization or WHO was recommend the use of traditional medicine, including herbs in the maintenance of public health, prevention and treatment of disease, especially for chronic diseases degenerative diseases and cancer (Patwardhan, 2005). The WHO also acknowledges the value of traditional medicine and the preservation and protection of this knowledge is one of their objectives (WHO, 2002). This traditional knowledge, however, is documented only to a limited extent, and is in danger of being lost. This is largely due to social changes within the communities, such as dislocation and westernization, and the death of the elders with this knowledge (Smith, 1991) as well as deforestation and environmental degradation (Giday et al., 2009). This trend in loss of traditional knowledge is being seen worldwide (Brouwer et al., 2005).

Indonesian society have long been familiar with and used plants as medicine in tackling health problems, including in rural communities. The local people of the rural areas have good knowledge about the uses of plants and they prefer medicinal plants due to their easy availability and cheap therapy as compared to costly pharmaceuticals. Data from Basic Medical Research (Riskesdas) on 2013 showed 35.2 % Indonesian society still retain and use traditional medicine for remedies (Shanthi et al., 2014). Inhabitants of the remote areas have discovered the therapeutic activity of medicinal plants against certain diseases through their indigenous experiences (Bibi et al., 2014).

Karangwangi village of Cianjur Regency, West Java, based on the classification Schmidt and Ferguson (1951), is included in type B with an average rainfall of 1840 mm/year. The type of vegetation in climate of type B (wet) is tropical rainforest. While the topography of the village has a height between 0 to 250 meters above the sea level (masl). The Karangwangi is a village that directly adjacent to the Bojonglarang Jayanti nature reserve area. The existence of this natural reserve affects the diversity of flora and fauna in the Karangwangi village, including the plant that used as herbal medicine. Karangwangi village was administratively about 27 years old and was a separation of the Cidaun village, but there was no health center. Otherwise, in rural communities of Sundanese, who inhabit West Java and are the second largest ethnic group in Indonesia, traditional herbal medicine has still played an important role in treatment of illnesses (Roosita et al., 2008). The advantages of traditional medicines include its widespread accessibility and relative cheapness, when most people in Indonesia pay for medicines out of their own pocket.

Therefore, it is necessary to inventory the kind of medicinal plants and their utilization by the community so that traditional knowledge of the medicinal plants can be documented and preserved. This study aimed (1) to identify plants collected for medical purposes by the local people as well as to document the local names, uses and preparation, as well as the location of these plants, (2) to characterize the plant by which them categorized as medicinal herbal; (3) to identify the transfer knowledge of medicinal plants to the younger generation, and (4) to count the enthusiasm of people to preserve their knowledge and skills to produce of herbal medicine. The results of this study are expected to document first hand traditional and contemporary knowledge as well as to provide information to communities that can be used for their cultural or educational purposes.

METHODS

This research was conducted in Karangwangi village, located in Cidaun subdistrict, Cianjur district, West Java Province, Indonesia. Geographically, this village is situated about 200-275 m above sea level. Temperature scarcely fluctuates in the year; with the mean monthly was 35^{O}C, and annual rainfall reaches 3500 mm/year. The village was bordered by Cimaragang village in the north, Indian Ocean in the south, Sindangbarang village in the west, and Ciringin village of Garut district in the east (Figure 1). Karangwangi Village, the land area of which is 2300 ha, was inhabited by 5587 people or 1817 households (Iskandar and Iskandar, 2016). The majority occupations of the Villagers were farming. In Karangwangi Village, there was limited access to a modern health center. Commercials drugs, however, were available to the Villagers at many retailers. On the other hand, there were "dukun" (traditional or herbalist healer) who recognized by the local people.

The method used in this research is qualitative approach with descriptive analysis and based on ethnobotanical approach (Martin, 1995; Cunningham, 2001; Newing et at, 2011).

Data was collected by semi-structured interviews with informants (local leader, "dukun") and direct observation in the field. Determination of the respondents used the snowball method (Bernard, 2004) and each respondent were requested information about medicinal plants, local name, utilization and processing method which has been used by communities in Karangwangi. Interview results were analyzed by cross-checking, summarizing and synthesizing from sources in order to build up a narrative account (Newing et al., 2011).

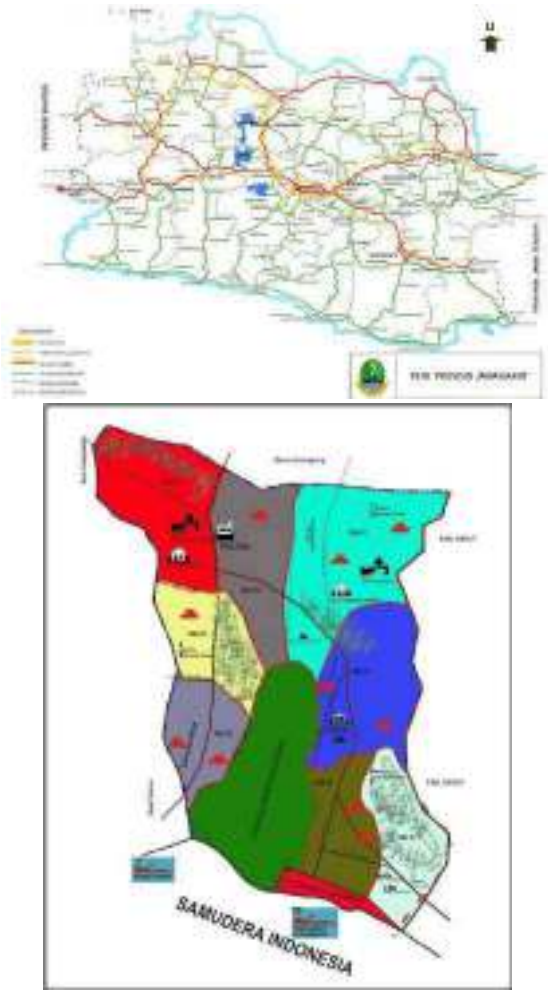

Figure 1. The Location of Karangwangi Village in Cidaun Subdistrict, Cianjur District, West Java Indonesia

On direct observation, each plant samples were found in Karangwangi village was collected, herbarium materials were prepared and the specimens were entitled. Plant identification process carried out directly in the field and a complete identification was carried out in the Laboratory of Botanical Taxonomy of Biology Department, Faculty of Mathematics and Natural Sciences, Padjadjaran University. Plant identification process was based on morphological characteristics of the plant (roots, stems, leaves, flowers, seeds and fruit) and was using *Buku Tumbuhan Obat Komersial* (Siswanto, 2004), *Atlas Tumbuhan Obat Indonesia, Jilid 6* (Dalimartha, 2009) and the book of Flora (Van Steenis, 2005). Ethno botanical data was descriptive analyzed.

RESULTS AND DISCUSSION

Medicinal Plants Reported

A total of 114 species belonging to 50 families were recorded from the study area, which categorized into herbs, shrubs, and trees (Table 1). The Family Zingiberaceae (7.9%) contributes the highest number of medicinal plants (nine species), followed by Asteraceae, Euphorbiaceae, and Fabaceae (seven species each). It was found that *Erythrina variegata, Annona muricata, Morinda citrifolia, Physialis angulata,* and *Artocarpus altilis* were the most commonly used species.

The families Zingiberaceae, Asteraceae, Euphorbiaceae, and Fabaceae have accounted for the highest number of Karangwangi medicinal plants which could probably be due to their high species and the compositions of secondary metabolites, for instance Zingeberaceae contained alkaloid, saponin, tannin, and flavonoid (Hartanto et al., 2014); Asteraceae contained triterpenoid, saponin, and steroid (Bhom et al., 2001); Euphorbiaceae contained diterpenes, triterpenes, flavonoids, saponin, and tannin (Mwine and Vam Damme, 2011), and Fabaceae contained flavonoid, alkaloid, terpenoid, steroid (Wink. 2013). Antioxidant properties from such secondary metabolites were not reduced when the plant was prepared using two traditional culinary and medicinal recipes (Tilak et al., 2004). The components of secondary metabolites correspond to the characteristic of the plants that usually categorized as medicinal herbal. High versatility of medicinal plants could also indicate higher diversity of active compounds contained by the species (Giday et al., 2009).

Most of the respondent said that Zingiberaceae was the most commonly used as medicinal plants because they were easily cultivated in the home garden and alternatively could be used as food spicy. The study that conducted by Roosita et al. (2008) showed that Zingiberaceae and Euphorbiaceae were the most common medicinal plants family that used by the villagers and herbalist healer in Sukajadi village located in Bogor district. Zingiberaceae was also commonly used by local people in Pangea, District

Table 1. Medicinal Plants Used by Karangwangi Villagers.

Family	Botanical name / latin name	Local name	Use	Parts of plants
Acanthaceae	*Graptophyllum pictum* (L.) Griff.	Handeuleum	Hemorrhoids	Leaves
	Sericocalyx crispus (L.) Bremek.	*Ki beling*	Low back pain	Leaves
Acoraceae	*Calamus* sp.	*Hoe*	Cough	Shoot
Amaranthaceae	*Amaranthus viridis* L.	*Bayem*	Anemia	Leaves
Anacardiaceae	*Anacardium occidentale* L.	*Jambu monyet*	Mouth sores	Leaves
Annonaceae	*Annona muricata* L.	*Manalika*	Low back pain, Fever, High blood pressure,	Leaves
Apiaceae	*Centella asiatica* (L.) Urb.	*Antanan*	Wounds, Gastritis	Leaves
	Apium graveolens L.	*Saledri*	High blood pressure	Leaves
Apocynaceae	*Alstonia scholaris* (L.) R. Br.	*Lame*	Toothache	Stem
Araceae	*Colocasia gigantea* (Blume)	*Kajar-kajar*	Cough	Stem
	Colocasia esculenta (L.) Schott	*Teleus lempong*	Cough	Shoot
Araliaceae	*Polyscias fruticosa* (L.) Harms	*Gordah*	Urinary disease	Leaves
	Polyscias scutellaria (Burm.f.) Fosberg	*Mamangkokan*	Low back pain	Leaves
Arecaceae	*Uncaria gambir* (Hunter) Roxb.	*Gambir*	Intestinal inflammation	Leaves
	Areca catechu L.	*Jambe*	Low back pain, Intestinal inflammation	Leaves
	Arenga pinnata (Wurmb) Merr.	*Kawung*	Low back pain	Root
	Cocos nucifera L.	*Kelapa hijau*	Low back pain, Diarrhea	Root
	Salacca zalacca (Gaertn.) Voss	*Salak*	Urinary disease	Shoot
Asparagaceae	*Cordyline fruticosa* (L.) A.Chev	*Hanjuang*	Cough	Shoot
Asteraceae	*Ageratum conyzoides* (L.) L.	*Babadotan*	Fever	Leaves
	Mikania scandens (L.) Willd.	*Capituheur*	Wounds	Leaves
	Erigeron linifolius Willd.	*Jalantir*	Eyes infection	Stem
	Chromolaena odorata (L.) R.M.King & H.Rob.	*Jatong / Nampong*	Wounds, Eyes infection	Leaves
	Eupatorium inulifolium Kunth	*Kirinyuh*	Wounds, Gastritis	Leaves
	Blumea balsamifera (L.) DC	*Sembung*	Low back pain	Leaves
	Elephantopus scaber L.	*Tapak liman*	Uric acid	Leaves
Athyriaceae	*Diplazium esculentum* (Retz.) Sw.	*Taruk paku*	Low back pain, Intestinal disorders	Leaves
Caricaceae	*Carica papaya* L.	*Gedang karayunan*	Malaria, Kidney disorder, Breastfeeding	Root
Clusiaceae	*Garcinia* x *mangostana* L.	*Manggu*	High blood pressure	Fruit peel
Convolvulaceae	*Ipomoea batatas* (L.) Lam.	*Hui boled*	High blood pressure	Leaves
Costaceae	*Cheilocostus speciosus* (J.Koenig) C.D.Specht	*Pacing*	High blood pressure, Wounds	Stem
Crassulaceae	*Bryophyllum pinnatum* (Lam.) Oken	*Buntiris*	Fever	Leaves
Cucurbitaceae	*Momordica charantia* L.	*Paria*	Fever, Diabetes	Leaves
	Sechium edule (Jacq.) Sw.	*Waluh*	Fever, Gastritis	Leaves

Dioscoreaceae	*Dioscorea hispida* Dennst.	*Gadung*	Labor-related condition	Leaves
Euphorbiaceae	*Abelmoschus manihot* (L.) Medik.	*Edi*	Fever	Leaves
	Jatropha curcas L.	*Jarak pager*	Toothache, Wounds	Leaves
	Ricinus communis L.	*Kaliki*	Labor-related condition	Leaves
	Euphorbia tirucalli L.	*Ki tulang*	Toothache	Stem
	Euphorbia hirta L.	*Nanangkaan*	Low back pain, Wounds	Stem
	Jatropha multifida L.	*Penisilin*	Wounds	Stem
	Manihot esculenta Crantz	*Sampeu*	Anemia, Gastritis	Leaves
Fabaceae	*Mucuna gigantea (Willd.) DC.*	*Areuy gongseng*	Cough	Stem
	Erythrina variegata L.	*Dadap minyak*	Cough, Low back pain, Fever, Eyes infection, Hookworm	Leaves
	Archidendron pauciflorum (Benth.) I.C.Nielsen	*Jengkol*	Diabetes	Fruit peel
	Vigna radiata (L.) R.Wilczek	*Kacang hejo*	Gastritis	Seed
	Glycine max (L.) Merr.	*Kacang kedelai*	Gastritis	Seed
	Albizia saman (Jacq.) Merr.	*Ki hujan*	Eyes infection	Stem
	Senna alata (L.) Roxb.	*Ki manila*	Skin infections	Leaves
	Le.ucaena leucocephala(Lam.) de Wi.t	*Petai selong (Lamtoro)*	Diabetes	Seed
Lamiaceae	*Tectona grandis* L.f.	*Jati*	Eye infections	Stem
	Plectranthus scutellarioides (L.) R.Br.	*Jawer kotok*	Eye infections, Bruised	Leaves
	Orthosiphon stamineus Benth.	*Kumis ucing*	Low back pain, Diabetes	Leaves
	Ocimum basilicum L.	*Surawung*	Itching	Leaves
Lauraceae	*Persea americana*	*Alpukat*	High blood pressure, Gastritis	Leaves
Lecythidaceae	*Barringtonia macrocarpa* Hassk.	*Songgom*	Labor-related condition	Leaves
Liliaceae	*Allium cepa* L.	*Bawang Beureum*	Fever	Bulb
	Allium sativum L.	*Bawang bodas*	High blood pressure	Bulb
Loranthaceae	*Scurrula atropurpurea* (Blume) Danser	*Mangandeuh*	Hemorrhoid	Leaves
Malvaceae	*Melochia umbellata* (Houtt.) Stapf	*Bintinu*	Toothache	Stem
	Hibiscus rosa-sinensis L.	*Kembang Gumatu*	Low back pain	Leaves
	Urena lobata L.	*Pungpurutan*	Low back pain, Dysentery	Leaves
	Ceiba pentandra (L.) Gaertn.	*Randu*	Fever, Urinary disease	Leaves
Marantaceae	*Donax canniformis* (G.Forst.) K.Schum.	*Bangban*	Eyes infection	Stem
Melastomaceae	*Melastoma polyanthum* Burm. f.	*Harendong*	Accelerate the loosening of umbilical cord	Leaves
Meliaceae	*Swietenia mahagoni* (L.) Jacq.	*Mahoni*	Diabetes	Seed

Menispermaceae	*Tinospora crispa* (L.) Hook. f. & Thomson	*Batrawali*	Gastritis, bronchitis	Stem
	Cyclea barbata Miers	*Cincau*	Supplement, Common cold	Leaves
	Arcangelisia flava (L.) Merr.	*Ki koneng*	Low back pain, Hepatitis	Stem
Moraceae	*Morus alba* L.	*Babasaran*	High blood pressure	Leaves
	Ficus septica Burm. f.	*Kuciat*	Itching	Leaves
	Artocarpus heterophyllus Lam.	*Nangka*	Gastritis, Cough	Leaves
	Artocarpus altilis (Parkinson ex F.A.Zorn)	*Sukun*	High blood pressure, Gastritis	Leaves
Muntingiaceae	*Muntingia calabura* L.	*Kersen*	Gastritis	Leaves
Musaceae	*Musa* x *paradisiaca* L.	*Cau ambon*	Wounds	Stem
	Musa x *paradisiaca* L.	*Cau beureum, Cau gembor, Cau mas*	Fever	Stem
	Musa x *paradisiaca* L.	*Cau raja siem*	Gastritis	Fruit
Myrtaceae	*Syzygium malaccense* (L.) Merr. & L.M.Perry	*Gulampo*	Headache	Leaves
	Psidium guajava L.	*Jambu batu*	Diarrhea	Leaves
	Syzygium polyanthum (Wight) Walp.	*Salam*	High blood pressure	Leaves
Oxalidaceae	*Averrhoa carambola* L.	*Balingbing*	High blood pressure	Fruit
Phyllanthaceae	*Sauropus androgynus* (L.) Merr.	*Katuk*	Eye infections	Stem
Piperaceae	*Piper aduncum* L.	*Ki seureuh*	Eye infections	Stem
	Piper nigrum L.	*Pedes*	Labor-related condition	Leaves
	Piper betle L.	*Seureuh*	Wounds	Leaves
Plantaginaceae	*Plantago major* L.	*Ki urat*	Wounds	Leaves
Poaceae	*Gigantochloa pseudoarundinacea* (Steud.) *Widjadja.*	*Awi gombong*	Cough	Stem
	Gigantochloa atroviolacea Widjadja.	*Awi hideung*	Cough	Stem
	Oryza sativa L.	*Beras*	Bruised	Seed
	Dinochloa scandens (Blume ex Nees) Kuntze	*Cangkoreh*	Eyes infection, Cough	Stem
	Imperata cylindrica (L.) Raeusch.	*Eurih*	Low back pain, Fever, Wounds	Root
	Bambusa vulgaris Schard.Ex var *striata*	*Haur koneng*	Cough	Stem
Rubiaceae	*Morinda citrifolia* L.	*Cangkudu*	Cough, Gastritis, High blood pressure	Fruit
	Gardenia jasminoides J.Ellis	*Kaca piring*	Low back pain	Leaves
Rutaceae	*Citrus aurantiifolia* (Christm.) Swingle	*Jeruk nipis*	Cough, Toothache	Fruit
	Clausena indica (Dalzell) Oliv.	*Ki baceta*	Cough, Fever , Asthma	Leaves
Gumotaceae	*Manilkara zapota* (L.) P.Royen	*Sawo*	Diarrhea	Leaves
Simaroubaceae	*Eurycoma longifolia* Jack	*Pasak bumi*	Anti-malaria	Rhizome

Solanaceae	*Capsicum annum* L.	*Cabe*	Wounds	Fruit
	Physalis angulata L.	*Cecendet*	Low back pain Diabetes, Anti-malaria	Root
	Solanum torvum Sw.	*Takokak*	High blood pressure	Fruit
	Solanum betaceum Cav.	*Terong walanda*	Toothache	Stem
Thymelaeaceae	*Phaleria macrocarpa* (Scheff.) Boerl.	*Mahkota dewa*	High blood pressure	Peel
Urticaceae	*Dendrocnide stimulans* (L.f.) Chew	*Pulus*	Cough	Stem
Vitaceae	*Tetrastigma lanceolarium* (Roxb.) Planch.	*Ki barela*	Cough, Wounds	Stem
Zingiberaceae	*Kaempferia galanga* L.	*Cikur*	Bruised	Rhizome
	Amomum maximum Roxb.	*Hangasa*	Eye infections, Diabetes	Stem
	Etlingera elatior (Jack) R.M.Sm.	*Honje*	Fever	Flower
	Zingiber officinale Roscoe	*Jahe*	Supplement	Rhizome
	Curcuma zanthorrhiza Roxb.	*Koneng gede*	Hepatitis, Gastritis	Rhizome
	Curcuma domestica Valeton	*Koneng temen*	Gastritis, Intestinal disorder	Rhizome
	Alpinia galanga (L.) Willd.	*Laja*	Cough	Rhizome
	Zingiber cassumunar Roxb.	*Panglay*	Itching	Leaves
	Amomum aculeatum Roxb.	*Parahulu*	Headaches	Stem

of Kuantan Senggigi Riau as traditional medicinal herbal, especially to cure diseases associated with pregnancy and heredity problem, that used individually or in combination (Hartanto et al., 2014). Ethnobotanical study on traditional treatment for women in the Surakarta Hadiningrat Royal Palace Community by Shanthi et al. (2014) showed that Zingiberaceae and Fabaceae were used mostly families that utilized as traditional medicine. Silalahi et al. (2015) reported that Zingiberaceae was the most commonly medicinal plants which have been traded in the Kabanjahe traditional market Karo Regency, North Sumatra Indonesia. Sukenti et al. (2016) also presented that Fabaceae contributed the highest number of species in ethnobotanical study on local cuisine of Sasak tribe in Lombok Island. Asteraceae is the largest medicinal plant family used by local people in district Mastung of Balochistan Province-Pakistan (Bibi et al., 2014). Asteraceae, Euphorbiaceae, and Fabaceae also contributed the highest number of medicinal plants of the Meinit ethnic group of Ethiopia (Giday et al., 2009).

Ailments treated

The reported of medicinal plants, most were used to treat human ailments and some for livestock ailments. Concerning human ailments, a total of 30 medicinal uses (remedies) were recorded, with the highest proportions of medicinal plants were used to treat cough (34.2%), gastritis (21%), high blood pressure (14%), low back pain (12.3%), wound (11.4%), as well as eyes infection (8.7%). Some were used against diabetes, malaria, anemia, skin-related disease, tooth ache, postpartum remedy, urinary disease, anti-hookworm and as food supplement. Eighteen species of medicinal plants were used to treat cough, whereas gastritis was treated using fifteen species of medicinal plants.

The most common ailments that suffer by Karangwangi people were respiratory disease (cough, asthma, common cold) and gastrointestinal diseases (gastritis, diarrhea, intestinal disesase), could be attributed to the major health problem in communities. Ethnopharmaecological studies have shown that in some parts of the world, the respiratory and gastrointestinal disorder is the first use category (Bibi et al., 2014). Due to poor dietary conditions and unsafe drinking water, this ailment is one of the most common problems in the areas studied and infecting other parts of the world (Nasab and Khosravi, 2014).

Plant parts used and modes of remedy preparation

The study showed that the medicinal plants frequently used of fresh materials, for example leaves, and with modes of preparation was decoction. Leaves and stems were the most frequently sought plant parts accounting for 51.8 and 22.9 % of claimed medicinal plants, respectively. Few were harvested for their roots, shoots, fruits, seeds, bulbs, rhizomes, fruit peels and gums. The majority of remedies were harvested for immediate uses with the modes of preparation included decoction (84%), poultice (6%), "dicincau" (5%; the leaves were squeezed and the filtrate was drink directly), directly eaten (2%), "dituak" (1%; the stem was cut and the water droplet was drink directly), and 2% with another mode ("dipopo"-the sample was grinded and attached into the wounded skin; "dikopi"-the sample was dry-fried, grinded, and added some hot water; "dibuhbui"- the sample put into hot ashes until wilted before eaten). This result showed that local people performed frequently used of leaves decoction as medicine for various ailments, thus agree with the result of Bibi et al. (2014), de Boer and Cotingting (2014).

Leaves was the botanical parts that most commonly used, because the villagers usually believe that leaves contained the highest medicinal properties and parts of plants that most easily harvested. A very high proportion of leaves was also observed in an ethnobotanical survey either in Sukajadi village, located in Tamansari subdistrict, Bogor district, Jawa Barat province (Roosita et al., 2008) or in Riau Province, Sumatra, Indonesia (Mahyar et al., 1991). The remedies are mostly prepared from newly harvested plant part could indicate the availability of copious plant materials in the vicinity to be picked at any time (Giday et al., 2009), for example in the house yard where the medicinal plants are cultivated or planted by the villagers or harvested freely from the immediate environment in which they are abundantly found. In otherwise, there was prohibition to enter the nature reserves for the villagers, so the location to obtain the medicinal plants was limited.

Route of administration and dosage

The most frequent routes of administration herbal preparations were oral (92%), while 6% were taken topical application, and for 2% with other modes, for examples to treat eyes infection, the stems gum was dropped directly to the eyes. More than half of the daily doses were administered once. In many cases, amounts of plant part/parts to be processed and doses to be used were roughly estimated and therefore, lacked precision. If patients did not show any sign of improvement over the treatment period, they were used commercial drugs or referred to nearby modern health centers. It was noted that dosage was influenced, among others, by the type of ailment, seriousness of the illness and age of the patient. According to few informants the dosage depends on the age and physical appearance of the individual and children are given less than adults. Same sort of conclusions have been observed in another studies (Roosita et al., 2008; Giday et al., 2009)

Location of medicinal plants

Great majority of medicinal plants were located in the house yard (55%) and fewer were located in the crop fields and paddy fields, as well as in the Bojong Larang Jayanti Nature reserve. The villagers raised medicinal plants, either cultivars or transplanted wild species, in their home gardens or in the fields. If they get some illness, usually they collected the plants from the home garden firstly, not only on their own but also from the neighbors, and then they were search in the fields or the nature reserve. Some of this medicinal plants reported to be occasionally cultivated primarily for its medicinal value. This result supported by Roosita et al. (2008) that in rural communities of Sundanese, many villagers raised and collected medicinal plant in their home garden. Therefore, the remedies were freely harvested from the immediate environment by those who needed them.

Characteristics of plant by which categorized as herbal medicines

Most of the respondent did not know how to categorize the plants as herbal medicines, but a number of respondent assumed that the plants usually have characteristics as watery, sticky, bitter, and abrasive. The watery plants could use as remedy for cough, cold, and fever; the sticky plants could use as remedy for infections because the gum was believed would kill the bacteria; otherwise the bitter and abrasive plants could use as remedy for internal diseases, such as gastrointestinal diseases, high blood pressure, diabetes, malaria, and etc.

Transfer of medicinal plants knowledge

Most of the respondent said that the knowledge of the using medicinal plant was obtained from parents (57%), or by directly observation from the community (30%), and fewer said from

1. Kaja-kaja (*Alocasia macrorrhiza*)

2. Talens lempong (*Colocasia esculenta*)

3. Hoe (*Calamus* sp.)

4. Hanjuang (*Cordyline fruticosa*)

5. Areuy gorgoseng (*Marsdenia gigantea*)

6. Dadap ungyak (*Erythrina variegata*)

7. Awi gombong (*Gigantochloa verticillata*)

8. Awi tali/ung (*Gigantochloa atroviolacea*)

9. Buluh kecung (*Bambusa vulgaris*)

10. Cangkudu (*Morinda citrifolia*)

11. Jeruk nipis (*Citrus aurantiifolia*)

12. Ki baceta (*Glaucosa indica*)

12. Pohe (*Dendrocnide stimulans*)

14. K. barela (*Tetracera Lanceolarium*)

15. Laja (*Alpinia galanga*)

16.Kalapa (*Cocos nucifera*)

17. Jambe (*Areca catechu*)

18. Kawung (*Arenga pinnata*)

19. Gedang karayunan (*Carica papaya*)

20. Manggu *Garcinia mangostana*)

21. Paria (*Momordica charantia*)

22. Jengkol (*Archidendron pauciflorum*)

23. Selong (*Leucaena leucocephala*)

24. Kumis ucing (*Orthosiphon stamineus*)

another information media, i.e. books, television, or health educator that came into the area. The heritage of medicinals plant relies on an oral tradition between parents and their children. Most of the informant described memories of being treated with herbs for illness as a child and said that they subsequently continued to learn from parents or knowledgeable elders. It was revealed that many ailments are diagnosed and treated at household or family level. The majority of the informants agreed that they kept their medicinal plant knowledge secret. This way of sharing knowledge has resulted in the loss of many drugs and prescriptions.

25. Mahoni (*Swietenia mahagoni*) 26. Ceremai (*Physalis angulata*) 27. Hanjeli (*Coix lacryma*)

The enthusiasm of people to preserve their knowledge and skills to produce of herbal medicine.

More than 80% of the respondent villagers still used the medicinal plants to cure their illnesses, however, the skill to produce herbal medicines was obtained by hit and trial methods, or get directly from their parents. In young generation, however, the enthusiasm to used herbal medicine was decreasing.

This study revealed that herbal medicine has played a significant role in treatment of illnesses in the study village. Some of the reasons of their high dependence on herbal medicine came from easily harvested and preparation, low cost expended, natural and low side effect, no expired time as well as more powerful that commercial drugs and closer location of the healer's house than the health center or hospital.

CONCLUSION

A total of 114 medicinal plants belonging to 50 families were identified in the region. The most common families were Zingiberaceae, Asteraceae, Euphorbiaceae, and Fabaceae. Various plant parts were used and the most common preparations were decoction, poultice and squeezed. A total of 30 medicinal uses (remedies) were recorded. Most of the plants were obtained from the home garden and usually characterized by watery, sticky, bitter, and abrasive surface of the plants. The knowledge of the using medicinal plant was mostly obtained from parents and the use of herbal medicine was still widespread among the people. In young generation, however, the enthusiasm to used herbal medicine was decreasing.

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REFERENCES

Bernard, H. R. (2004). *Research Methods in Anthropology: Qualitative and quantitative methods*, 3rd ed. AltaMira Press,Walnut Creek, California.

Bhom, B. A. & Stuessy, T. F. (2001). Introduction to the Sunflower family – Biology and distribution. In *Flavonoids of the Sunflower Family (Asteraceae),* Springer-Verlag: Wien, 2001; pp 3-17.

Bibi, T., Ahmad, M., Tareen, R. B., Tareen, N. M., Jabeen, R., Rehman, S. U., ... & Yaseen, G. (2014). Ethnobotany of medicinal plants in district Mastung of Balochistan province-Pakistan. *Journal of ethnopharmacology*, 157, 79-89.

Brouwer, N., Liu, Q., Harrington, D., Kohen, J., Vemulpad, S., Jamie, J., ... & Randall, D. (2005). An ethnopharmacological study of medicinal plants in New South Wales. *Molecules*, 10(10), 1252-1262.

Cunningham, A. B. (2001). *Applied Ethnobotany: People, World Plant Use & Conservation.* Earthscan Publication, London and Sterling.

Dalimartha, S. (2009). *Atlas Tumbuhan Obat Indonesia,* Jilid 6. Jakarta: Pustaka Bunda. h:89-94.

de Boer, H. J. & Cotingting, C. (2014). Medicinal plants for women's healthcare in southeast Asia : a meta-analysis of their traditional use, chemical constituents, and pharmacology. *Journal of Ethnopharmacology*,

151(2), 747–767.

Giday, M., Asfaw, Z., & Woldu, Z. (2009). Medicinal plants of the Meinit ethnic group of Ethiopia: an ethnobotanical study. *Journal of Ethnopharmacology*, 124(3), 513-521.

Hartanto, S., Fitmawati, & Nery S. (2014). Studi Etnobotani Famili Zingiberaceae dalam Kehidupan Masyarakat Lokal di Kecamatan Pangean Kabupaten Kuantan Singingi, Riau. *Biosaintifika: Journal of Biology & Biology Education,* 6(2), 98-108.

Iskandar, J. & Iskandar, B. S. (2016). Etnoekologi dan Pengelolaan Agroekosistem oleh Penduduk desa Karangwangi Kecamatan Cidaun Cianjur Selatan Jawa Barat. *Jurnal Biodjati,* 1(1), 1-12.

Kumalasari, L. O. (2006). Pemanfaatan Obat Tradisional Dengan Pertimbangan Manfaat Dan Keamanannya. *Majalah Ilmu Kefarmasian*, 3(1), 1-7.

Mahyar, U. W., Burley, J. S., Gyllenhaal, C., & Soejarto, D. D. (1991). Medicinal plants of Seberida (Riau Province, Sumatra, Indonesia). *Journal of ethnopharmacology*, 31(2), 217-237.

Martin, G. J. (1995). *Ethnobotany: A Methods Manual*. Chapman & Hall, London.

Mwine, J. T. & Damme, P. V. (2011). Why do Euphorbiaceae tick as medicinal plants? A review of Euphorbiaceae family and its medicinal features, *Medicinal Plants Research,* 5(5), 652-662

Nasab, F. K., & Khosravi, A. R. (2014). Ethnobotanical study of medicinal plants of Sirjan in Kerman Province, Iran. *Journal of ethnopharmacology*, 154(1), 190-197.

Newing, H., Eagle, C.W., Puri, R. K. & Watson, C. W. (2011). *Conducting Research in Conservation: Social methods and practice*. Routledge, London and New York.

Patwardhan, B. (2005). Traditional Medicine: Modern Approach for Affordable Global Health. World Health Organization (WHO) and Commission on Intellectual Property, Innovation, and Public Health (CIPIH) Study Nine on TM, Draft Report.

Roosita, K., Kusharto, C. M., Sekiyama, M., Fachrurozi, Y., & Ohtsuka, R. (2008). Medicinal plants used by the villagers of a Sundanese community in West Java, Indonesia. *Journal of ethnopharmacology*, 115(1), 72-81.

Schmidt, F. H. & Ferguson, J. H. A. (1951). Rainfall Types Based on Wet and Dry Period Ratios for Indonesia and Western New Guinea. Verh. Djawatan Met. dan Geofisik, Jakarta.

Shanthi, R.V., Jumari, & Munifatul I. (2014). Studi Etnobotani Pengobatan Tradisional untuk Perawatan Wanita di Masyarakat Keraton Surakarta Hadiningrat. *Biosaintifika: Journal of Biology & Biology Education,* 6(2), 61-69.

Silalahi, M., Walujo, E. B., Supriatna, J., & Mangunwardoyo, W. (2015). The local knowledge of medicinal plants trader and diversity of medicinal plants in the Kabanjahe traditional market, North Sumatra, Indonesia. *Journal of ethnopharmacology*, 175, 432-443.

Siswanto, Y. W. (2004). *Penanganan Hasil Panen Tanaman Obat Komersial*. Penebar Swadaya. Jakarta.

Smith, N. M. (1991). Ethnobotanical Field Notes from the Northern Territory. *J. Adelaide Bot. Gard. 14*, 1-65.

Sukenti, K., Hakim, L., Indriyani, S., Purwanto, Y., & Matthews P. J. (2016). Ethnobotanical study on local cuisine of the Sasak tribe in Lombok Island, Indonesia. *Journal of Ethnic Foods*, 3(3), 189-200

Tilak, J. C., Banerjee, M., Mohan, H., & Devasagayam, T. P. A. (2004). Antioxidant availability of turmeric in relation to its medicinal and culinary uses. *Phytotherapy Research*, 18(10), 798-804.

Van Steenis, C. G. G. J. (2005). *Flora.* Penerjemah: M. Suryowinoto, S.Hardjosuwarno, S.S. Adisewojo, Wibisono, M. Partodidjojo, S. Wirjohardjo. Jakarta: PT Pradnya Paramita.

Webb, C. O., Cannon, C. H., Davies, S. J. (2008). Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds) Tropical Forest Community Ecology. Wiley-Blackwell, New York. (8 pt).

Wink, M. (2003). Evolution of secondary metabolites in legumes (Fabaceae). *Phytochemistry*. 64(1), 3-19.

World Health Organisation. (2002). *Traditional Medicine Strategy 2002-2005*; WHO: Geneva, http://www.who.int/medicines/library/trm/trm_strat_eng.pdf.

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The Expression of mRNA LMP1 Epstein-Barr Virus from FFPE Tumour Biopsy: a Potential Biomarker of Nasopharyngeal Carcinoma Diagnosis

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Abstract

Nasopharyngeal carcinoma (NPC) is a multifactorial disease that is endemic geographically in the world. Indonesian population has a highly incidence rate that is 6.2/100,000 people year. The pathogenesis of NPC is more directly reflected by carcinoma-specific viral transcriptional activity at the site of primary tumour. Epstein-Barr virus (EBV) infection in NPC is reflected by the expression of EBV latent and lytic gene. In fact, mRNA Latent Membrane Protein 1 (LMP1) EBV expression was an important latent infection biomarker. The aim of this study was to determine a potential use of relative expression of mRNA LMP1 EBV from formalin-fixed paraffin embedded (FFPE) tumour biopsy in NPC as a tumour biomarker. This reseach design was a cross sectional study. The samples were the archived specimens of FFPE tumour biopsy from NPC WHO-3 patient which were collected from untreated patients from 2014 in the Department of Pathology Anatomy, Prof. dr. Margono Soekarjo Hospital, Purwokerto. The expression of mRNA LMP1 EBV expression was determined by RT-PCR technique. The positivity of mRNA LMP1 EBV expression was 51.9%, indicating a moderate positivity. The result proved that the expression of mRNA LMP1 EBV from FFPE NPC WHO-3 tumour biopsy was a potential biomarker of NPC diagnosis. The molecular methods would improved the management of NPC, particularly in the histopathological diagnosis of NPC.

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INTRODUCTION

Nasopharyngeal carcinoma (NPC) has a significant difference in the geographical distribution and ethnicity (Yu & Yuan, 2012; Chang & Adami, 2006). NPC is endemic in certain regions of the world, especially in Southeast Asia, and has a poor prognosis. In Indonesia, the recorded mean prevalence is 6.2/100 000, with 13 000 yearly new NPC case (Soeripto, 1998; Adham et al., 2012). Etiology of NPC is multifactorial including host genetic factor, Epstein-Barr virus (EBV) infection and environmental factors. EBV infection has been shown to be consistent with the onset of NPC (Zur Hauzen, et al., 1970; Old et al., 1996; Roezin, 1999; Munir, 2006).

EBV has two phases in the cycle of infection, i.e., latent and lytic phase. In the latent phase, few EBV latent genes are expressed, so the number copies of viral DNA is maintained in a relatively low level and does not produce virions. In the lytic phase, a series of lytic gene is expressed caused by genome replication and virion production (Chang et al, 2004; Kieff & Rickinson 2001). EBV infection shows a pattern of latent infection phase II that will express Epstein-Barr virus Nuclear Antigen (EBNA-1) and Latent Membrane Protein I (LMP1). The direct measurement of activities mRNA EBV in primary tumour location on nasopharyngeal region needs to be done, because the activity of mRNA EBV reflects better virtually the pathogenesis of NPC than a serologic diagnosis and a measurement of EBV DNA in circulation (Steven et al., 2006). The advantages of using FFPE NPC tumour biopsy as samples were they was confirmed as NPC World Health Organization-3 (WHO-3) histopathologically and non invasive specimen. In prevoius study, the expression of mRNA LMP1 EBV genes have been proved only in fresh NPC tumour biopsy and is performed by Reverse Transcriptase Polymerase Chain Reaction technique (RT–PCR) (Wahyono et al, 2016). Reverse transcription process is a complement DNA (cDNA) synthesis by reverse transcriptase enzyme. PCR is an alternative method to identify the Avian Influenza (AI) virus, although the viral genomes in few quantities (Payungporn et al., 2004; OIE 2005; Wibowo et al., 2012). The research aimed at knowing potensial use for mRNA LMP1 expression from FFPE tumour biopsy in NPC WHO-3 as a biomarker of NPC diagnosis. Regarding to NPC management, it could be recommended to the clinician using a molecular technique, because the mRNA LMP1 expression method from FFPE tumour biopsy increased the accuracy of NPC diagnosis. It was the first study to analysis the mRNA expression of LMP1 EBV from FFPE NPC WHO-3 tumour biopsy as a tumour biomarker.

METHODS

This reseach design was a cross sectional study. The research was done from February 2015 until August 2015. All NPC formalin-fixed paraffin embedded (FFPE) tumour biopsies were collected from untreated patients who where histopathologically confirmed as NPC WHO-3 at Department of Pathology Anatomy, Prof. dr Margono Soekatjo Hospital/Faculty of Medicine, Universitas Jenderal Soedirman, Purwokerto. The histological diagnosis was confirmed by the pathologist involved in the study. Total of research subject were 27 people for untreated NPC WHO-3 patients from 2014. All research subjects were given informed consent before taking part in this research.

The 8-10 slices of NPC formalin-fixed paraffin embedded (FFPE) tumour biopsies were performed by PureLink FFPE RNA isolation kit protocol (Invitrogen) to obtain 50-100µL RNA solution. By 10 µL RNA solution, it could be directly used for the analysis of Reverse Transcriptase PCR (RT-PCR) or stored for long periods at a temperature of -80°C. cDNA was synthesis by cDNA Synthesis Super Script III First Strand Systemprotocol (Invitrogen) to obtain 20 µL cDNA solution.

Two steps of RT-PCR technique were performed to detect mRNA LMP1 EBV expression from FFPE tumour biopsy in NPCWHO-3 patients. A set of primer used to amplify the cDNA of LMP1 EBV gene resulted in a cDNA amplicon 142 bp consisting of a forward primer of 5'-GGAGATTCTCTGGCGACTTG-3' and a reverse primer of 5-GAGCCAAAGGAGATCAACCA-3'. Primer was designed by Primer3 software from the NCBI GenBank Sequence Database (accession number NC_009334.1, GeneID: 5176215). The composition of 15 µL was 7.5 µL Dream Taq PCR MasterMix – Thermo Scientific (Dream Taq DNA Polymerase, 2X Dream Taq Buffer, 4 mM $MgCl_2$, 0,4 mM dGTP, 0,4 mM d*ATP*, 0,4 mM dCTP, 0,4 mM dTTP). The PCR mix was runon a Thermocycler (Primus 25, PeqLab) by 35 cycles. The PCR condition of first step was pre-denaturation at 94°C for 5 min, denaturation at 94°C for 60 sec, annealing at 55°C for 60 sec, elongation at 72°C for 60 sec, and post- elongation at 72°C for 7 min. The amplicon was visualized by the electrophoresis on 2.5% agarose gel.

RESULTS AND DISCUSSION

Positivity expression of mRNA LMP1 EBV

Figure 1 shows the result of mRNA Latent Membrane Protein 1 (LMP1) Epstein-Barr Virus (EBV) from Formaline-Fixed Paraffin Embedded (FFPE) in Nasopharyngeal Carcinoma World Health Organization-3 (NPC WHO-3) tumour biopsy detected by RT-PCR technique on 2 % Agarose gel. Samples that expressed mRNA LMP1 EBV showed a 142 bp amplicon which represented on sample no. 1646, 713, 2822, 3028 and 2106. However, the sample no. 677 and 3274 did not express mRNA LMP1 EBV.

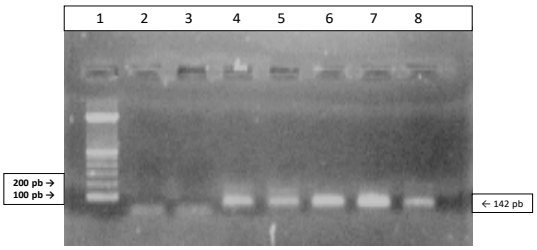

Figure 1. The results of mRNA LMP1 EBV from FFPE tumour biopsy in 3 NPC WHO-3 patients by RT-PCR technique as follow: no. 1. DNA marker (100 bp), no. 2 & 3 NPC WHO-3 patients who did not expressed mRNA LMP1 EBV (no. 677&3274), no.4-8NPC WHO-3 patients who expressed mRNA LMP1 EBV (no. 1646, 713,2822,3028 & 2106).

The positivity of mRNA LMP1 EBV was calculated by the proportion of NPC WHO-3 patient who expressed mRNA LMP1EBV group and all NPC WHO-3 patients (Table 1.) The positivity of mRNA LMP1 EBV from FFPE NPC WHO-3 tumour biopsy that were 14 of 27 samples (51.9%). The results of this research indicated that the RT-PCR technique could be used to detect the expression of EBV latent gene in NPC WHO-3patients. In this study, the positivity of mRNA LMP1 EBV expression from FFPE tumour biopsy in NPC WHO-3 patients (51.2%) was higher than the previous study in LMP1 expression analysis by Immunohistochemistry technique (10 - 50%) (Middeldorp et al, 2003). However, it was lower than that of mRNA LMP1 EBV expression from the fresh biopsy in NPC WHO-3 patients (91.3%) (Wahyono et al., 2016), that of LMP1 protein expression in NPC by Immunohistochemistry (IHC) staining methods (65%) (Niedobitek, 2000). LMP1 protein expression was detected 75% cases with Immunohistochemistry staining methods and has a significant relationship to locoregional progressivity at the young age (Adham et al., 2012).

The analysis of formalin-fixed paraffin-embedded (FFPE) often has difficulty because of total RNA yielded from FFPE tissue often significantly degraded. Only 3% total RNA is able to isolate from paraffin blocks to obtain cDNA synthesis, compared with the use of fresh biopsy or fresh frozen biopsy (Gouveia et al., 2014). Therefore, fresh biopsy or fresh frozen biopsy more often to be used to analyse mRNA expression than formaline-fixed paraffin-embedded (FFPE) biopsy. However, formalin-fixed paraffin-embedded (FFPE) biopsies have been collected as an archived specimen abundantly at Departement of Pathology Anatomy, Prof. dr. Margono Soekarjo Government Hospital, Purwokerto. Detection of mRNA LMP1 EBV expression from FFPE tumour biopsy in NPC WHO-3 patients by RT-PCR technique is the novelty of the research because of the first research used FFPE tumour biopsy specimens in the analysis of mRNA EBV in NPC.

Table 1. Molecular characteristic and clinical pathologyof research sample

Parameter	Number of Samples
Sample size	27
mRNA LMP1 EBV expression of NPC WHO-3 patient	
Positive	14
Negative	13
Positivity of mRNA EBV of NPC WHO-3 LMP1	51.9%

Identification of the substance that gives information of malignant tumours such as EBV gene expression is expected to improve diagnosis, determine prognosis, and predict NPC pathology (Kresno, 2011). More than 90 percent of the world's population has been infected by EBV that began in the first year after birth (Thompson & Kurszrock, 2004). NPC is a malignancy associated with EBV having epithelial cells as target cell (Young & Rickinson, 2004). LMP1 is more potential than that of VCA-IgA serology and detection of LMP-1 EBV with PCR technique in the nasopharyngeal swab. It can be used as a good diagnosis of NPC pathogenesis (Hao et al., 2004; Cho, 2007). At the time, some of mRNA EBV gene has been used to be a molecular marker in pathogenesis of NPC, such as Epstein-Barr virus Encoded RNA (EBER), Epstein-Barr

virus Nuclear Antigen (EBNA1), Latent Membrane Protein 1 (LMP1), Latent Membrane Protein 2A/2B (LMP2A/2B), BamHI A Fragment Rightward Reading Frame (BART), BamH1 A Fragment Rightward Reading Frame 1 (BARF1), and BamH1 Z Fragment Leftward Reading Frame 1 (BZLF1). However, study exploring the expression of mRNA EBV gene from FFPE tumour biopsy in NPC WHO-3 patients in the pathogenesis of NPC is rarely done (Hirankarn et al., 2004).

Several molecular techniques have been used to detect EBV genes in KNF before, such as in situ hybridization of EBV DNA (1991-1992), hybridization of EBV DNA Blots (1992), PCR DNA EBV (1993-1994), in situ hybridization EBERs (1995 – at present), immuno histochemistry LMP1 (1995), immuno histochemical EA (early antigen difuse) and gp350/220 (2002) (Barnes et al., 2005). RT-PCR technique is the development of Northern blotting techniques that have been commonly used to detect mRNA EBV. The advantage of the RT-PCR technique is that it is more sensitive to detect mRNA and requires a small sample (Brink et al., 1997; Middeldorp et al., 2003). The multiprime RT-PCR technique is a variation of conventional RT-PCR techniques to simultaneously detect mRNA expression of several EBV genes EBNA1, EBNA2, LMP1, LMP2A, LMP2B, BZLF1, BARTs, and U1A snRNP (house keeping gene) (Steven et al., 2005). Therefore, RT-PCR-based molecular technique is the preferred technique for the analysis of mRNA LMP1 EBV expression on NPC pathogenesis.

LMP1 EBV as the parameter of NPC diagnosis is more potential than serological detection by VCA-IgA. The detection of LMP-1EBV by PCR technique on Nasopharyngeal swab can be used as a good diagnostic for NPC pathology (Cho, 2007; Hao et al., 2004). Constitutive activation of NF-kB by the viral oncogene (LMP1) has an important role in persistence, but is a risk factor for EBV-associated lymphomas. Endogenous LMP1 escapes degradation upon accumulation within intraluminal vesicles of multivesicular endosomes and secretion via exosomes. LMP1 associates and traffics with the intracellular tetraspanin CD63 into vesicles that lack MHC II and sustain low cholesterol levels, even in 'cholesterol-trapping' conditions. The lipid-raft anchoring sequence FWLY, nor ubiquitylation of the N-terminus, controls LMP1 sorting into exosomes. Rather, C-terminal modifications that retain LMP1 in Golgi compartments preclude assembly within CD63-enriched domains and/or exosomal discharge leading to NF-kB overstimulation. Interference through shRNAs further proved the antagonizing role of CD63 in LMP1-mediated signalling. Thus, LMP1 exploits CD63-enriched microdomains to restrain downstream NFjB activation by promoting trafficking in the endosomalexosomal pathway. CD63 is thus a critical mediator of LMP1 function in- and outside-infected (tumour) cells (Verweij et al., 2011). mRNA LMP1 EBV that is a transcript of BNLF1 gene are detected abundantly in cell culture (Boos et al., 1987; Middeldorp et al., 2003). mRNA LMP1 EBV is used to be a marker of EBV infection in NPC (Yu & Yuan, 2012), because LMP1 EBV plays an important role for transformation from normal cells into tumour cells and metastatic tumour cells (Barnes et al., 2005; Cho, 2007). Therefore, the expression of LMP1 mRNA can be used to perform NPC screening and as a marker of NPC diagnostic molecules. The positivity of mRNA LMP1 EBV expression is 10-50 percent (Middeldorp et al., 2003). In previous studies, free EBV DNA in plasma were detected in 3 of 17 healthy people as control sample (18%) and the expression of EBV lytic genes could be detected in healthy career tissue biopsies (Martel-Renoir et al, 1995; Feng et al., 2000; Gulley, 2001).

FFPE is routinely used for the histopathological diagnosis like cancer in the hospital. The process of fixation and paraffin block embedded is able to change the structure of DNA as formaline fixation process. Fixation process of the network tissue using the formalin and stored it for long periods can cause changes in the structure of cells and also causing DNA degradation. Fixation should be used for optimum results is the use of liquid neutral buffered formalin, compared with 10% of non-buffered formalin as it will slow down the process of degradation caused by formaldehyde (Rezeki et al., 2014). The advantages of using FFPE NPC tumour biopsy in this researchs i.e. FFPE NPC tumour biopsy have a potensial tissue bank which it has been collected by Pathology Anatomy Department of Prof. dr. Margono Soekarjo Hospital for many years, FFPE NPC tumour biopsy was a non-invasive clinical sample because it was an archived specimen, FFPE NPC tumour biopsy needed not an ethical approval, the molecular histopathological diagnosis used by FFPE NPC tumour biopsy was rarely used until at present. Therefore, the expression of mRNA LMP1 EBVFFPE tumour biopsy in NPC WHO-3 patients can be used to be an NPC diagnosis.

The research result indicated that mRNA LMP1 EBV from FFPE tumour biopsy in NPC WHO-3 patients were expressed moderately (51,2

%). The expression of mRNA LMP1 EBV from FFPE NPC tumour biopsy has potential as a tumour biomarker of NPC diagnosis in that NPC management. Therefore, the molecular methods of NPC diagnosis would improved the management of NPC.

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REFERENCE

Adham, M., Kurniawan, A. N., Muhtadi, A. I., Roezin, A., Hermani, B., Gondhowiardjo, S., & Middeldorp, J. M. (2012). Nasopharynngeal Carcinoma in Indonesia : Epidemiology, Incidence, Sign & Symptom at Presenation. *Chinese Journal of Cancer,* 31(4), 185-196.

Barnes, L., Eveson, J. W., Reichart, P., Sidransky, D. (2000). (Eds.): *World Health Organization Classification of Tumours. Pathology and Genetics of Head and Neck Tumours*. Lyon : IARC Press, 85-97.

Boos, H., Berger, R., Kuklik-Roos, C., Iftner, T., & Mueller-Lantzsch, N. (1987). Enhancement of Epstein-Barr virus membrane protein (LMP) expression by serum, TPA, or n-butyrate in latently infected Raji cells. *Virol,* 159(1), 161-65.

Brink, A. A., Oudejans, J. J., Jiwa, M., Walboomers, J. M., Meijer, C. J., & van den Brule, A. J. (1997). Multiprimed cDNA synthesis followed by PCR is the most suitable method for Epstein-Barr virus transcript analysis in small lymphoma biopsies. *Molecular and cellular probes*, 11(1), 39-47.

Chang, E. T., & Adami, H. O. (2006). The enigmatic epidemiology of nasopharyngeal carcinoma. *Cancer Epidemiology and Prevention Biomarkers*, 15(10), 1765-1777.

Chang, Y., Chang, S.S., Lee, H.H., Doong, S.L., Takada, K., Tsai, C.H. (2004). Inhibition of the Epstein-Barr virus lytic cycle by Zta-targeted RNA interference. *Journal of general virology,* 85(6), 1371-1379.

Cho, W. C. S. (2007). Nasopharyngeal carcinoma: molecular biomarker discovery and progress. Molecular cancer, 6(1).

Feng, P., Ren, E. C., Liu, D., Chan, S. H., & Hu, H. (2000). Expression of Epstein–Barr virus lytic gene BRLF1 in nasopharyngeal carcinoma: potential use in diagnosis. *Journal of General Virology*, 81(10), 2417-2423.

Gouveia, G. R., Ferreira, S. C., Ferreira, J. E., Siqueira, S. A. C., & Pereira, J. (2014). Comparison of two methods of RNA extraction from formalin-fixed paraffin-embedded tissue specimens. *BioMed research international*, 2014.

Gulley, M. L. (2001). Molecular diagnosis of Epstein-Barr virus-related disease. *The Journal of Molecular Diagnostics,* 3(1), 1-10.

Hao, S. P., Tsang, N. M., Chang, K. P., & Ueng, S. H. (2004). Molecular diagnosis of nasopharyngeal carcinoma: detecting LMP-1 and EBNA by nasopharyngeal swab. *Otolaryngology—Head and Neck Surgery*, 131(5), 651-654.

Hirankarn, N., Kimkong, I., & Mutirangura, A. (2004). HLA-E polymorphism in patients with nasopharyngeal carcinoma. *HLA*, 64(5), 588-592.

Kieff, E. & Rickinson, A. B. (2001). *Epstein-Barr virus and Its Replication*. In : Virology. 4th ed. Fields BN, Knipe DM, Howley PM, editors. Philadelphia : Lippincott-Raven, 2511-2573.

Kresno, S. B. (2011). *Ilmu Dasar Onkologi*. Edisi Kedua. Jakarta: Penerbit FKUI.

Martel-Renoir, D., Grunewald, V., Touitou, R., Schwaab, G., & Joab, I. (1995). Qualitative analysis of the expression of Epstein—Barr virus lytic genes in nasopharyngeal carcinoma biopsies. *Journal of general virology*, 76(6), 1401-1408.

Middeldorp, J. M., Brink, A. A., van den Brule, A. J., & Meijer, C. J. (2003). Pathogenic roles for Epstein–Barr virus (EBV) gene products in EBV-associated proliferative disorders. *Critical reviews in oncology/hematology*, 45(1), 1-36.

Munir, D. (2006). Beberapa Aspek Karsinoma Nasofaring pada Suku Batak di medan dan sekitarnya. *J Med School* USU, 39(3), 223-226.

Niedobitek, G. (2000). Epstein-Barr virus infection in the patogenesis of nasopharyngeal carcinoma. *Molecular Pathology,* 53(5), 248.

[OIE] Office international des Epizooties. (2005). Update on avian influenza viruses, including highly pathogenic H5N1 from poultry in live bird market in Hanoi, Vietnam in 2001. *J Virol,* 79: 4201- 4212.

Old, L. J., Boyse, E. A., Oettgen, H. F., De Harven, E., Geering, G., Williamson, B., & Clifford, P. (1966). Precipitating antibody in human serum to an antigen present in cultured Burkitt's lymphoma cells. *Proceedings of the National Academy of Sciences*, 56(6), 1699-1704.

Payungporn, S., Phakdeewirot, P., Chu-tinimitkul, S., Theamboonlers, A., Keawcharoen, J., Oraveerakul, K., Amonsin, A., & Poovorawan, Y. (2004). Single-step multiplex reverse transcription-polymerase chain reaction (RT-PCR) for influenza A virus subtype H5N1 detection. *Vi-*

ral Immunol, 17(4), 588-593.

Rezeki, M., Parwati, I., Bethy., & Tjandrawati, A. (2014). Validitas Multiplex Real Time Polymerase Chain Reaction untuk Diagnosis Limfadenitis Tuberkulosis pada Spesimen Blok Parafin. *MKB,* 46(3), 162-167.

Roezin, A. (1999). Berbagai faktor penyebab dan predisposisi karsinoma nasofaring. *Maj Kedokt Indon*, 49(3), 85-88.

Soeripto. (1998). Epidemiology of nasopharyngeal carcinoma. Berita Kedokteran Masyarakat, XIII : 207-11.

Stevens, S. J., Verkuijlen, S. A., van den Brule, A. J., & Middeldorp, J. M. (2005). Comparison of quantitative competitive PCR with LC-based PCR for monitoring of Epstein-Barr virus (EBV) DNA load in clinical specimens. *J Clin Microbiol*, 40, 4105-13.

Stevens, S. J., Verkuijlen, S. A., Hariwiyanto, B., Paramita, D. K., Fachiroh, J., Adham, M., ... & Middeldorp, J. M. (2006). Noninvasive diganosis of nasopharyngeal carcinoma : nasopharyngeal brushing reveal high Epstein-Barr virus (EBV) DNA load and carcinoma-specific viral BARF1 mRNA. *International journal of cancer*, 119(3), 608-614.

Thompson, M. P. & Kurzrock, R. (2004). Epstein-Barr virus and cancer. *Clin Cancer Res*, 10(3), 803-821.

Verweij, F. J., Van Eijndhoven, M. A., Hopmans, E. S., Vendrig, T., Wurdinger, T., Cahir-McFarland, E., ... & Middeldorp, J. M. (2011). LMP1 association with CD63 in endosomes and secretion via exosomes limits constitutive NF-κB activation. *The EMBO journal*, 30(11), 2115-2129.

Wahyono, D. J., Musa, Z., Lisnawati, Suryandari, D. A., Hermani, B., & Soeharso, P. (2016). Host-patogen study : The relative expression of mRNA BRLF1 Epstein-Barr virus : a potensial use for biomarker of tumour progresivity &polymorphisms of *TCRBC* and *TCRGC2* host genes related to genetic susceptibility on nasopharyngeal carcinoma. *BMC Proceeding2016 : 7th Biannual International Symposium on Nasopharyngeal Carcinoma 2015, Yogyakarta, Indonesia.*

Wibowo, A. A., Susanti R,. Umum, F., & Nugrahningsih, W. H. (2012). Isolasi dan identifikasi virus Avian Influenza subtype H5N1 di pertenakan tradisional, Kecamatan Gunungpati, Semarang. *Biosaintifika: Journal of Biology & Biology Education*, 4(2), 62-69.

Young, L. S. & Rickinson, A. B. (2004). Epstein-Barr virus : 40 years on. *Nat Rev Cancer,* 4(10), 757-68.

Yu, M. C. & Yuan, J. M. Epidemiology of nasopharyngeal carcinoma. (2002). Epidemiology of nasopharyngeal carcinoma. In *Seminars in cancer biology* (Vol. 12, No. 6, pp. 421-429). Academic Press.

Zur Hauzen, H. (1970). EBV DNA in biopsies of Burkitt tumors and anaplastic carcinomas of the nasopharynx. *Nature*, 228, 1956-1058.

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The Effect of Water Content of Medium Containing *Oryctes rhinoceros* Larvae on *Metarhizium anisopliae* Pathogenicity

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Abstract

The entomopathogenic fungus, *Metarhizium anisopliae* (Metschnikoff) Sorokin (Ascomycota: Hypocreales) would effectively infect the target host on the appropriate medium water content. The aim of this study was to analyze the influence of water content of medium on the effectiveness of *M. anisopliae* fungus infection on *O. rhinoceros* larvae in the laboratory. Fifty healthy third instar larvae of *O. rhinoceros* were obtained from field. The *M. anisopliae* obtained from Estate Crop Protection Board in Salatiga. The conidia density and viability of *M. anisopliae* were examined before used. The medium for maintaining the larva was the sawdust that had been sterilized. A total of 50 plastic cups were prepared to place 50 larvae (1 larva/cup). Each cup was filled with 100 g medium of sawdust plus 2 g of *M. anisopliae* which was then stirred until mixed, with different water content: P1 (20%), P2 (40%), P3 (60%), P4 (80%) and P5 (98%). The result indicated that the water content of the medium affected the effectiveness of *M. anisopliae* fungus infection on *O. rhinoceros* larvae. The water content influenced the duration of larval mortality at each treatment. An important finding in this study is that controlling *O. rhineceros* larvae with *M. anisopliae* can be done by manipulating the water content of medium. The benefit of this study may be used for the recommendation of *O. rhinoceros* pest control using *M. anisopliae* with an effective water media content.

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INTRODUCTION

Coconut is one of the important plantation commodities in the Indonesian (Mulyono, 2007). In coconut cultivation, pest and disease are factors that limiting the coconut production. One of the most commonly found destructive pest of coconut plants is *Oryctes rhinoceros*. The imago of *O. rhinoceros* attacks the coconut shoots with the unopened young leaves, while the larval phase lives in the soil containing organic matter or on rotten coconut trunk (Indriyanti et al., 2016; Abidin et al., 2014). *Oryctes rhinoceros*, commonly known as the rhinoceros beetle is an important agricultural pest that is known to inflict serious damage on young oil palm trees (Manjeri et al., 2014).

There are different methods to control the insect pests. This includes chemical, mechanical, physical and biological control of pest. Chemical control can be done with the use of pesticides (Tarwotjo & Rahardian, 2017) and pheromones (Chakravarthy et al., 2014). Mechanical and physical control can be done by searching and destroying the existing larvae in the soil. Biological control can be done with the use of biological controlling agents. One of the insect pathogens that has been utilized for the control of *O. rhinoceros* is the fungus *Metarhizium anisopliae* (Harjaka, 2011; Manurung et al., 2012). *M. anisopliae* is a soil borne entomopathogen, found worldwide. It is an interesting fungus for biological control (Chen et al., 2014; Simamora et al., 2013). It can infected larvae and adult *Oryctes agamemnon arabicus* (Coleoptera: Scarabaeidae) (Ibrahim, 2017).

Many factors that influence the success of parasitic fungi in infecting insects, including temperature, humidity (Athanassiou et al., 2017; Subhathma et al., 2013). Efficacy *M. anisopliae* in the fields is significantly affected by enviromental conditions, particularly moisture (Bidochka, 2000; Chen et al., 2014; Moslim & Kamarudin, 2014). Water content media where fungus grow is very important. The fungus *M. anisopliae* will effectively infect the host if the water content of the media is suitable for conidia germination.

Water is the main component needed by the fungus in order to keep growing. The water content is an indicator of the presence of water in the environment. Entomopathogenic fungi require a high water content for growth. The optimum growth of fungi increase the possibility of infecting the insect pests effectively (Gupta & Gopal, 2002).

One of the places used as a habitat of *O. rhinoceros* larva is a pile of sawdust. Sawdust media has a hollow structure so that there is more oxygen in there. In controlling *O. rhinoceros* using *M. anisopliae*, it is important to note the conformity of abiotic factors of medium where larvae live including the water content and humidity of the media. The pathogenicity of *M. anisopliae* against *O. rhinoceros* larvae on sawdust media was 86.24%, higher than in manure media (68.27%) (Mulyono, 2007), but it was not known how much water content in these media. Therefore, it is necessary to examine the appropriate water content in media for the optimum fungi germination. The aim of this study was to analyze the influence of water content of medium on the effectiveness of *M. anisopliae* fungus infection on *O. rhinoceros* larvae in the laboratory. This study provides an information the effect of water content of medium *against M. anisopliae* pathogenicity on *O. rhinoceros* larvae. The benefit of this study was used for the manipulation of environmental factors in control *O. rhinoceros* in the field.

METHODS

Larvae *O. rhinoceros*

Fifty healthy third instar larvae of *O. rhinoceros* with length of 7-12 cm and weight of 9-13 g were obtained from field, in Demak Central Java Indonesia. This study was carried out in the laboratory during the dry season with the temperature ranges from 29.8 - 35°C, pH media of 6.9, room light intensity of 70-77 lux and RH of 58-83%.

Fungi *M. anisopliae*

The fungi *M. anisopliae* obtained from Estate Crop Protection Board in Salatiga, Central Java Province, Indonesia. It was cultured on the corn medium. The conidia density and viability of *M. anisopliae* was examined before the use. Conidia density was calculated under microscope using *Haemocytometer*. The viability of conidia was observed after 10 hours incubation on PDA (potato dextrose agar) media. Density and density calculations are performed four repetitions. The formula for calculating the density and viability of conidia were based on the formula recommended by BPTBUN (2012).

Treatment

The medium for maintaining the larva was the sawdust that had been sterilized for 8 hours.

During the treatment, the larvae were placed in plastic cups (diameter = 8.4 cm, height = 11 cm) with the cap section given a small hole for air ventilation. A total of 50 plastic cups are prepared to place 50 larvae (1 larva/cup). Each cup was filled with a medium of 100 g of sawdust

with different water content P1 (20%), P2 (40%), P3 (60%), P4 (80%) and P5 (98%) plus 2 g of *M. anisopliae* which was then stirred until mixed. The *O. rhinoceros* larvae were then fed into prepared media. The observations were conducted every two days for 28 days (estimated death of all larvae). This study used a Randomized Block Design technique with 5 water treatments (P1-P5) and 10 repetitions.

Data analysis

The data obtained including: 1) density and viability of *M. anisopliae* conidia, 2) morphology of *O. rhinoceros* due to *M. anisopliae* application, 3) Mean survival time of *O. rhinoceros* larvae treated with *M. Anisopliae*, data were analyzed statistically using SPSS version 16.0 and further testing by Tukey's test.

RESULTS AND DISCUSSION

The density of conidia greatly affects the speed of the *M. anisopliae* in killing *O. rhinoceros* larvae . The calculation of the conidial density of *M. anisopliae* used is presented in Table 1.

Table 1. The Density of *Metarhizium anisopliae* Conidia

Repetition	Result on the counting area of *Haemocytometer*					Total	Conidia density per gram of sample
	a	b	c	d	e		
1	10	15	9	14	10	58	2.90×10^8
2	10	14	12	10	9	55	2.75×10^8
3	11	12	14	9	11	57	2.85×10^8
4	10	14	11	12	10	57	2.85×10^8
Mean							2.84×10^8

The mean conidial density of 2.84×10^8 includes in a good category according to biological agent quality standard, because the number of conidia was more than 10^6 (BPTBUN, 2012). The result of viability calculation of *M. anisopliae* Conidia is presented in Table 2.

The viability of *M. anisopliae* was 94.6% which included in a very good category (in range of 86-100%) according to the biological agent quality standard from BPTBUN (2012). Conidia growth is listed in the Figure 1.

The Survival Time of *O. rhinoceros* Larvae

Figure 2 shows that there is a difference in the survival time of *O. rhinoceros* larvae in 5 different treatments.

Table 2. The Viability of *M. anisopliae* Conidia

Repetition	Amount of Observed Conidia			Viability (%)
	Not Growing	Growing	Total	
1	3	61	64	95.3
2	5	64	69	92.7
3	2	58	60	96.6
4	4	60	64	93.8
Mean				94.6

Figure 1. Conidia of *M. anisopliae* (A). Conidia germination (B), at magnification of 400x.

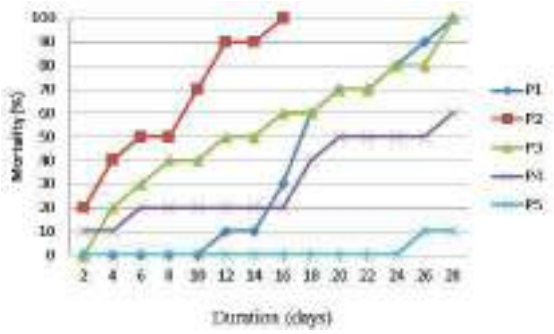

Figure 2. The Percentage of *O. rhinoceros* Larvae Mortality treated by *M. anisopliae* on the Sawdust Medium with Different Water Content (P1= 20%; P2= 40%; P3= 60%; P4= 80%; P5= 98%)

The mean survival time of *O. rhinoceros larvae* treated with *M. anisopliae* is presented in Table 3.

Table 3. Mean survival time of *O. rhinoceros* larvae treated with *M. anisopliae*

Treatment	Mean survival time
P1	19.6 [b]
P2	7.8 [a]
P3	15.0 [ab]
P4	23.6 [b]
P5	34.8 [c]

Note: Numbers in the same column, followed by the same letter, are not significantly different (Tukey's test , $\alpha = 0.05$)

In P2 treatment, the *M. Anisopliae*-treated

larvae had a shortest mean survival time (7.8 days) among all treatments, while larvae in P5 treatment had a longest mean survival time (34.8 days) (Table 3). The result of ANOVA test (F = 15.119; df = 4; P = 0.000; P <0.005), indicated that there is a difference of larvae survival time at each treatment (Table 3). Water content in P1 (20%) treatment was considerably less than the ideal one. It caused the fungus could not germinate and the mortality of treated larvae due to the lack of water occurred (Figure 2). Therefore, the water content greatly affects the effectiveness of fungi *M. anisopliae* in infecting *O. rhinoceros* larvae.

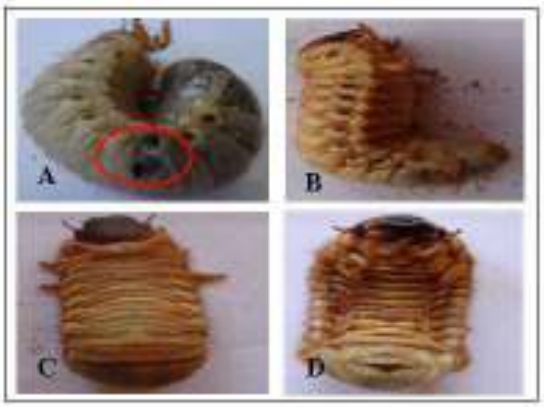

Figure 3. The Morphological Changes of *O. rhinoceros* Larvae in P1 (20%) Treatment

Note:
A: The symptom of *M. anisopliae* infection, indicated by the appearance of brown spot on the cuticle (day 2)
B: The larvae begin to dwarf (day 8)
C: The body of the larvae becomes more shrinkage (day 10)
D: The dead larval with the absence of the symptoms of *M. anisopliae's* infection (day 12)

At the P1 (20%) treatment, there were no larvae died due to *M. anisopliae* infection. Initially, all the larvae in this treatment showed the symptoms of infection such as melanization and the slower movement on the 2nd day of treatment, however, on the 8th and 10th days the larvae morphology became dwarf and the larvae were on the surface of medium. The death of the larvae begins on the 12th day with the condition of dwarf and shrink. This is because the larvae were lost a lot of water or dehydrated (Figure 3).

Insects mortality will occur when the water content drops below the tolerance limit. The reduced water content results in the dwarf growth and low metabolic rate. The content of water in the insect body varies, generally ranging from 50-90% of body weight. In thick skinned insects, the body content of the water is lower. Insects should try to get the right water balance in order to maintain their life (Sodiq, 2009).

The larvae maintained on sawdust media containing *M. anisopliae* fungi with a moisture content of P2 (40%) had the shortest survival time compared to four other media. It was indicated that treatment P2 is the most effective treatment to control the *O. rhinoceros* population. In this treatment, conidia of *M. anisopliae* can grow well so that it can infect large numbers of larvae. Mortality in the treated larvae began on day 2, and reached 100% mortality by day 16 (P2, Figure 2), The average survival time is 7.8 days (Table 3). Statistically, the result of P2 and P3 treatment did not significantly different, so it can be said that the effective water content to maintain the *O. rhinoceros* larvae and *M. anisopliae* growth ranges from 40-60%.

In the treatment of P4 (80%) and P5 (98%), conidia did not grow well due to the high water content resulting in many conidia to die from long waterlogged. In these treatments, the fungi failed to infect the larvae. Treatment of P5 (98%) showed significantly different results compared with all other treatments. This is due to the very high amount of water contained in the P5 treatment. In this treatment, the *M. anisopliae* fungus will not be able to infect the larvae, because the conidia were drowned and eventually dead. The larvae began to die on day 26 and reached 100% mortality by the day 40. The dead of larvae was because they could not last long in water. The interesting phenomenon on high water content of the media was that the larvae tend to be uncomfortable, as evidenced by the larvae tried to get out of the place of maintenance.

In nature, the phenomenon of high water content occurs during the high rainfall for a long time. According to Susanti et al. (2013) rainfall greatly affects the effectiveness of *M. anisopliae* infection because it is related to water content in a medium. In a high rainfall, *M. anisopliae* cannot infect the larvae because it drifts with water or rottenness of germination fungus, because it was submerged in water for a long time.

The higher the water content of the media, the longer mean survival time of the treated larvae. It can be explained that in the medium with moisture content of 80-98%, *M. anisopliae* conidia is submerged in water for a long time, the possible condition of conidia was rotten and die after the germination, so that the larvae were failed to be infected by the fungus. As a result, the mean survival time of the larvae became 23.6 - 34.8

days (P4 & P5, Table 3).

Temperature, humidity, light intensity and pH are factors that also affect the effectiveness of *M. anisopliae* in infecting the *O. rhinoceros* larvae. The appropriate temperature and humidity will reduce the dehydration process in the fungus' body (Prayogo et al., 2005). The study was conducted during the dry season, temperatures ranging from 29.8 - 35°C, so that the temperature was relatively higher.

The development of *M. anisopliae* conidia causes the larvae's body to become weak and end with the larvae mortality (Simamora et al., 2013). According to Susanti et al. (2013), conidia germinate in air humidity above 90% and achieve the optimum germination at a high air humidity (100%). In this study, in air humidity of 58-83%, *M. anisopliae* was able to infect larvae effectively. It is proven with the results of P2 treatment (mean survival time of 7.8 days). *M. anisopliae* can grow and proliferate optimally in places with relatively low light intensity.

In this study, the pH obtained at the time of observation was in the range of 6.9 (relatively neutral). The appropriate pH range supports the activity of enzymes contained in *M. anisopliae* (lipase, chitinase, amylase, proteinase, phosphatase, and esterase). According to Prayogo et al. (2005), chitinase and proteinase are two enzymes that have a very important role. Chitinase serves to assist the activity at the beginning of growth of the fungus, conidia formation, and conidiospore sporulation while protease enzymes play a role in degradation of the cuticle and chitinase stimulation.

The larva undergoes numerous sequential morphological changes since the first until the last stage of infection which was indicated by the appearance of green fungi in the body's surface of the larvae (Figure 4).

The melanization process was first seen in P2 (40%) treatment larvae, indicated by the appearance of a dark brown spot on the larval cuticle. The brown spots mostly seen on the folds between segments adjacent to the spiracles. *M. anisopliae* attach more strongly to the folds of the larval cuticle. It is more easier to penetrate into the body of the *O. rhinoceros* larvae. It is in accordance with the opinion of Manurung et al. (2012) that the infection of *M. anisopliae* on the larvae are characterized by larval cuticles that turn into dark brown color. The cuticle was covered by the hyphae from the fungus which then turns into green which means that the conidia has grown. According to Prayogo et al. (2005), hypha from the conidia of *M. anisopliae* get into the cavities within the host's body due to enzyme aids and mechanical stresses. The infected larvae will undergo the mummification phase because all the tissues and body fluids of the larvae are used up by the fungus for its reproduction (Permadi, 2012). Finally, the whole body of the host insect is full of propagules and the soft part of its body will be pierced out. It shows the growth of hyphae outside the body of the host insect. The growth of external hyphae will produce conidia which then being disseminated to the environment and infect the other healthy insect pests.

Figure 4. The Morphological Changes of *O. rhinoceros* Larvae due to the Infection of *M. anisopliae* on P2 Treatment.
Note:
A: A healthy *O. rhinoceros* larva (day 1)
B: The infection of *M. anisopliae* indicated by the appearance of brown spot on the cuticle (melanization) (day 2)
C: Dead larvae infected with *M. anisoliae* with the stiff and pale body (day 4)
D: The appearance of white hyphae in the surface of larva's body (day 6)
E: The sporulation of fungi outside the larva's body indicated by the hyphae that turns to green (day 10).

The symptoms of *M. anisopliae*-infected larvae are loss of appetite, slow movement, and then the mortality followed by stiff and pale dead larvae. The green hyphae in the body of the larvae will appear later. According to Herlinda et al. (2005), the length of time required by entomopathogenic fungi isolates ranging from fungal infections to dead larvae can range from 2-10 days. In this study, the mean of survival time of larvae range from 7.8 - 34.8 days (Table 3.)

The results of this study can be suggested that, controlling of the *O. rhineceros* larvae with the *M. anisopliae* fungus, will be optimum if carried out in during the transition season (the beginning of the rainy season) the field. At the be-

ginning of rainy season, usually the intensity of the rain is not too high, thus allowing the fungus to grow and infect the larvae well. *O. rhineceros* larvae controlling will not be optimal if it is conducted in dry season, because conidia will not be able to germinate. The controlling also will not be optimal if it is conducted in the rainy season, because too much water conidia will die, so the infection will not happen.

An important finding in this research is that controlling *O. rhineceros* larvae with *M. anisopliae* can be done by manipulated the environmental, such as water content of the media where the *O. rhineceros* larvae live. The benefit of this study may be used for the recommendation of *O. rhinoceros* pest control using *M. anisopliae* in an effective water media content (40-60%).

CONCLUSION

The water content of the medium affects the effectiveness of the fungus *M. anisopliae* in infecting the *O. rhinoceros* larvae. Medium with 40-60% water content is the suitable medium for *M. anisopliae* growth to infect *O. rhinoceros* larvae. The very low water content (20%) causes the fungus of *M. anisopliae* unable to germinate. The very high water content (98%) causes the conidia died from being submerged in water.

REFERENCES

Abidin, C. M. R. Z., Ahmad, A. H., Salim, H., & Hamid, N. H. (2014). Population Dynamics of Oryctes rhinoceros in Decomposing Oil Palm Trunks in Areas Practising Zero Burning and Partial Burning. *Journal of Oil Palm Research*, 26(2), 140-145.

Athanassiou, C. G., Kavallieratos, N. G., Rumbos, C. I., Kontodimas, D. C. (2017). Influence of Temperature and Relative Humidity on the Insecticidal Efficacy of *Metarhizium anisopliae* against Larvae of *Ephestia kuehniella* (Lepidoptera: Pyralidae) on Wheat. *Journal of insect science* 17(1), 22

[BPTBUN] Balai Proteksi Tanaman Perkebunan. (2012). Laporan Perbanyakan APH dan Pesnab. Dinas Perkebunan Provinsi Jawa Tengah. Salatiga

Bidochka, M. J., Kamp, A. M. & Decroos, J. N. A. (2000). *Insect Pathogenic Fungi: From Genes to Populations.* In *Fungal Pathology*. (Ed) Kronstad J. W. Kluwer Academic Publisher Group, Dordrecht, Netherlands. 171-193p.

Chen, Z. H., Xu, L., Yang, F. L., Ji, G. H., Yang, J., & Wang, J. Y. (2014). Efficacy of Metarhizium anisopliae isolate MAX-2 from Shangri-la, China under desiccation stress. *BMC microbiology*, *14*(1), 4.

Chakravarthy, A. K., Chandrashekharaiah, M., Kandakoor, S. B., & Nagaraj, D. N. (2014). Efficacy of aggregation pheromone in trapping red palm weevil (Rhynchophorus ferrugineus Olivier) and rhinoceros beetle (Oryctes rhinoceros Linn.) from infested coconut palms. *Journal of Environmental Biology*, *35*(3), 479-484.

Gupta, A., & Gopal, M. (2002). Aflatoxin production by Aspergillus flavus isolates pathogenic to coconut insect pests. *World Journal of Microbiology and Biotechnology*, *18*(4), 329-335.

Harjaka. (2011). Potensi Jamur *Metarhizium anisopliae* untuk Pengendalian Uret Perusak Akar Tebu. Fakultas Pertanian UGM. Yogyakarta.

Herlinda, S., Pujiastuti, Y., Pelawi, J., Riyanta, A., Nurnawati, E. & Suwandi. (2005). Patogenisitas isolat-isolat *Beauveria bassiana* (Bals.) Vuill. Terhadap larva *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) di rumah kaca. *Inovasi*, 2(2), 85-92.

Ibrahim, R. A. (2017). Laboratory Evaluation of Entomopathogenic Fungi, Commercial Formulations, against the Rhinoceros Beetle, *Oryctes agamemnon arabicus* (Coleoptera: Scarabaeidae). *Egyptian Journal of Biological Pest Control*, 27(1), 49-55.

Indriyanti D. R., Putri, R.I.P., Widiyaningrum, P. & Herlina, L. (2017). Density, Viability conidia and symptoms of *Materhizium anisopliae* infection on *Oryctes rhinoceros* larvae. *Journal of Physics*: Conf.Series 824(2017)012058.

Manurung, E. M., M. C. Tobing, L. Lubis, dan Priwiratama. (2012). Efikasi Beberapa Formulasi *Metarhizium anisopliae* Terhadap Larva *Oryctes rhinoceros* L. (Coleoptera: Scarabaeidae) di Insektarium. *Jurnal Agroekoteknologi,* 1(1), 47-60.

Manjeri, G., Muhamad, R., & Tan, S. G. (2014). *Oryctes rhinoceros* Beetles, an Oil Palm Pest in Malaysia. *Annual Research & Review in Biology*, 4(22), 3429-3439.

Mulyono. (2007). Kajian Patogenisitas Cendawan *Metarhizium anisopliae* Terhadap Hama *Oryctes rhinoceros* L. Tanaman Kelapa Pada Berbagai Waktu Aplikasi. *Thesis.* Program Pascasarjana Universitas Sebelas Maret Surakarta.

Moslim, R. & Kamarudin, N. (2014). The use of palm kernel cake in the production of conidia and blastospores of *Metarhizium anisopliae* var. *Major* for control of the *Oryctes rhinoceros. Journal of Oil Palm Research*, 26(2), 133-139.

Permadi, M. A. (2012). Patogenisitas Beberapa Isolat Cendawan Entomopatogen *Metarhizium* spp Terhadap Kumbang Predator *Menochilus sexmaculatus* F. (Coleoptera: Coccinelidae). Prodi Agroekoteknologi, BKI Perlindungan Tanaman. Fakultas Pertanian. Universitas Andalas, Kampus Limau Manis, Padang.

Prayogo, Y., Tengkano, W., & Marwoto. (2005). Prospek cendawan entomopatogen metarhizium anisopliae untuk mengendalikan ulat grayak *Spodoptera litura* pada kedelai. *Jurnal litbang pertanian*, 24(1), 19.

Susanti, U., Salbiah, D. J., Loah, H. (2013). Uji Beberapa Konsentrasi *Metarhizium anisopliae* (Metsch) Sorokin Untuk Mengendalikan Hama Kepik Hijau (*Nezara viridula* L.) Pada Kacang Panjang (*Vigna sinensis* L.). *Jurnal Universitas Riau.*

Subhathma, W. G. R., Suwandharathna, N. I., Fernando, L. C. P., & Ahangama, D. (2013). Effect of temperature on lokal and Philippine isolates of Metarhizium anisopliae and their virulence on larvae of *Oryctes rhinoceros*, a pest of coconut in Sri Lanka. Research Article. Cocos 20: 49-58.

Sodiq, M. (2009). Ketahanan Tanaman Terhadap Hama. Universitas Pembangunan Nasional "Veteran". Fakultas Pertanian. Jawa Timur.

Simamora, L. O., Bakti, D. & Oemry, S. (2013). Kajian Epizootik *Metarhizium anisopliae* pada Larva Tritip (*Plutella xylostella* L.) (Lepidoptera: Plutellidae) Di Rumah Kaca. *Jurnal Online Agroekoteknologi*. 1(2), 171.

Tarwotjo, U. & Rahardian, R. (2017). Resistance Inheritance of *Plutella xylostella* Population to Residual of Emamectin Benzoat. *Biosaintifika: Journal of Biology & Biology Education*, 9(1), 19-25.

Biosaintifika 9 (2) (2017) 370-379

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Correlation of Physical-Chemical Parameters to Total Coliform Value in Jawi River, Pontianak, West Kalimantan

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Abstract

Coliform bacteria can be used as an indicator of the presence of pathogenic bacteria, such as *E.coli* bacteria that cause diarrhea. The aimed of this study is to determine the relationship between physical-chemical parameters namely temperature, pH, DO and BOD to the density of coliform bacteria in Jawi River, Pontianak. The sampling was conducted at one point each in the upstream, midstream and downstream area of the Jawi River during two tidal conditions of the Kapuas River in September 2016 at 09:40 (at low tide) and at 15:40 (at high tide). The correlation of physical-chemical parameter to coliform value was tested Pearson Product Moment. The results showed that coliform bacterial density increased from upstream to downstream with 150-1500 MPN/100 ml at high tide and 930-11000 MPN/100 ml at low tide. The results showed that the coliform bacterial density value had a positive relation with pH and BOD parameters and negative relation with temperature and DO parameters. So, it can be concluded that there is a correlation between physical parameters, such as temperature and chemical parameters such as pH, DO and BOD to microbiological parameters especially Coliform bacterial density. The benefit of this study is to give information about water quality of Jawi River and its correlation with density of Coliform bacterial, so that people are expected to pay more attention to the use of clean water to avoid the disease caused by coliform.

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INTRODUCTION

Water quality is influenced by changes in land use, such as an increase in domestic, agricultural and industrial activities which have an impact on BOD concentrations (Priyambada et al., 2008). This is similar to the idea of Agarwal et al. (2011) stated that most rivers in urban areas of developing countries are industrial waste, such as in Africa and Asia which have rapid industrial growth and affect environmental conservation conditions. Anhwange et al. (2012) and Abida et al. (2008) suggest that the accumulation of discharges such as urea, animal manure and vegetable cuts into aquatic bodies became the growth factor of algae and other aquatic plants as a result of an increase in the number of microbial activity. Uncontrolled distribution of waste discharges may lead to degradation of water quality such as a decrease in dissolved oxygen content and the death of aquatic animals.

Jawi River in Pontianak is the primary channel which lies between the West Pontianak District and the Pontianak District as well as being the estuary for the secondary channel from the surrounding residential areas. According to Ramadhani (2012), the continuous discharge of domestic waste in the Jawi River affects water quality in waterways such as the consumption of dissolved oxygen. Based on the results of research of Khotimah (2009), it is showed that the highest coliform bacterial density was found at the mouth of the Jawi River. The use of polluted river water may affect the health status of the users with the emergence of water-borne disease, one of which is diarrhea. Contaminated water affects the health of human populations around the world, especially for developing countries (Cabral, 2010). In developing countries, 43 cases of the disease are caused by water-borne diseases, with diarrheal diseases as the first leading cause of death especially in children (Rahman et al., 2013). The same thing is expressed by Kumar et al. (2011) and Farkas et al. (2013) that pollution in rivers may spread diseases such as cholera, typhoid fever and diarrhea.

Generally, the presence of bacteria in the water is influenced by abiotic factors such as temperature, pH, oxygen demand and moisture and biotic factors that include negative and positive interactions between populations. Water to be used for drinking, domestic, agricultural and industrial purposes is essential to be tested first on the physical, chemical, and microbiological parameters of the water. Some physical parameters tested are temperature, color, odor, turbidity and TDS (Total Dissolved Solid), chemical parameters include pH (potential of Hydrogen), BOD (Biological Oxygen Demand), COD (Chemical Oxygen Demand), DO (Dissolved Oxygen), alkalinity and hardness (Patil et al., 2012), as well as microbiological parameters in the form of microbial content, the presence of coliform bacteria as an indicator of pathogenic microbes. Therefore, it is necessary to investigate relationship of density of coliform bacteria with physical-chemical parameters, particular parameters of temperature, pH, DO and BOD along Jawi River channel region. Selection of these parameters based on the growth factor of Coliform bacteria that adjust the conditions of temperature, pH, oxygen levels and nutrients in the water it occupies.

Pujiastuti et al. (2013) stated that water temperature affects the process of metabolic exchange of living things and affects the dissolved oxygen level and the growth of fish populations. The pH of water affects plants and aquatic animals, so it is often used for the condition of the good or poor state of the water as the living environment of water biota. Dissolved oxygen is one of the dissolved gases in the waters with varying levels and influenced by temperature, salinity, water turbulence and atmospheric pressure. Oxygen is also needed in the process of decomposition of organic compounds into inorganic compounds. The source of dissolved oxygen comes from the diffusion of oxygen present in the atmosphere, either directly in stagnant conditions or due to agitation (mass water upheaval) due to the presence of water or wind waves. According to Varale & Yashodhara (2012) BOD is the average amount of oxygen used by bacteria when decomposing organic substances into inorganic substances in the aerobic phase. The process of decomposition and activity of bacterial metabolism use dissolved oxygen. Dissolved oxygen is used to calculate the amount of BOD content in the tested sample.

Coliform bacteria is used as indicator of fecal pollution or human and animal feces in the waters because coliform bacteria are intestinal bacteria derived from human intestines and other warm-blooded animals, such as chickens, cows and pigs (Badiamurti, 2010; Rajendra et al., 2012). Thus, the bacteria should not be present in the used water, in terms of health, aesthetics, hygiene and the possibility of dangerous infections (Pujiastuti et al., 2013). This became the basic purpose of this study, which is to determine the relationship between physical-chemical parameters such as temperature, pH, DO and BOD against the density of coliform bacteria in the ri-

ver Jawi, Pontianak.

METHODS

This research was conducted in Jawi River, Pontianak City (Figure 1). The study was conducted from September 2016 to November 2016 covering sampling, physical and chemical factor measurements, calculation of Coliform bacterial density, and data analysis. Analysis of DO and BOD samples was conducted at the Laboratory of Land Quality and Health, Faculty of Agriculture and Coliform analysis was conducted at Microbiology Laboratory, Faculty of Engineering, Tanjungpura University, Pontianak.

The tools used for this research on the field were the measuring beam, the ball that has been given ballast, measuring tape, rope, sample bottles of 250 ml and 1 L, Winkler bottle, thermometer, pH-meter. Tools used in laboratory were beaker glass, Erlenmeyer glass, test tube, test tube rack, petri dish, ose needle, bunsen, micropipette, dropper drops, hot plate, plastic wrapping, incubator, Laminar Air Flow, autoclave, durham tube, analytic balance and spatula. The materials used are aquades, carbolic, alcohol 70%, LB (Lactose Broth) media, BGLB (Brilliant Green Lactose Broth) and EMB (Eosin Methylene Blue) agar.

Sampling was conducted on September 16, 2016 at the highs and lows of the Kapuas River, at 09.40 WIB (low tide) and at 15:40 (high tide) at the upstream (Parit Haruna), midstream (Suwignyo) and downstream (Gertak I Jawi River). Sampling DO and BOD were taken using size 1 L bottles and Winkler bottles and were duplicated. The first stage of sampling for the DO and BOD parameters was rinsing the sample bottle with the sample water to be taken three times to homogenize the condition of the bottle with the sample water. Then, the water sample was taken by dipping the bottle under the water surface and closed until it was airtight. Microbiological sampling used 250 ml sample bottles that have been sterilized by using autoclave. The sample bottle was dipped below the water level (± 20 cm), the position of mouth of the bottle is opposite to the flow direction. The mouth of the bottle was immediately closed and put in the cool box. Microbiological sampling was done in 3 times repetition (Khotimah, 2009).

Temperature measurements were made by inserting a thermometer into the sample water for ± 5 minutes until the temperature figure on the thermometer was stable. The pH meter measurements were made by entering the pH meters into the sample and the results was recorded when the numbers indicated on the device were stable. Measurements of DO and BOD parameters using iodometric titration method were performed at the Laboratory of Land Quality and Health.

Calculation of total value of coliform used MPN test with sample volume value that is 1 ml, 0,1 ml and 0,01 ml on 3 series of test tube (WHO, 1996). The coliform test refers to SNI 01-2332.1-2006, including estimation test, assertion test and complementary test. The formula used to find the value of the density of Coliform is (APHA, 2005):

$$MPN/100ml = value\ of\ MPN\ table/100\ ml \times \frac{first\ column\ of\ treatment\ (1\ ml)}{smallest\ tube\ dilution\ (0,1\ ml)}$$

(Equation 1)

The analysis of temperature, pH, DO and BOD parameters to coliform bacterial density was using Pearson Product Moment correlation test, so that correlation value and direction of relationship between parameters have been known to be analyzed. If the value of R approaches 1 or -1, then there is a relationship between the variables, but if the value of R is 0, then there is no relationship between variables tested. Pearson Product Moment correlation test is using the following formula (Sugiyono, 2016):

$$r = \frac{\Sigma xy}{\sqrt{\Sigma x^2 \times \Sigma y^2}}, \text{ with } \alpha = 5\%$$

, with α = 5% (Equation 2)

Thus, the hypothesis used for Pearson Product Moment correlation test is:

1) $H_0 : R = 0$; 2) $H_a : R \neq 0$

Figure 1. Sampling point in Jawi River, Pontianak City

RESULTS AND DISCUSSION

The value of DO and BOD Jawi River water varied at each point and affects water quality standards as shown in Table 1 and Table 2. The temperature value at low tide was lower than during high tide, this is because of differences in sampling time that affect the ambient temperature (Saputri, 2014). In addition, the temperature difference at each point is also influenced by the seasons, geographical location, weather differences, air humidity and the intensity of sunlight

(Ahinpathi & Puttaiah, 2006; Romanto, 2013). The water temperature of Jawi River were ranged from 28 - 31°C and classified in class I, II, III standard quality. Berutu (2001) said if the water temperature ranges from 27 - 29°C even 30 - 31°C, it is still in the normal temperature for tropical waters. According to Sunardi (2017), changing in water temperature may affect the freshwater condition as it links directly to water cycle, such as changing water column stratification and resources availability, mainly nutrients, light or intensified grazing by heterotrophs.

The pH of Jawi River was in the range of 6,8 - 8 and classified in class I, II and III quality standards. At the upstream and middle point, the pH value was lower than the downstream point because in the upstream area, there are still some people who use the water of Jawi River for the purpose of washing clothes, tableware and even bathing, so that the waste water from these activities went directly into the water flow. According to Romanto (2013), the remainder of these activity are allegedly carrying organic material to be decomposed by water microbes.Wardhana (2014) stated that pH values are also influenced by waste discharges that convert the concentration of hydrogen ions in water to acid or base due to the chemical content contained therein. Atobatele et al. (2008) suggested that the decrease in pH is also associated with increased rain intensity. Decrease in pH in the rainy season can reduce the quality of dissolved oxygen due to the entry of organic matter carried by the rain. Adeyemo et al. (2008) stated that the optimal pH for the survival of aquatic organisms is 6.5 – 8.2.

The content of dissolved oxygen depends on temperature, the presence of plants for photosynthesis, physiological activity and physiological processes of plankton, the degree of light penetration dependent on depth and turbidity of water, degree of water hardness and the amount of organic matter described in water (Astel et al., 2006; Vikal, 2009; Rosli et al., 2010). The water flow of the Jawi River gets runoff from the housing complex, market and shop. Son (2013) stated that generally the concentration of DO in a waters is temporary or seasonal and fluctuates.

The decrease in the BOD value in water was caused by the effective sedimentation and deoxygenation process of river water or waste materials which were affecting the river environment and the characteristics of the waste without prior treatment which directly discharged into water bodies (Fardiaz cit. Saputri, 2014). Differences in BOD values in each point were also caused by the influx of pollutants received by water bodies from surrounding areas such as residential areas, shops and open land (Trofisa, 2011). River with low BOD values had low nutrient levels and included as a part of DO concentrations. Unspoiled and uncontaminated waters have a BOD value of less than 5 mg/L (Agbaire & Oyibo, 2009). BOD value is usually higher in the rainy season than in the dry season (Ezekiel, 2001). In this study, the sample analysis for BOD parameters was performed at high and low tide, marked with higher BOD values at high tide due to inclusion of loads of contamination from the Kapuas River into the Jawi River. Most of the organic waste that can be broken down by microorganisms is in the water, but there are some organic components that are difficult to decompose such as lignin and cellulose. The component will cover the water area, degrade the water area and cause the decrease in dissolved oxygen concentration (Usman, 2015). Organic materials containing carbon, nitrite, phosphate, ammonia and some minerals are nutrients for the growth and breeding of pathogenic microorganisms (Sidharta, 2000). In addition, the presence of nutrients also affects the amount of algae production (Varunprasath & Nicholas, 2010). High consuming rate of nutrition make biomass and microorganisms primary productivity in the ecosystem were inversely to pH, nitrate concentration, temperature and other hydrobiological parameters (Setiabudi, 2016).

The value of the density of the Coliform bacteria of the Jawi River water varied at each point and affected the water grade standard as shown in Table 1 and Table 2. After a complementary test using EMB media, Jawi River water positively contains *E.coli* bacteria at each sampling point during high tide and low tide. Some people who live close to the Jawi River still use the water for bathing and washing purposes, either used directly or aspirated using a pump to the people's home. The existences of these activities also affect the water quality of the Jawi River and have the potential to have an impact on public health. The research result Cahyaning et al. (2009) shows the link between river water utilization for bathing and washing kitchen utensils with increasing cases of diarrhea and skin diseases.

The temperature and DO parameters are related to the total of Coliform bacteria with the direction of inverse relationship, where, if the Coliform bacterial density value continues to increase at each point, the temperature and DO values will decrease, so the curves shown in Figures 2 and Figures 4 are mutually inversely proportional. This also occurs in the resulting correlation

Table 1. Results of Jawi River Water Quality Analysis

Location	Result									
	High Tide					Low Tide				
	Temperature (°C)	pH	DO (mg/L)	BOD (mg/L)	Coliform (MPN/100 ml)	Temperature (°C)	pH	DO (mg/L)	BOD (mg/L)	Coliform (MPN/100 ml)
Parit Haruna (upstream)	28	7	5.67	15.17	150	30	6.8	6.78	13.64	930
Jl. Suwignyo (midstream)	29	7.8	5.51	13.05	430	31	6.9	7.96	9.49	2400
Gertak I Sungai Jawi (downstream)	28	8	1.14	27.96	1500	28	8	3.98	16.52	11000

Table 2. Quality Standard of Jawi River Water Class

Location	Water Quality Standard of Government Regulation No. 82 Year of 2001								Decree of The Directorate General of PPM and PLP	
	High Tide				Low Tide				High Tide	Low Tide
	Temperature	pH	DO	BOD	Temperature	pH	DO	BOD	Coliform	
Parit Haruna (upstream)	I - III	I - III	II	IV	I - III	I - III	I	IV	C	C
Jl. Suwignyo (midstream)	I - III	I - III	II	IV	I - III	I - III	I	III	C	D
Gertak I Sungai Jawi (downstream)	I - III	I - III	III	IV	I - III	I - III	III	IV	D	E

values of -0.32 during high tide and -0.892 at low tide for temperature parameters, as well as -0.986 at high tide and -0.91 at low tide for DO parameters. Eisakhani & Malakahmad (2009) stated that the number of Coliform bacteria would be lower when the temperature level, and DO are higher. This is due to the pollutant source of Coliform which is related to the activity of human secretion. The same is consistent with the research of Bensig et al. (2012) and Singh et al. (2014) which states that the DO parameter is negatively correlated to the total Coliform.

The pH and BOD parameters are related to the total of Coliform bacteria with the direction of proportional relationship, if the Coliform density value continues to increase at each point, the pH and BOD values also rise, so that the curves shown in Figures 3 and Figure 5 are directly proportional. This also occurs on the resulting correlation value of 0.7904 at high tide and 0.9982 at low tide for pH parameters, as well as 0.9462 at high tide and 0.7227 at low tide for BOD parameters. This is consistent with research by Bensig et al. (2012) which also concluded that the BOD parameter correlated positively with total Coliform bacteria. Singh et al. (2014) also concluded that the pH parameter was positively correlated with total Coliform. The pH, BOD or total coliform values continue to increase from upstream to downstream at high and low tide. The organic exhaust that enters the waters affects the value of BOD and Coliform bacteria that degrade the organic material. The existence of degradation activity causes the pH content to change into base or acid due to changes in hydrogen ion concentration value.

The existence of coliform bacteria in surface water comes from the point source and diffuse source which has been concentrated in a long time. Point source pollution includes urban waste and contaminated tributaries. While diffuse source pollution includes pollution from agriculture and rainwater flows. The burden of water-borne

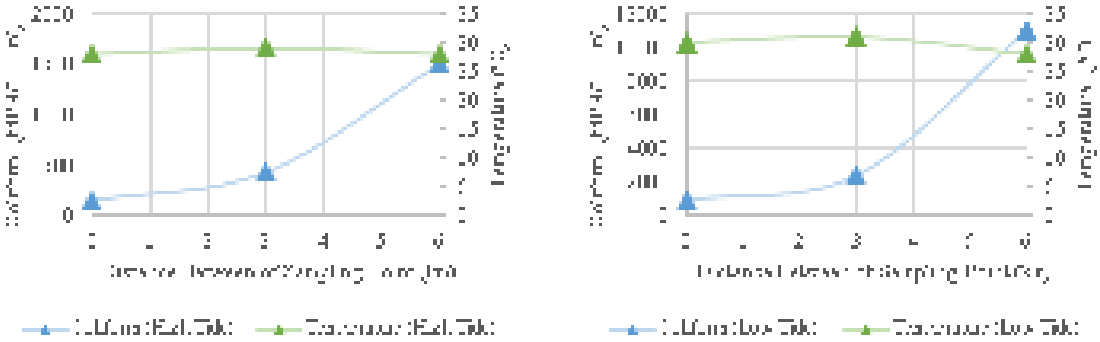

Figure 2. Relation of Coliform and Temperature Parametes at High and Low Tide

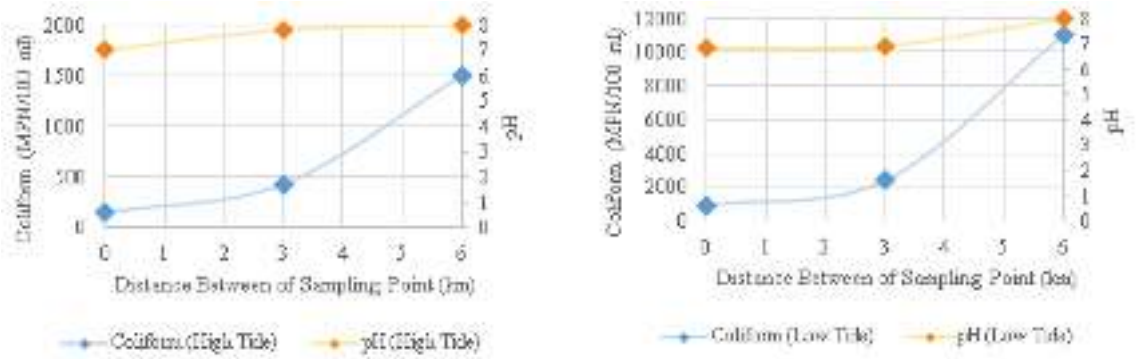

Figure 3. Relation of coliform and pH parameters at high and low tide

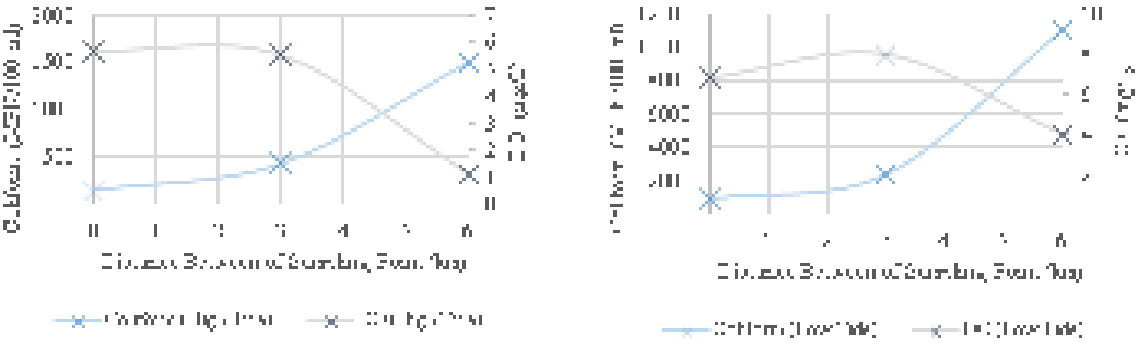

Figure 4. Relation of Coliform and DO Parameters at High and Low Tide

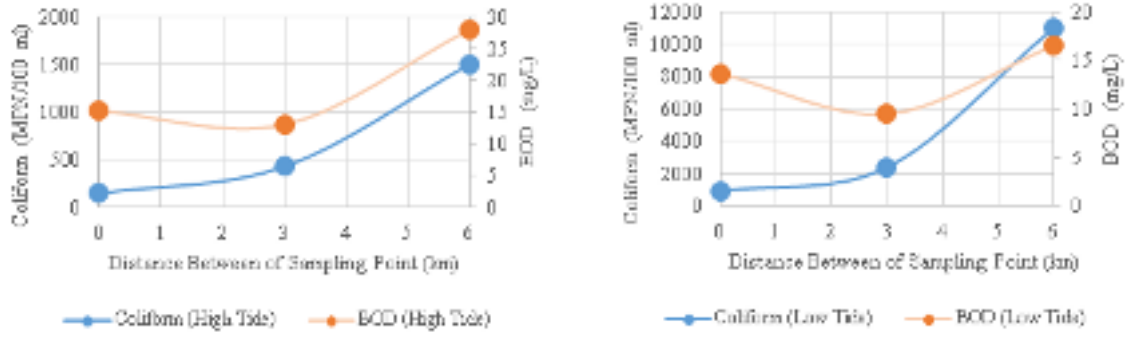

Figure 5. Relation of Coliform and BOD Parameters at High and Low Tide

bacteria around the catchment area is a natural factor due to the effects of weather (rain, sunlight, temperature), hydrology and topography (Kinzelman et al., 2004; Mills & Thurman, 1994). Rainwater flows also carry bacterial contamination from peripheral environments such as animal waste and pavement (Karlaviciene et al., 2009; Sidhu et al., 2013).

Rivers play an important role as the assimilation of urban and industrial waste discharges from rainwater flow (Sigua & Tweedale, 2003). Improper water quality can be hazardous to health, so efforts should be made to improve the management of water quality in water catchment areas (Astrom et al., 2007; Won et al., 2013). The existence of physical, chemical and microbiological parameters in waters are interconnected, as described in the preceding paragraph. Usman (2015) stated that the parameters of temperature, pH, dissolved oxygen and BOD are included as biotic factors that affect the existence of coliform bacteria. The result of the analysis shows that there was correlation between the biotic factors on the growth of coliform bacteria. Temperature and pH parameters act as indicators of certain bacterial species, such as coliform bacteria are tolerant to mesophyll temperature range (25 - 37^{o}C) and neutrophil pH (6.7 – 7.5) as shown in the results of this study.

Astrom et al. (2002) stated that Coliform bacteria are not too dependent on dissolved oxygen demand, so it is characterized as anaerobic facultative that plays a role in the process of respiration and electron acceptor. In addition to oxygen, bacteria also require nutrients for metabolic processes obtained from organic matter in the waters. Decomposition analysis of organic matter in waters by bacteria can be seen from the result of BOD parameter measurement. The higher the organic material in water causes the dissolved oxygen content to be smaller because it is used by bacteria to oxidize organic matter. The principle of restructuring the organic materials in the water by means of aeration is used to increase the dissolved oxygen, so that the bacteria can perform the decomposition of organic matter in the water treatment process.

The surface water which will be used should be free from contaminants either physically, chemically or microbiologically. Optimization of processing and improvement of water quality related to the types of parameters aimed to be minimized (Han et al., 2012 & Sedmak et al., 2005). Control and elimination of sources of bacteria contaminating water sources can be done in several ways (Okoh et al., 2007), among which is an integrated liquid waste treatment system, which is responsible for collecting waste from residential, commercial and industrial pollutants (Ritter et al., 2002), The abundance of water in urban areas from both drainage and rainfall runoffs connected to the area of the waste treatment system (Even et al., 2007), and the use of septic tanks to collect and process waste from settlements (Cheung & Venkitachalem, 2004). In addition, the surface water management of the technical aspect comprises boil processing method by heating the water temperature up to 100^{o}C for 1 minute. Any area with an altitude above 1000 feet above sea level should add water heating time to effectively kill bacteria. Chlorination treatment uses chemicals for the disinfection process. In this method, Cl_2 or NaOCl is added to water and produces hypochlorous acid (Richardson, 2002). Filtration treatment uses shards of ceramics, sand, gravel or zeolite (Makutsa, 2001). The bacteria will be separated from the water from the top filter compartment to the lowest compartment and water will go to the shelter (Iijima, 2001). The benefit of this study is to give information about water quality of Jawi River and its correlation with density of coliform bacterial, so that people are expected to pay more attention to the use of clean water to avoid the disease caused by Coliform.

CONCLUSION

The density of Coliform bacteria in the Jawi River at high tide continued to increase from upstream to downstream, ranging from 150-1500 MPN/100 ml at high tide and 930-11000 MPN/100 ml at low tide. If the water is associated with the classification of water class according to Decree of Directorate General of PPM and PLP No. 1/PO.03.04.PA.91 the water quality of Jawi River is classified C and D class at high tide and class C, D and E at low tide. Based on the correlation analysis, the pH and BOD parameters are related to the direction of directly proportional, the temperature and DO parameters are also related to the direction of inversely proportional. So, it can be concluded that there is a correlation between physical parameters, such as temperature and chemical parameters such as pH, DO and BOD to microbiological parameters especially Coliform bacterial density.

REFERENCE

Abida, B., Harikrishna, S., Irfanulla, K., Ramaiah, M., Veena, K., & Vinutha, K. (2008). Analysis of Flouride Level in Wa-

ter and Fish Species of Sankey, Bellandur and Madivala Lakes of Bangalore, Rasayan. *Journal of Chemistry*, 1(3), 595-601.

Adeyemo, O.K., Adedokum, O.A., Yusuf, R.K., & Adeleye, E.A. (2008). Seasonal Changes in Physico-Chemical Parameter and Nutrient Load of River Sediments in Ibadan City, Nigeria. *Global NEST Journal*, 10(3), 326-336.

Agarwal., Animesh and Manish., & Saxena. (2011). Assesment of Pollution by Physicochmecial Water Parameters Using Regression Analysis: A Case Study of Gagan River at Moradabad-India. *Applied Science Research*, 2(2), 185-189.

Agbaire, P.O., & Oyibo, I.P. (2009). Seasonal Variation of Some Physico-Chemical Properties of Borehole Water in Abraka, Nigeria. *African Journal of Pure and Applied Chemistry*, 3(6), 116-118.

Ahinpathi, M.V., & Puttaiah, E.T. (2006). Ecological of Vrishabhavathi River in Bangalore (India). *Environmental Geology*, 49, 1217-1222.

Anhwange, B.A., E.B. Agbaji., & E.C. Gimba. (2012). Impact Assesment of Human Activities and Seasonal Variation on River Benue, Within Makurdi Metropolis. *International Journal of Science and Technology*, 2(5), 248-254.

APHA. (2005). *Standard Methodes for The Examination of Water and Wastewater. 20th Ed*. American Public Health, Association, American Water Works Association Water Environment Federation.

Astel, A., Biziuk, M., Przyjazny, A., & Maiesnik, J. (2006). Chemometrics in Monitoring Spatial and Temporal Variations in Drinking Water Quality. *Water Research*, 40(8), 1706-1716.

Astrom, K., Petterson, S., Bergstedt, O., Petterson, T.J., & Stenstrom, T.A. (2007). Evaluation of The Microbial Risk Reduction Due to Selective Closure of Raw Water Intake Before Drinking Water Treatment. *Journal of Water and Health*, 5, 81-97.

Atobatele, Oluwatosin E., Ugwumbe., & Alex, O. (2008). Seasonal Variation in The Physicochemistry of A Small Tropical Reservoir (Aiba Reservoir, Iwo, Osum, Nigeria). *African Journal of Biotechnology*, 7(12), 1962-1972.

Badiamurti, G.R. (2010). *Korelasi Kualitas Air dan Insidensi Penyakit Diare Berdasarkan Keberadaan Bakteri Coliform di Sungai Cikapundung*. Jurnal Program Studi Teknik Lingkungan. Institut Teknologi Bandung.

Bensig, E.O., Mary, J.L.F., & Fleurdeliz, F.M. (2012). Relationship of Coliform and Physicochemical Factors of Buhisan, Bulacao and Lahug Riers, Cebu, Philippines. *WALIA Journal*, 32(S1), 28-30.

Berutu, P. (2001).*Kajian Parameter Fisika, Kimia dan Biologi dalam Kaitannya dengan Kebersihan Ikan di Kawasan Perairan Danau Toba di Sumatra Utara*. Tesis Program Studi Ilmu Lingkungan. Universitas Gadjah Mada.

Cabral, J.P. (2010). Water Microbiology Bacterial Pathogens and Water. *Int. J. Environ. Res. Pub. Helath*, 7(10), 3657-3703.

Cahyaning, N., Aras, M., & Thamrin. (2009). Pengaruh Pemanfaatan Air Sungai Siak Terhadap Penyakit Diare dan Penyakit Kulit pada Masyarakat Pinggiran Sungai Siak (Kasus Kecamatan Rumbai Pesisir Pekanbaru). *Journal of Environmental Science*, 3(1), 46-57.

Cheung, K.C., & Venkitachalam, T.H. (2004). Assesment of Contaminant by Percolation of Septic Tank Effluent Through Natural and Amended Soils. *Environmental Geochemistry and Health*, 26 (2-3), 157-168.

Eisakhani, M., & Malakahmad, A. (2009). Water Quality Asessment of Bertam River and Its Tributaries in Cameron Highlands, Malaysia. *World Applied Sciences Journal*, 7, 769-776.

Even, S., Mouchel, J.M., Servais, P., Flipo, N., Puolin, M., Blanc, S., Chabanel, M., & Paffoni, C. (2007). Modelling The Impacts of Combined Sewer Overflows on The River Seine Water Quality. *Science of The Total Environment*, 375(1-3), 140-151.

Ezekiel, E.N., Hart., & Abowei, J.F.N. (2011). The Physical and Chemical Condition of Sombreiro River, Niger Delta, Nigeria. *Research Journal of Environmental and Earth Sciences*, 3(4), 327-340.

Farkas, A., Dragan-Bularda, M., Muntean, V., Ciataras, D., & Tigan, S. (2013). Microbial Activity in Drinking Water Associated Biofilms. *Central European Journal of Biology*, 8(2), 201-214.

Han, M., Zhao, Z.W., Cui, F.Y., Gao, W., Lui, J., & Zeng, Z.Q. (2012). Pretreatment of Contaminated Raw Water by A Novel Double-Layer Biological Aerated Filter for Drinking Water Treatment. *Desalination and Water Treatment*, 37(1-3), 308-314.

Iijima, Y., Karama, M., Oundo, J.O., & Honda, T. (2001). Prevention of Bacterial Diarrhea by Pasteurization of Drinking Water

in Kenya. *Journal of Microbial Immunology*, 45(6), 413-416.

Karlaviciene, V., Svediene, S., Marciulionene, D.E., Randerson, P., Rimerika, M., & Hoglan, W. (2009). The Impact of Storm Water Runoff on A Small Urban Stream. *Journal of Soils and Sediment*, 9(1), 6-12.

Khotimah, S. (2009). Kepadatan Bakteri *Coliform* di Sungai Kapuas Kota Pontianak. *ProsidingSemirata FMIPA Universitas Lampung 2013*. 339-349.

Kinzelman, J., McLellan, S.L., Daniels, A.D., Cashin, S., Singh, A., Gradus, S., & Bagley, R. (2004). Non-Point Source Pollution: Determination of Replication Versus Persistence of Escherichia coli in Surface Water and Sediments With Correlation of Levels to Readily Measureable Environmental Parameters. *Journal of Water and Health*, 2(2), 103-114.

Kumar, Rita, N., Rajal S., & Nirmal, K.J.I. (2011). An Assesment of Seasonal Variation and Water Quality Index of Sabarmati River and Kharicut Canal at Ahmedabad, Gujarat. *Electronic Journal of Environmental and Food Chemistry*, 10(8), 2771-2782.

Makutsa, O., Nzaku, K., Ogutu, P., Barasa, P., Ombeki, S., Mwaki, A., & Wuick, R.E. (2001). Challenges in Implementing a Point-of-Use Water Quality Intervention in Rural Kenya. *American Journal of Public Health*, 91, 10.

Mills, M.S., & Thurman, E.M.(1994). Reduction of Nonpoint-source Contamination of Surface Water and Groundwater by Starch Encapsulation of Herbicides. *Environmental Science and Technology*, 28(1), 73-79.

Okoh, A.L., Odjadjaran, E.E., Igbinosa, E.O., & Osedo, A.N.(2007). Wastewater Treatment Plants as A Source of Microbial Pathogens in Receiving Watershed. *African Journal of Biotechnology*, 6(25), 2932-2944.

Patil, P.N, Sawant, D.V., & Deshmukh, R.N. (2012). Physico-Chemical Parameters for Testing of Water – A Review. *International Journal of Environmental Sciences,* 3(3), 1194-1207.

Priyambada, I.B., Oktiawan, W., & Suprapto, R.P.E. (2008). Analisa Pengaruh Perbedaan Fungsi Tata Guna Lahan Terhadap Beban Cemaran BOD Sungai (Studi Kasus Sungai Serayu Jawa Tengah). *Jurnal Presipitasi*, 5(2), 55-62.

Pujiastuti, P., Bagus,I., & Pranoto. (2013). Kualitas dan Beban Pencemaran Perairan Waduk Gajah Mungkur. *Jurnal EKOSAINS*, 5(1), 59-75

Rahman, S., Anoar, K.P., & Rezwanul, I. (2013). Identification and Quantification of Escherichia coli From Drinking Water in Bangladesh. *International Journal of Microbiology and Immunology Research*, 1(4), 47-51.

Rajendra, S., Dasgupta, R., & Malik, A. (2012). Microbiological Quality of Potable Water in Dehradun City. *International Research Journal of Pharmacy*, 3(6), 130-137.

Ramadhani, N.R. (2012). *Analisis Sebaran Oksigen Terlarut Saluran Sungai Jawi*. Skripsi Program Studi Teknik Lingkungan. UniversitasTanjungpura.

Richardson, S.D. (2002). The Role of GC-MS and LC-MS in The Discovery of Drinking Water Desinfection by Products. *Journal of Environmental Monitoring*, 4(1), 1-9.

Ritter, L., Solomon, K., Sibley, P., Hall, K., Keen, P., Mattu, G., & Linton, B.(2002). Sources, Pathways, and Relative Risk of Contaminants in Surface Water and Groundwater: A Perspective Prepared for The Walkerton Inquiry. *Journal of Toxicology and Environmental Health-Part a Current Issues*, 65(1), 1-142.

Romanto (2013). *Status Kualitas Air Sungai Ciambulawung, Banten*. Skripsi Fakultas Perikanan dan Ilmu Kelautan. Institut Pertanian Bogor.

Rosli, N., Gandaseca, S., Ismail, J., & Jailan, M.I.(2010). Comparative Study of Water Quality at Different Peat Swamp Forest of Batang Igan, Sibu Sarawak. *American Journal of Environmental Sciences*, 6(5), 416-421.

Saputri, A. (2014). *Analisis Sebaran Oksigen Terlarut Pada Sungai Raya*. Skripsi Program Studi Teknik Lingkungan. UniversitasTanjungpura.

Sedmak, G., Bina, D., McDonald, J., & Couillard, L. (2005). Nine-Year Study of The Occurence of Culturable Viruses in Source Water for Two Drinking Water Treatment Plants and The Influent and Effluent of A Wastewater Treatment Plant in Milwauke, Wisconsin (August 1994 through July 2003). *Applied and Environmental Microbiology*, 71(2), 1042-1050.

Setiabudi, G.I., Dietriech, G.B., Hefni, E., & Ocky, K.R. (2016). The Community Structure of Phytoplankton in Seagrass Ecosystem and Its Relationship with Environmental Characteristic. *Biosaintifika: Journal of Biology & Biology Education*, 8(3), 257-269.

Sidharta, R.B. (2000). *Pengantar Mikrobiologi Ke-*

lautan. Universitas Atma Jaya: Yogyakarta.

Sidhu, J.P.S., Ajmed, W., Gernjak, W., Aryal, R., McCarthy, D., Palmer, A., Kolotelo, P., & Toze, S. (2013). Sewage Pollution in Urban Stormwater Runoff as Evident From The Widespread Presence of Multiple Microbial and Chemical Source Tracking Markers. *Science of Total Environment*, 463, 488-496.

Sigua, G.C., & Tweedale, W.A.(2003). Watershed Scale Assesment of Nitrogen and Phosphorus Loadings in The Indian River Lagoon Basin, Florida. *Journal of Environmental Management*, 67(4), 363-372.

Singh, M., & Singh, S.K. (2014). Correlative Study of Physico-Chemical and Microbiological Parameters of Radha and Shyam Kund-Govardhan, Mathura. *The Experiment, International Journal of Science and Technology*, 25(3), 1726-1735.

SNI 01-2332.1-2006 tentang *Penentuan Coliform dan Escherichia coli pada Produk Perikanan*.

Sugiyono. (2016). *Statistika untuk Penelitian*. Bandung: Penerbit Alfabeta.

Sunardi., Rina, F., Budi, I., & Mutia, S.S. (2017). The Dynamic of Phytoplankton Community Structure in Face of Warming Climate in A Tropical Man-Made Lake. *Biosaintifika: Journal of Biology & Biology Education*, 9(1), 140-147.

Trofisa, D. (2011). *Kajian Beban Pencemaran dan Daya Tampung Pencemaran Sungai Ciliwung di Segmen Kota Bogor*. Skripsi Fakultas Kehutanan. Institut Pertanian Bogor.

Usman, W.S. (2015). *Bakteri Asosiasi Karang yang Terinfeksi Penyakit Brow Band (BRB) di Perairan Pulau Barranglompo Kota Makassar*. Skripsi Fakultas Ilmu Kelautan dan Perikanan.

Varale, A., & Yashodhara, V. (2012). Determination of BOD in The Underground Water. *Journal of Chemical and Pharmaceutical Research*, 4(10), 4601-4603.

Varunprasath, K., & Nicholas, A.D. (2010). Comparison Studies of Three Freshwater Rivers (Cauvery, Bhavani and Noyyal) in Tamilnadu, India. *Iranica Journal of Energy & Environment*, (14), 315-320.

Vikal, P. (2009). Multivariant Analysis of Drinking Water Quality Parameters of Lake Pichhola in Udaipur, India. *Biological Forum – An International Journal*, 1(2), 97-102.

Wardhana, W.A. (2014). *Dampak Pencemaran Lingkungan*. Yogyakarta: Penerbit Andi.

Won, G., Kline, T.R., & LeJeune, J.T. (2013). Spatial-Temporal Variations of Microbial Water Quality in Surface Reservoirs and Canals Used for Irrigation. *Agriculture Water Management*, 116, 73-38.

World Health Organization. (1996). *Water Quality Monitoring – A Practical Guide to The Design and Implementation of Freshwater Quality Studies and Monitoring Programmes*. Edited by Jamie Bartram and Rizhard Balance. Geneva.

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