JTBB
Journal of Tropical Biodiversity and Biotechnology

Journal of Tropical Biodiversity and Biotechnology

online http://jtbb.or.id

ISSN 2540-9581 (online)

Volume 5, Issue 2, August 2020

Published by:

Faculty of Biology
Universitas Gadjah Mada

In collaboration with:

KOBI

KONSORSIUM BIOTEKNOLOGI INDONESIA
Indonesian Biotechnology Consortium

J.Trop. Biodiv.Biotech.	Volume 5	Issue 2	Page 78 - 166	Yogyakarta August 2020	ISSN 2540-9581

Table of Contents

Research Articles

Journal of Tropical Biodiversity and Biotechnology
Volume 05, Issue 02 (2020): 78 — 88
DOI: 10.22146/jtbb.53795

Research Article

Diversity of Epiphytic Orchids, Hoya, Dischidia and Phorophytes (Host Trees) in Bawean Island Nature Reserve and Wildlife Reserve, East Java, Indonesia

Trimanto[1]*, Setyawan Agung Danarto[1]
1) Purwodadi Botanic Garden, Research Center for Plant Conservation and Botanic Garden, Indonesian Institute of Sciences (LIPI)

Submitted: 27 January 2020; Accepted: 28 March 2020; Published: 15 August 2020

ABSTRACT
Bawean Island is a small island located between two islands (Java and Borneo). Geographically, the diversity of plants, especially epiphytic plants on this island is very interesting to be studied. This research aims to investigate the diversity of epiphytic plants, focussing on epiphytic orchids, Hoya and Dischidia in Bawean Island Nature Reserve and Wildlife Reserve. It was conducted through an inventory of epiphytic orchids and hoya growing on host trees. The results showed there were 10 species of epiphytic orchid and 3 species of epiphytic Hoya, and 1 species of Dischidia growing on-location studies. The epiphytic orchids which found in location studies included *Phalaenopsis amabilis, Aerides odorata, Cymbidium aloifolium, Dendrobium anosmum, Rhynchostylis retusa, Liparis condylobulbon, Taeniophyllum biocellatum, Cymbidium* sp., *Eria* sp. Orchid species that most often found in the study location was *Phalaenopsis amabilis.* In addition, this study recorded *Taeniophyllum biocellatum* as an endemic orchid from Java that was found on this island. The epiphytic Hoya recorded in there, i.e. *Hoya diversifolia, H. verticillata,* and *H. amoena*, and also only found 1 species of Dischidia was *Dischidia imbricata.* There were 12 species of trees as the host trees of epiphytic, i.e. *Irvingia malayana, Tectona grandis, Diospyros buxyfolius* were the host trees frequently found as the host of the epiphytic plant. Zone 3 as an area of 1/3 basal part of a total length of the branches was the most preferred zone by epiphytic orchids and hoya. The epiphytic orchid and hoya hardly found in Zone 5.

Keywords: Bawean, dischidia, epiphyte, hoya, orchid

INTRODUCTION
Bawean island is one of the small islands located between Borneo and Java islands which leads to the unique biogeography of the island. The diversity of flora and fauna in the Bawean Island consists of Wildlife Reserve with an area of 3,836.6 Ha and Nature Reserve with an area of 725 Ha (Ministry of Agriculture of Indonesia, 1979). The topography of Bawean Island Wildlife Reserve and Nature Reserve is hilly, mountainous and bumpy. The altitude of the region ranges from 200-687 m above sea level. Based on the observation of the altitude, the highest altitude of Gunung Lumut Forest was 687 m above sea level.

The discovery of endemic species such as Bawean deer *Axis kuhlii* (Semiadi *et al.*, 2015), new species of butterfly *Atrophaneuracoon* sub. sp. *sangkapurae* (Maurizio & Salla, 1992), some endemic birds of *Falconiformes* and *Strigiformes* (Nijman, 2004), Javan warty pig *Sus verrucosus* (Blouch, 1995), Bawean warty pig (*Sus blouchi*) (Rademaker *et al.*, 2016), indicated that Bawean Island, which is rich with bioresources, can be an interesting subject to be studied. Some inventory studies and assessments on flora biodiversity in Bawean Island have been conducted sporadically in a few past years. Most important and common trees species found in some montane forests are *Irvingia malayana, Ficus variegata, and Myristica guatteriaefolia* (Danarto and Rahadiantoro, 2015; Trimanto, 2014; Trimanto and Hapsari, 2016).

There is a paucity of data and information on the diversity of epiphytes, especially epiphytic orchids and Hoya in Bawean Island Nature Reserve. Epiphytic plants are plants that grow on the other plants (host trees) but are not parasitic, they can

***Corresponding author**
Email: triman.bios08@gmail.com

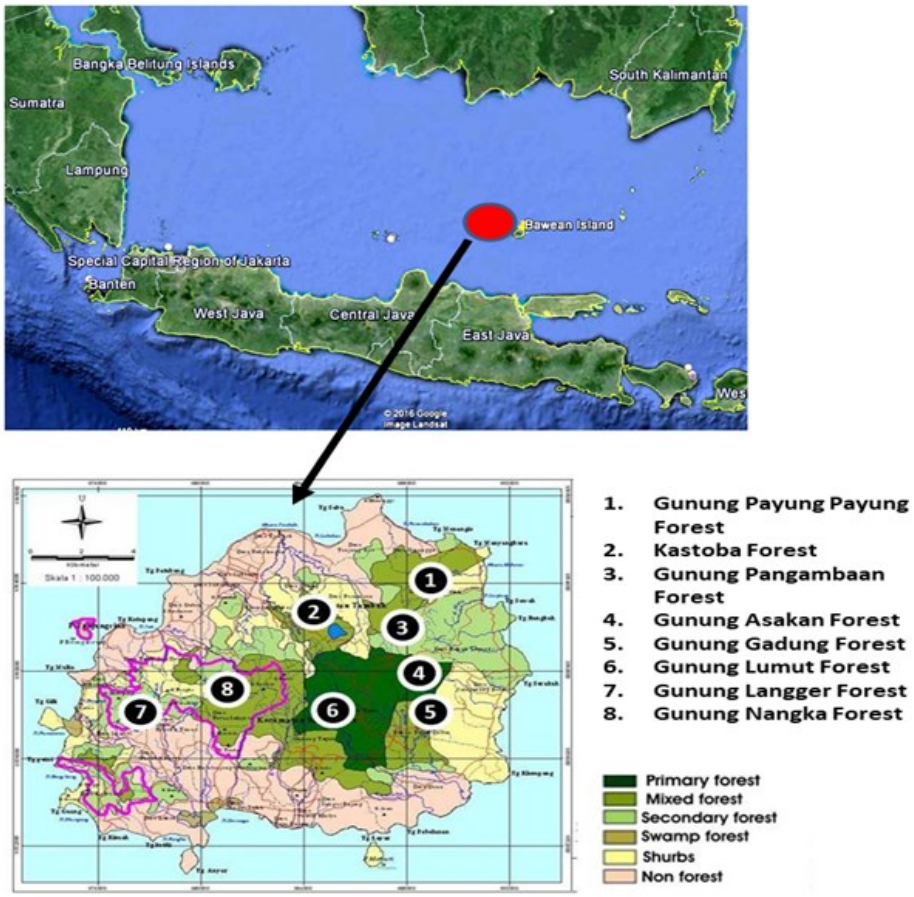


Figure 1. Location of epiphytic plants in Bawean Island, East Java. (Source: Bawean Island Natural Resource Conservation Center and Google Earth 2003).

photosynthesize to provide nutrients for themselves. Ecologically, epiphytes have important roles as they can be used as a bioindicator for the condition of abiotic factors such as humidity, temperature and light intensity in an area. The diversity of epiphytic plants is greatly influenced by microclimates and tree stands (Setyawan, 2000)

This study aims to investigate the epiphytic orchids, Hoya and Dischidia in Bawean Island Nature Reserve and Wildlife Reserve while also can contribute to the scientific documentation and data of the diversity of plants in Bawean Island. This information can be developed into a guide and basis for recommendations on plant conservation policies at the local level as well as modeling for plant conservation strategies on small islands.

METHODS

Location and Materials

The study was conducted in 2014 and located in Bawean Island Nature Reserve and Wildlife Reserve (Figure 1). This research used a purposive sampling method covering eight Gunung forest, they are Gunung Langger forest, Gunung Gadung forest, Gunung Asakan forest, Gunung Nangka forest, Gunung Payung-payung forest, Gunung Pangambaan forest, Kastoba forest, and Gunung Lumut forest with 20 plot for each Gunung forest (each plot's size is 10m x 10m) were used in this study. Materials used for this study i.e. tally sheets, alcohol 70%, labels, and herbarium sheet while the tools used in this study i.e. GPS, binoculars, and magnifying glasses.

Data Collection

The method used in this orchid inventory is exploratory. Observations on the epiphytic orchids, Hoya and Dischidia growing on the host trees were conducted. The vertical distribution of the epiphytic orchids and hoya was recorded based on Johansson's methods (1974), which divide host trees into 5 zones (Figure 2):

- Zone 1: Area covering the base of the tree (1/3 of the main trunk)
- Zone 2: Area from the main trunk of the tree to the first branch (2/3 of the main trunk)
- Zone 3: The area covering the basal part of the branching (1/3 part of the total length of the branch)
- Zone 4: The area covering the middle part of the branch (1/3 of the following middle part)
- Zone 5: Outermost area of branching (1/3 of the

outermost branching)

The data recorded are the species name of epiphytic plant, the species name of host tree, the number of epiphytic species on host tree, and the number of host tree. For each orchid, Hoya and Dischidia that unidentified in location study needs to be made its herbarium and then the identification process was carried out in Herbarium of Purwodadiensis. An environmental condition such as altitude measured around the habitat of epiphytic plants found at the study site and then recorded on tally sheets.

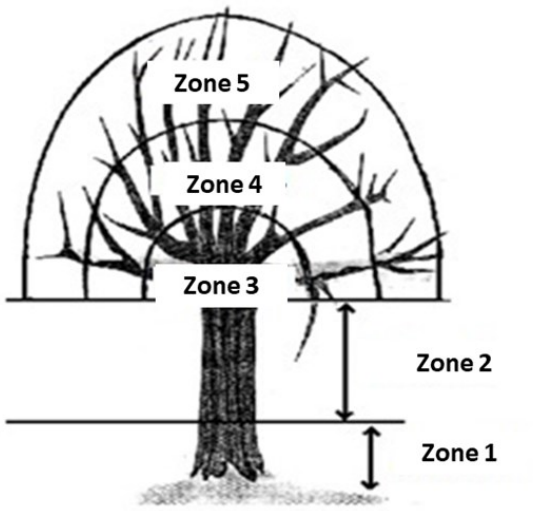


Figure 2. Illustration of host zoning of epiphytic plants by Johansson, 1974. (Source Figure: Aulia and Hakim, 2019).

Data Analysis

The research measured and analyzed some parameters with a relative abundance of epiphytic (% Fo), frequency of phorophyte (% Ft), the average number of individuals of orchids of each phorophyte species (Ji/Jt), the average number of epiphytic orchid species on a phorophyte species (Js/Jt), and the vertical distribution of the orchids on the phorophytes (Nurfadilah, 2016; Yulia & Budiharta, 2011; Yulia & Budiharta, 2012).

1) Relative Frequency of Host trees (%Ft)

$$\% \text{ Ft} = \frac{\text{Nt}}{\text{Total number of all phorophytes}} x\ 100\%$$

where: Nt = the number of trees in the plot hosting a particular epiphytic species.

2) Abundance of epiphytic plants (% Fo)

$$\% \text{ Fo} = \frac{\text{No}}{\text{Total number of all epiphytic species}} x\ 100\%$$

where: No = the number of individuals of a particular ephypitic species.

3) Average number of individual epiphytic on each host plant (phorophytes)

$$= \frac{\text{Ji}}{\text{Jt}}$$

where: Ji = the number of epiphytic individuals.
Jt = the number of individuals of each phorophyte species.

4) The average number of epiphytic species on a phorophyte species

$$= \frac{\text{Js}}{\text{Jt}}$$

where: Ji = the number of epiphytic species.
Jt = the number of individuals of each phorophyte species.

Vertical distribution of orchids (zoning) on the host tree was calculated from the number of species in zone, orchid species on the phorophyte, from the trunk to outer branches in five zones (zone 1, zone 2, zone 3, zone 4, and zone 5) and by calculating the average number of individuals of epiphytic orchids in each zone. Percentage of epiphytic on every zone was calculated from number present of epiphyte species in each zone/total of present in all zone.

RESULTS AND DISCUSSION

There were 10 species of epiphytic orchid, 3 species of Hoya and 1 species of Dischidia (Table 1). The results of the present study showed that the number of phorophyte species hosting each epiphytic varied from 1 to 3 species of phorophyte (host trees). Epiphytic plants occurred on a single phorophyte and multiple phorophytes. Some epiphytic plants occurred on a single phorophyte such as *Aerides odorata*, *Dendrobium anosmum, Liparis condylobulbon, Pholidota imbricata, Taeniophyllum biocellatum, Hoya diversifolia, Eria* sp. Other Epiphytic plants occurred on multiple phorophytes such as *Phalaenopsis amabilis, Cymbidium aloifolium, Rhynchostylis retusa, Hoya verticillata* and *Dischidia imbricata.* The results of the present study were similar to other studies which showed that the number of phorophyte species hosting epiphytic orchids varied from a single to multiple phorophyte species (Nurfadilah, 2016; Adhikari *et al.,* 2012). *P. amabilis* is an epiphytic orchid with the highest phorophyte species. Some species of epiphytic have not specific phorophyte. Umiyah *et al.* (2011) reported that epiphytic orchids in Sempu Island have not specific species of host trees but generally grow under canopy cover.

Table 1. The epiphytic orchids, Hoya, Dischidia and the phorophytes in the Bawean Island Nature Reserve and Wildlife Reserve.

No	Species	Number of Host Trees Species	Host Trees	Zone	Location	Altitude
1	*Phalaenopsis amabilis*	3	*Euonimus javanicus*	Zone 3	Gunung Langgar Forest	167 m asl
			Tectona grandis	Zone 1,2	Gunung Langgar Forest	167 m asl
			Tectona grandis	Zone ,1, 2, 3 and 4	Gunung Gadung Forest	195 m asl
			Garcinia dioica	Zone 3 and 4	Gunung Gadung Forest	195 m asl
2	*Aerides odorata*	1	*Leea angulata*	Zone 4 and 5	Gunung Langgar Forest	167 m asl
3	*Cymbidium aloifolium*	2	*Antidesma petandrum*	Zone 2	Gunung Asakan Forest	178 m asl
			Schleichera oleosa	Zone 2	Gunung Nangka Forest	214 m asl
4	*Cymbidium* sp.	2	*Tectona grandis*	Zone 3	Gunung Gadung Forest	195 m asl
			Schleichera oleosa	Zone 1	Gunung Lumut Forest	423 m asl
			Schleichera oleosa	Zone 2	Gunung Lumut Forest	423 m asl
5	*Dendrobium anosmum*	1	*Tectona grandis*	Zone 4 and 5	Kastoba Forest	256 m asl
6	*Rhynchostylis retusa*	2	*Diospyros buxifolia*	Zone 2, 3	Gunung Nangka Forest	215 m asl
			Diospyros buxifolia	Zone 3	Gunung Nangka Forest	215 m asl
			Syzygium sp	Zone 1	Gadung Mount Forest	195 m asl
7	*Liparis condylobulbon*	1	*Irvingia malayana*	Zone 3 and 4	Gadung Mount Forest	195 m asl
8	*Pholidota imbricata*	1	*Irvingia malayana*	Zone 1,3,4	Pangambaan Mount Forest	266 m asl
9	*Taeniophyllum biocellatum*	1	*Canarium hirsutum*	Zone 3 and 4	Hutan Kastoba	285 m asl
10	*Eria* sp.	1	*Tectona grandis*	Zone 4	Pangambaan Mount Forest	266 m asl
11	*Hoya diversifolia*	1	*Ficus variegate*	Zone 1, 2 and 3	Payung-payung Mount Forest	197 m asl
12	*Hoya amoena*	1	*Ficus variegata*	Zone 1 and 2	Gunung Nangka Forest	207 m asl
13	*Hoya verticillata*	2	*Euria nitida*	Zone 3	Kastoba Forest	295 m asl
			Ficus variegata	Zone 4	Kastoba Forest	295 m asl
14	*Dischidia imbricata*	2	*Antidesma petandrum*	Zone 3	Gunung Nangka Forest	207 m asl
			Diospyros buxifolia	Zone 4	Gunung Nangka Forest	207 m asl
			Diospyros buxifolia	Zone 3	Gunung Nangka Forest	207 m asl

Epiphytic Orchids, Hoya and Dischidia in Bawean Island Nature Reserve and Wildlife Reserve

The most abundant epiphytic plant in Bawean Island Nature Reserve and Wildlife Reserve is *Phalaenopsis amabilis* that most often found in *Tectona grandis*. There were 3 species of host tree of *P. amabilis*, i.e. *Tectona grandis*, *Euonimus javanicus,* and *Garcinia dioica*. Besides that this species can grow in zone 1, 2, 3, 4 of host trees. The presence of *Phalaenopsis amabilis* is a species of epiphytic orchid species (Fig. 3a) indicates that the orchids are not only found on Borneo and Java islands. There is an abundance level of orchid species availability both in distribution and population on Bawean Island Nature Reserve. *P. amabilis* is a species of orchid having the most significant relative abundance (22.5%) compared to other epiphytic plants (Table 2). *Phalaenopsis amabilis* as known as *Anggrek Bulan* in local names as the most preferable orchid by the community due to the beauty of flowers. This genus has a wide distribution in tropical forests (Fandani *et al*., 2018), which is found in several areas including Sempu Island (Yulia, 2010), Batuputih Nature Park in Sulawesi with an altitude of 52-102 m above sea level (Yubu *et al*., 2018), but can also be found in the middle altitude such as Petungkriyono Forest in Pekalongan with an altitude of 700-900 m above sea level (Mardiyana *et al*., 2019).

Rhynchostylis retusa and *Dischidia imbricata* is the second abundant epiphytic plant in the study locations with relative diversity was 10%. *R. retusa* that can grow on zone 1,2,3, and well adapt in 2 species of host tree i.e. *Diospyros buxifolia* and *Syzygium* sp. This species can be found in several locations that indicated it has well adaptation in different host trees and habitat. *Dischidia imbricata* also can grow in 2 species of host trees (*Antidesma*

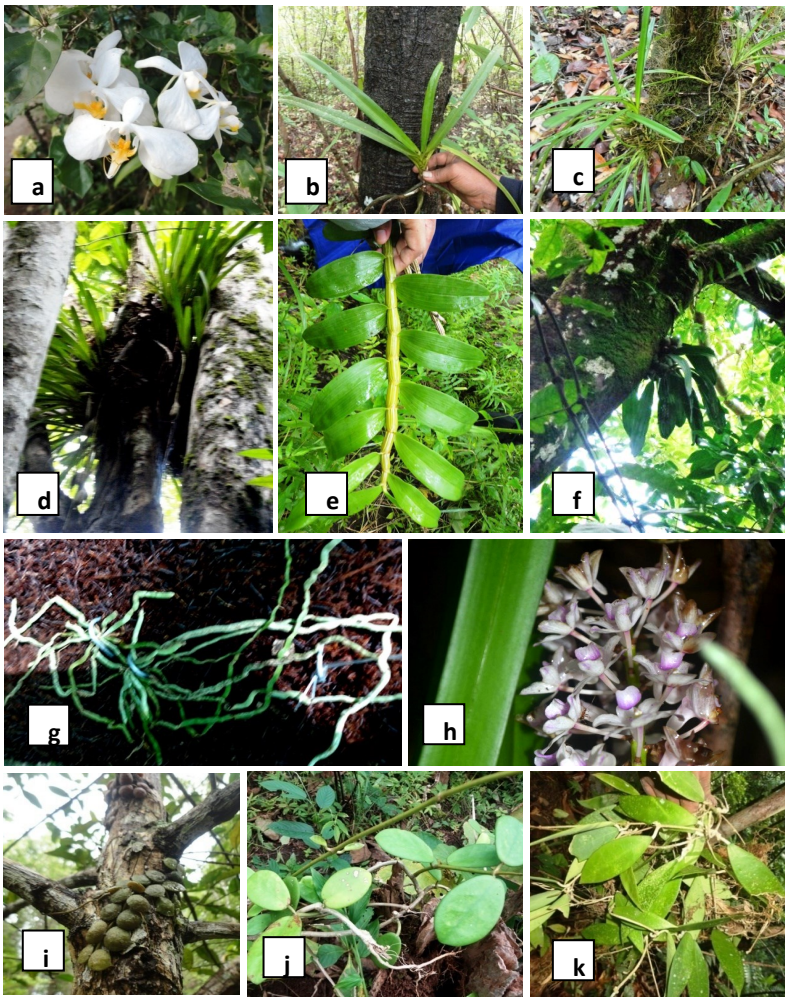


Figure 3. Diversity of Orchid, Hoya and Dischidia epiphyte. a) *Phalaenopsis amabilis*, b) *Aerides odorata*, c) *Cymbidium aloifolium*, d) *Cymbidium* sp., e) *Dendrobium anosmum*, f) *Pholidota imbricata,* g) *Taeniophyllum biocellatum,* h) *Rhynchostylis retusa, i) Dischidia imbricata*, j) *Hoya diversifolia*, k) *Hoya verticillata.*

petandrum and *Diospyros buxifolia*) on zone 3 and 4. *D. imbricata* is an adaptable plant that has a small leaf to reduce transpiration which supports its growth in low humidity and low water availability.

Some other species of epiphytic orchids found on Bawean Island were *Aerides odorata, Cymbidium aloifolium, Dendrobium anosmum, Pholidota imbricata,* and *Pholidota imbricata*. Compared with other studies of diversity epiphytic orchids on Java, the richness of epiphytic orchids on Bawean Island has almost the same number species of epiphytic orchids compared with other location on Java such as Sempu Island Nature Reserve which consists of 10 species, Gunung Lamongan forest area consisting of 7 species, Penanggungan forest area consisting of 9 species (Yulia, 2010), and Gunung Tukung Gede Nature Reserve consisting of 9 species (Sulistiarini and Djarwaningsih, 2017). Sadili (2019), reported that there were only 4 species which found on Sempu Island. However, the species of epiphytic orchids on Bawean Island have lower numbers than the number of orchid species found in the high altitude such as Semarang Gebugan Nature Reserve which consists of 11 species of orchids (Farokhah *et al*., 2018), and the Conservation Area of Senduro which has 39 species of epiphytic orchids (Febriandito and Soetopo, 2019). This illustrates that Bawean Island has unique environmental conditions due to its location in a unique geographical location between Java and Borneo. Orchids on Bawean Island are priceless germplasm so that it need attention and preservation from the government.

One of the endemic orchids found on Java Island is *Taeniophyllum biocellatum* (Figure 3g.). On Bawean Island Nature reserve this species only found in 1 host tree (*Canarium hirsutum*) with 5% abundance. The population of this species is threatened due to forest exploitation. The discovery of *T. biocellatum* indicates that the presence of endemic orchids in Java is also spread in Bawean Island so that it can be seen that Bawean Island reflects as Java lowland forests. This species is dominants in Sempu Island and grow optimally in

sheltered condition (Sadili, 2019). Leaves of *T. biocellatum* are absent but they have green roots that contain chloroplasts. The presence of chloroplasts confirmed in the anatomical roots characters of *T. biocellatum* (Nurfadilah *et al*., 2016). There must be conserve of the *T. biocellatum* in its natural habitat while maintaining the presence of trees serving as the host for the epiphytic orchid. *T. biocellatum* is very difficult to conserve outside its habitat (*ex situ*) that was indicated by poor adaptation in the acclimatization process in Purwodadi Botanic Garden.

Table 2. Abundance of epiphytic species in Bawean Island Nature Reserve and Wildlife Reserve.

No	Species of Epiphyte	Nt	No	% Ft	% Fo
1	*Phalaenopsis amabilis*	4	9	16	22.5
2	*Aerides odorata*	1	2	4	5
3	*Cymbidium aloifolium*	1	1	4	2.5
4	*Cymbidium* sp.	3	3	12	7.5
5	*Dendrobium anosmum*	1	2	4	5
6	*Rhynchostylis retusa*	3	4	12	10
7	*Liparis condylobulbon*	1	2	4	5
8	*Pholidota imbricata*	1	3	4	7.5
9	*Taeniophyllum biocellatum*	1	2	4	5
10	*Eria* sp.	1	1	4	2.5
11	*Hoya diversifolia*	1	3	4	7.5
12	*Hoya amoena*	1	2	4	5
13	*Hoya verticillata*	2	2	8	5
14	*Dischidia imbricata*	4	4	16	10

Nt = The number of treesin the plot hosting a particular epiphytic species.
No = The number of individuals of a particular epiphytic species within the plot.
% Ft = Relative frequency of phorophytes.
% Fo = Abundance of epiphytic plants.

There are only 3 species of Hoya and 1 species of Dischidia that recorded in Bawean Island i.e. *Hoya diversifolia* (Figure 3j), *H. verticillata* (Figure 3k), *H. amoena*, *Dischidia imbricata* were found in the forest of Bawean Island with abundance ranges between 5-10%. Compared with other locations, the diversity of Hoya in Bawean Island is relatively low. The number of Hoya species in other location including Bodogol Conservation Area recorded 6 species (Sulaeman *et al*., 2019), Belitung Island recorded 5 species (Rahayu *et al*., 2018), Gunung Gede Pangrango recorded 10 species (Rahayu, 2012), Sumatra included 41 species and 2 subspecies (Rahayu and Rodda, 2019), and Borneo included 34 species (Lamb and Rodda, 2016).

Hoya is a succulent plant having the ability to grow on less moist or dry conditions. Hoya can also live as terrestrial plants, but most of them were found in nature as epiphytic plants. It has a sticky root on the trunk, which is used to attach itself to the host tree. Hoya plants are also called "wax plants" due to the waxy appearance of their leaves or flowers (Panajon *et al*., 2016). The leaf characteristic of the three species of hoya is succulent, which means it can store water in its organs (Fahn, 1991), which function to adapt to extreme conditions (Keraudren, 1990). Succulence also associated with ecophysiological strategies and occurs in plants that have evolved in many different environments condition (Griffiths & Males, 2017). A previous study from Sulaeman *et al.* (2019) in Bodogol Conservation Area reported that air humidity and canopy cover is abiotic factors that most influencing *Hoya's* existence. The presence of *Hoya campalunata* has the highest correlation with wind speed and air temperature. *Hoya multiflora*, *Hoya vitellinoides*, *Hoya hasseltii*, and *Hoya imperalis* have positively correlated with air humidity and canopy cover.

Host Trees (Phorophyte) Species

There were 12 species recorded as host plants of epiphytic species. The preferable tree species as a host tree by the epiphytic species are *Tectona grandis, Irvingia malayana* and *Diospyros buxifolia* (Table 3). *T. grandis* had the highest species richness of epiphytic orchids, 4 species had been recorded growing on this phorophyte. *Tectona grandis* or often referred to as teak trees is also an orchid host tree in Bawean Nature Reserve. *T. grandis* is cultivated by people surrounding the forest as a community forest (*hutan rakyat*). Teak (*Tectona grandis*) is one of the most economic tropical hardwood which has naturally distributed from India through Myanmar, Laos, and Thailand (Deb *et al*., 2017). There were 4 species of orchids attaching on teak trees, i.e. *Phalaenopsis amabilis, Dendrobium anosmum* and *Cymbidium* sp., and *Eria* sp. Among other species, teak is the preferable tree by epiphytic plants. In teak trees, there are often found seedlings of *P. amabilis* and *D. anosmum* in their trunk. This proves that *T. grandis* is a suitable host for epiphytic orchid growth. At the Meru Betiri National Park in East Java, *T. grandis* is a tree that is often seen as a host for epiphytic orchids among other trees (Puspitaningtyas, 2007). Orchid seeds require special conditions to germinate because of mycoheterotrophic character which during the growth stage, the seeds have a depend on fungi as a source of carbohydrates for orchid growth (Dearnaley *et al*., 2016).

Table 3. Host trees (phorophytes) in Bawean Island with these parameters.

No	Host Trees	Jt	Js	Ji	Js/Jt	Ji/jt
1	*Antidesma petandrum*	3	2	3	0.67	1.00
2	*Canarium hirsutum*	1	1	1	1.00	1.00
3	*Diospyros buxifolia*	4	2	5	0.50	1.25
4	*Euonimus javanicus*	1	1	1	1.00	1.00
5	*Euria nitida*	1	1	1	1.00	1.00
6	*Ficus variegate*	2	2	3	1.00	1.50
7	*Irvingia malayana*	2	2	5	1.00	2.50
8	*Leea angulata*	1	1	1	1.00	1.00
9	*Schleichera oleosa*	3	2	3	0.67	1.00
10	*Syzygium* sp.	1	1	1	1.00	1.00
11	*Tectona grandis*	5	4	10	0.80	2.00
12	*Garcinia dioica*	1	1	1	1.00	1.00

Jt = Number of host trees for epiphytic plants.
Js = Number of epiphytic plant species.
Ji = Number of individual epiphytic species.
Js / Jt = Average number of epiphytic species in host plants.
Ji / Jt = Average number of epiphytic individuals in the host plants.

The second preferable tree species as a host tree is *Diospyros buxifolia*. This tree is the host of the *Rhynchostylis retusa* and *Dischidia imbricata*. *D.buxifolia* is suitable for the growth of orchids and hoya due to its trunk surface has a rough character which makes it easy for epiphytic roots to stick to stem surface. *Dischidia imbricata* is an epiphytic plant that has a small root character, so it requires a suitable stem to be able to attach the roots. Genus of Diospyros is the largest, most widely distributed, and economically trees of Ebenaceae family (Tang *et al*., 2019). *D. buxifolia* has branching with a circular arrangement at interval 15-20 cm. This species has black coloured in the stem, smooth surface, and shallow grooved (Kinho, 2013). This circular branching arrangement allows sunlight to enter the canopy and affect the air humidity level around the canopy that suitable for the growth of *R. retusa* and *D. imbricata*.

The third preferable tree species as a host tree is *Irvingia malayana*. The largest individual numbers of epiphytic orchids was also found in *I. malayana*. This species is suitable for epiphytic growth, especially orchids, i.e. *Liparis condylobulbon* and *Pholidota imbricata*. *I. malayana* is the dominant tree in the Bawean Island forest with a large diameter. Its called in local name as "red wood" (*bahasa: kayu merah*). In addition, this species also has a reasonably large wood density of 0.84 g cm-3 (Zanne *et al*., 2009). *Irvingia malayana* fruit is a favorite feed of endemic deer (*Axis kuhlii*) of Bawean Island. The presence of endemic deer (*Axis kuhlii*) is expected to play a role in the distribution of seeds of the species of *Irvingia malayana* (Trimanto, 2014). This deer knew as Critically Endangered status on the IUCN Red List (Rahman *et al*., 2017a; 2017b). Based on the inventory of orchid in East Borneo, *Irvingia malayana* is the most tree for a host of epiphytic plants. There are at least 10 species of epiphytes growing in *Irvingia malayana*. It indicates that *I. malayana* has characteristics of having a suitable trunk surface for epiphytic growth (Trimanto & Sofiah, 2018).

One of the host trees is *Euonimus javanicus* found in Gunung Lumut Forest. Bark surface of *E. javanicus* becomes a growing place of moss making it suitable for epiphytic plants. *P. amabilis* grew on this bark. In other studies, the condition of the stem that stuck by *Hoya purpureofusca* has a rather to very rough skin stem. The surface of the bark of the host tree covered 20-70% by moss so that it can support the growth of *H. Purpureofusca* (Hidayat *et al*., 2012). *Schleicera oleosa* (Kesambi) is a host tree by *Cymbidium aloifolium*. *S. oleosa* can reach up to 40 m in height, up to 2 m in diameter. Skin bark was smooth, gray, and wrinkled (Suita, 2012), that suitable for the growth of epiphytic orchids. Morphology of trees influencing the presence of epiphytic plants such as stem diameter, crown and branch shape. The presence of epiphytic plants is also influenced by microclimate, while the microclimate of the forest depends on vegetation conditions such as crown density and shape crown. Skin bark of a tree with rough and cracked surface, and has many indentation and scar will be filled by humus which suitable for epiphytic plants (Sujalu, 2017).

Vertical Distribution of Epiphytic Orchids, Hoya and Dischidia on Host Trees (Phorophyte) Species

The present study showed a range of vertical distribution of epiphytic orchid, Hoya and Dischidia from zone 1 to zone 5 (Table 4). The epiphytic orchid grew on zone 1 to zone 5, while epiphytic Hoya grew on zone 1 to zone 4, and epiphytic Dischidia grow on zone 3 to zone 4. The most abundant orchid *Phalaenopsis amabilis*, was found to have the widest vertical distribution ranging from zone 1 to zone 4. Most epiphytic orchid species grew on zone 3 and zone 4. A small number of orchid species occurred on the trunk (only two orchids species grew on zone 5). The epiphytic Hoya most grew on zone 1-3, but only 1 species grew on zone 4. Hoya was not found in zone 5. The

epiphytic Dischidia only grew on zone 3 and 4. Based on other studies epiphytic orchid in Coban Trisula and Mexico, where vascular epiphyte abundance was higher in zone 3-5 (tree crown) than in zone 1-2 (along the trunk) due to enter light intensity nearby the tree crown (Nurfadilah, 2016; Manzano *et al*., 2014), but in Bawean Island forest, the abundance of epiphytic orchid is the least on zone 5 compare to other zones. Two species of orchid that grew on zone 5 is *Aerides odorata* and *Dendrobium anosmum.*

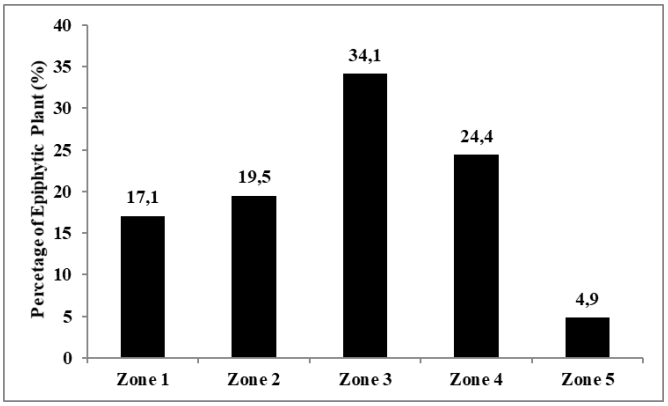


Figure 4. Comparison of the frequency of epiphytic presence in the phorophytes zone.

The presence of epiphytes was often grown on zone 3 and 4 (Figure 4). Zone 3 is the central area or in the main trunk branching area of phorophytes that has an optimum level of light intensity for epiphytic plants growth. Zone 3 is the first branching of a tree, which provides sufficient surface area to stick the epiphytic roots. Epiphytic orchids were mostly found in this zone. The highest frequency of epiphytic plant (Orchids, Hoya, and Dischidia) were present in zone 3 with percentage was 34.1 %. There were 9 species that grew on this zone. Zone 3 provides optimum environmental conditions for epiphytes growth. In the branching area, it usually contains more humus subtract and other epiphytic plants such as ferns so it provides enough moisture for epiphytes growth. Skin roughness and substrate fields serve as important factors that influence the abundance level of epiphytic orchids (Manzano, 2014). The second largest percentage zone is zone 4, it was also about 9 species grew on zone 4 with percentage were 24.4 %. The presence of epiphytic plants on phorophytes is determined by branches containing humus and moisture substrate that are accumulated from other epiphytic plants such as fern. Humus and moisture substrate in phorophytes supporting the growth of epiphytic plants. In this case, *Discidhia imbricata* only grew on zone 3 and zone 4. Zone 1 is the lowest or base area of the tree (1/3 of the main trunk). This zone has a low intensity of light and very close to the soil surface. Zone 1 is the habitat transition for epiphytic plants that previously as terrestrial plants and then change as epiphytic plants. At the beginning of its growth, Hoya grows and develops on the soil surface and then spread to the trunk surface of host trees. This zone is very suitable for Hoya, which starts to adapt from terrestrial habitat (soil) to an epiphyte habitat. In Zone 1, it is also found 4 species of orchids and 2 species of Hoya, i.e. *Pholidota imbricata, Cymbidium* sp., *Rhynchostylis retusa, Phalaenopsis amabili, Hoya diversifolia* and *H. amoena*. Zone 2 has moderate light intensity so that some of the species can growth optimum in this zone. Zone 2 also has a greater surface area for epiphyte root. In Zone 2, the percentage of

Table 4. Zones of the occurrence of epiphytic orchids, Hoya and Dischidia on their phorophytes and the number of individuals in each zone.

No	Species of Epiphyte	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5
1	*Phalaenopsis amabilis*	2	3	2	2	
2	*Aerides odorata*				1	1
3	*Cymbidium aloifolium*		2			
4	*Cymbidium* sp.	1	1	1		
5	*Dendrobium anosmum*				1	1
6	*Rhynchostylis retusa*	1	1	2		
7	*Liparis condylobulbon*			1	1	
8	*Pholidota imbricate*	1		1	1	
9	*Taeniophyllum biocellatum*			1	1	
10	*Eria* sp.				1	
11	*Hoya diversifolia*	1	1	1		
12	*Hoya amoena*	1	1			
13	*Hoya verticillata*			1	1	
14	*Dischidia imbricate*			3	1	
	Total number of epiphytic plant	7	8	14	10	2

epiphytes presence is large with a percentage of 19.5%. In location studies, there were 3 species of orchids and 2 species of hoya grows on zone 2 i.e. *Phalaenopsis amabilis, Cymbidium aloifolium, Rhynchostylis retusa*, *Hoya diversifolia* and *Hoya amoena*. Zone 2 is also a suitable habitat for *Cymbidium aloifolium* which is proven that orchid species were rarely found in the upper zone of the phorophytes.

The smallest presence of epiphytes in zone 5 (the outermost area of the branching) due to this zone has the smallest surface area. There were only 2 species of epiphytes present in this zone due to this zone is not sheltered by a crown of phorophytes, i.e. *Aerides odorata* and *Dendrobium anosmum*. This zone has high light intensity and low air humidity level because of the character forest of Bawean Island is lowland and dry so that not supporting for growth of epiphytic plants in this zone. Zone 5 is actually the safest area to avoid epiphytic plants from human exploitation. This zone is blocked by small branches and leaves.

Implication for Conservation

It is necessary to maintain the sustainability of forest trees in order to protect it from exploitation because trees are host plants of various epiphytic plants in the forest. Preservation of forest trees and epiphytic plants can involve the local community. Orchids, Hoya, and Dischidia on Bawean Island are sources of germplasm that must be conserved because these epiphytic plants have genetic sources that may be different from similar epiphytic plants in other regions.

CONCLUSION

There were 10 species of epiphytic orchids, 3 species of Hoya, and 1 species of Dischidia that have been recorded in Bawean Island Nature Reserve and Wildlife Reserve. The most commonly found of epiphytic orchid in location studies is *Phalaenopsis amabilis*. There were 12 species of trees as the hosts of epiphytic plants. *Tectona grandis, Irvingia malayana,* and *Diospyros buxifolia* were the trees that often found as the host of epiphytic plants. Zone 3 is the area covering the basal part of the branching (1/3 part of the total length of the branch) serves as the zone which is the most often found epiphytic plants. The least epiphytic presence is found in Zone 5.

REFERENCES

Adhikari, Y.P., Fischer, H.S., Fischer, A., 2012, Host tree utilization by epiphytic orchids in different land-use intensities in Kathmandu Valley, Nepal, *Plant Ecology* 213, 393–412.

Aulia, A. N., & Hakim, L., 2019, Technique for sustainable development along trisula waterfall (Coban Trisula)-Ranu Pani, Bromo Tengger Semeru National Park: a case of epiphytic orchid conservation, *Journal of Environmental Engineering and Sustainable Technology* 6(1), 16-22.

Blouch R.A., 1995, Conservation and research priorities for threatened Suids of South and Southeast Asia, IBEX J.M.E. 3, 21-25.

Danarto S.A., & Rahadiantoro, A., 2015, Eksplorasi tumbuhan di Pulau Bawean, Gresik, Jawa Timur [Plant exploration in Bawean Island,Gresik-East Java]. *Proceedings of National Conference of Indonesian Biodiversity Community* 1 (5), 974-979.

Dearnaley, J., Perotto, S., & Selosse, M. A., 2016, 'Structure and development of orchid mycorrhizas', in F.Martin (eds), *Molecular Mycorrhizal Symbiosis*, pp.63-86, John Wiley & Sons, Canada.

Deb, J. C., Phinn, S., Butt, N., & McAlpine, C. A., 2017, Climatic-induced shifts in the distribution of teak (*Tectona grandis*) in tropical Asia: implications for forest management and planning, *Environmental management* 60(3), 422-435.

Farokhah, T., Utami, S., & Jumari, J., 2018, Keragaman dan kelimpahan anggrek di Cagar Alam Gebugan, Semarang, Indonesia [Diversity and abundance of orchids at Gebugan Nature Reserve in Semarang, Indonesia]. *Biosaintifika* 10(2), 284-290.

Fandani, H. S., & Mallombasang, S. N., 2018, Keragaman anggrek pada beberapa penangkaran di Desa Ampera dan Desa Karunia, Kecamatan Palolo, Kabupaten Sigi [Diversity of orchid in some of captivity in Ampera and Karunia Village, Palolo Sub-district, Sigi District]. *Jurnal Warta Rimba* 6(3), 14-20.

Febriandito, P.D., & Soetopo, L., 2019, Exploration of epiphytic orchid diversity at Senduro Conservation Area II, Ireng-Ireng Block, Bromo Tengger Semeru National Parks, East Java, *Plantropica* 3(2), 68-76.

Griffiths, H., & Males, J., 2017, Succulent plants, *Current Biology* 27(17), 890-896.

Hidayat, S., Rahayu, S., & Ningtyas, K, 2012, Analisis vegetasi habitat hoya *Hoya purpureofusca* hook.f. di Resort Selabintana, Taman Nasional Gunung Gede Pangrango [Vegetation analysis of habitat *Hoya purpureofusca* hook.f. in Selabintana Resort, Gunung Gede-Pangrango National Park]. *Berita Biologi* 11(1), 103-110.

Johansson D.R., 1975, Ecology of epiphytic orchids in West African rainforests. *Am Orchid Soc Bull* 44, 125-136.

Keraudren-Aymonin, M., 1990, 'Epidermal and anatomical features of the succulent xerophytic cucurbitaceae of Madagascar: adaptive and phylogenetic aspects' in D.M. Bates, R.W. Robinson, & C. Jeffrey (eds.), *Biology and Utilization of the Cucurbitaceae*, pp. 239-247, Cornell University Press, London.

Kinho, J., 2013., *Restoring the glory of ebony in North Sulawesi*, Balai Penelitian Kehutanan Manado, Manado.

Lamb, A. & Rodda, M., 2016, *A guide to hoyas of Borneo*, Natural History Publications Borneo, Kota Kinabalu.

Manzano, E.D.L., Andrade, J.L., Zotz, G., Reyes, G.C., 2014, Epiphytic orchids in tropical dry forests of Yucatan, Mexico – Species occurrence, abundance and correlations with host tree characteristics and environmental conditions, *Flora* 209, 100–109.

Mardiyana, M., Murningsih, M., & Utami, S., 2019, Inventarisasi anggrek epifit (Orchidaceae) di hutan Peungkriyono, Pekalongan, Jawa Tengah [Inventory of epiphytes orchids (Orchidaceae) in Petungkriyono Forest, Pekalongan, Central Java]. *Jurnal Akademika Biologi* 8(2), 1-7

Ministry of Agriculture. 1979. Decree number 762/ Kpts/um/12/1979 about the appointment of Bawean Island forests covering an area of 4556.6 Ha located in Gresik regency, East Java Province as forest in EastJava as Nature Preserve Forests Cq. Nature Reserve covering an area of 725 ha and Wildlife area of 3381.6 hectares.

Maurizio, B. & Salla, G., 1992., A new sub species of *Atrophoneura coon* from Bawean Island (Indonesia) Lepidoptera – Papilionidae, Tropical Lepidoptera 3(2), 119-128.

Nijman, V., 2004, Survey on birds of prey and owls (*Falconiformes* and *Strigiformes*) on Bawean Java Sea with records of three species new to the island, Raffles Bull Zool 52(2), 647-651.

Nurfadilah, S., 2016, Diversity of epiphytic orchids and host trees (phorophytes) in secondary forest of Coban Trisula, Malang Regency, East Java, Indonesia, *Biotropia* 22(2), 120-128.

Nurfadilah, S., Yulia, N. D., & Ariyanti, E. E., 2016, Morphology, anatomy, and mycorrhizal fungi colonization in roots of epiphytic orchids of Sempu Island, East Java, Indonesia, *Biodiversitas* 17(2), 592-603.

Panajon, N.M., Aurigue, F.B., Shen, C.C., Ragasa, C.Y., 2016, Triterpenes and sterols from *Hoya diversifolia* Blume, *J App Pharm Sci* 6(6), 79-82.

Puspitaningtyas, D. M., 2007, Inventarisasi anggrek epifit dan inangnya di Taman Nasional Meru Betiri [Orchid Inventory and the host in Meru Betiri National Park, East Java]. *Biodiversitas* 8 (3), 210-214.

Rademaker, M., Meijaard, E., Semiadi, G., Blokland, S., Neilson, E. W., & Rode-Margono, E. J., 2016, First ecological study of the Bawean warty pig (*Sus blouchi*), one of the rarest pigs on earth, *PloS one* 11(4), 1-15.

Rahayu, S., Fakhrurrozi, Y., & Putra, H. F., 2018, Hoya species of Belitung Island, Indonesia, utilization and conservation, *Biodiversitas* 19(2), 369-376.

Rahayu, S., & Rodda, M., 2019, Hoya of Sumatra, an updated checklist, three new species, and a new subspecies, *European Journal of Taxonomy* 508, 1-23.

Rahman, D. A., Gonzalez, G., & Aulagnier, S., 2017, Population size, distribution and status of the remote and critically endangered Bawean deer *Axis kuhlii*, *Oryx* 51(4), 665-672.

Rahman, D. A., Gonzalez, G., Haryono, M., Muhtarom, A., Firdaus, A. Y., & Aulagnier, S. 2017, Factors affecting seasonal habitat use, and predicted range of two tropical deer in Indonesian rainforest, *Acta Oecologica* 82, 41-51.

Sadili, A., 2019, Struktur, distribusi, dan pola tata ruang anggrek epifit (Orchidaceae) hutan pantai Cagar Alam Pulau Sempu [Structure, distribution, and spatial patterns of epiphytic orchids (Orchidaceae) at coastal forest of the Sempu Island Nature Reserve, Malang, East java]. *Jurnal Ilmu Kehutanan* 13(1), 38-47.

Semiadi, G., Duckworth, J.W., Timmins, R., 2015, '*Axis kuhlii*' in The IUCN Red List of Threatened Species 2015, viewed 24 March 2020 from http://dx.doi.org/10.2305/IUCN.UK.2015-2.RLTS.T2447A73071875.en. [26 August 2016].

Suita, E, 2012, *Forest crop seed technology series: kesambi* (*Schleichera oleosa* MERR.), Balai Penelitian Teknologi Perbenihan Tanaman Hutan, Bogor.

Sujalu, A. P. 2018. Identifikasi pohon inang epifit di hutan dataran rendah klimaks, Kabupaten Malinau [Identification of epiphytes host trees in climax Dipterocarpaceae lowland forest, Malinau District]. *Jurnal Hutan Tropis* 1(2), 196-201.

Sulaeman, M., Sulistijorini, S., & Rahayu, S., 2019, Habitat suitability for Hoya spp. (Apocynaceae) in the Bodogol Conservation Area, West Java. *Biosaintifika* 11(1), 91-99.

Sulistiarini, D., & Djarwaningsih, T., 2017, Keragaman anggrek di Cagar Alam Gunung Tukung Gede, Serang, Banten [Diversity of orchids in Gunung Tukung Gede Nature Reserve, Serang, Banten]. *Jurnal Biodjati* 2(1), 72-82.

Tang, D., Zhang, Q., Xu, L., Guo, D., & Luo, Z., 2019, Number of species and geographical distribution of *Diospyros* L.(Ebenaceae) in China, *Horticultural Plant Journal* 5(2), 59-69.

Trimanto & Hapsari, L., 2016, Botanical survey in thirteen montane forests of Bawean Island Nature Reserve, East Java Indonesia: flora diversity, conservation status, and bioprospecting, *Biodiversitas* 17(2), 832-846.

Trimanto, 2013, Keragaman pohon sekitar mata air di Pulau Moyo, Nusa Tenggara Barat [Diversity of trees around springs flow in the Moyo Island Region of West Nusa Tenggara]. *Proceedings of National Conference of Biology* 10(2), 15-23.

Trimanto. 2014. Analisis vegetasi dan estimasi stok karbon 7 gunung di suaka alam Pulau Bawean [Vegetation analysis and estimation of tree carbon stock biomass in seven mountain forests, Bawean Island Nature Reserve, East Java]. *Berita Biologi* 13 (3), 321-332.

Trimanto T., & Sofiah, S., 2018, Exploration of flora diversity and recommending species for reclamation of coal mining with biodiversity concept in Besiq Bermai Forest, East Borneo, *Journal of Tropical Life Science* 8(2), 97-107.

Umiyah, Yulia, N.D., and Prasetyani, D., 2011, Inventarisasi anggrek epifit dan pohon inangnya di Cagar Alam Pulau Sempu, Kabupaten Malang, Jawa Timur [Inventory of epiphytic orchid species and its host trees in Sempu Island Nature Reserve, Malang District, East Java]. *Proceeding of Na-tional Conference "Tropical Plant Conservation: "Current condition and the next challenge"*, 475-478.

Vincen, N., 2004, Survey on birds of prey and owls (Falconiformes and Strigiformes) on Bawean Java Sea with records of three species new to the island, *The Raffles Bulletin of Zoology* 52 (2), 647-651.

Yubu, A., Pollo, H. N., & Lasut, M. T., 2019, Inventarisasi anggrek hutan di taman wisata alam batuputih, kota bitung, sulawesi utara [Orchid inventory in Taman Batuputih Nature Tourism, Bitung City, North Sulawesi]. *Eugenia* 24(3), 116-122.

Yulia, N. D., 2010, Evaluasi keragaman anggrek di tiga kawasan hutan alam Jawa Timur [Evaluation of orchid diversity on three forest area of East Java]. *Berkala Penelitian Hayati Special Edition* 4A, 41-44.

Yulia, N.D., Budiharta, S., 2011, Epiphytic orchids and host tree diversity at Gunung Manyutan Forest Reserve, Wilis Mountain, Ponorogo, East Java, *Biodiversitas* 12(1), 22-37.

Yulia, N.D., & Budiharta, S., 2012, The diversity of epiphytic orchid and its phorophyte along cemoro sewu hiking pathway, Lawu Mountain, District of Magetan, East Java, Indonesia. *Journal of Nature Studies* 10(2), 26 -31.

Zanne, A.E., Lopez, G., Gonzalez , D.A., Coomes., J., Ilic., S., Jansen., S.L., Lewis., R.B., Miller., N.G., Swenson, Wiemann., M.C., and Chave, J., 2009, '*Irvingia malayana*' in *Global Wood Density Database*, viewed 30 February 2020, from https://datadryad.org/stash/dataset/doi:10.5061/dryad.234.html.

Journal of Tropical Biodiversity and Biotechnology
Volume 05, Issue 02 (2020): 89 — 99
DOI: 10.22146/jtbb.53830

Research Article

The Plant Species Diversity of Lasitae Protected Nature Forest and Nearby Area, District of Barru, South Sulawesi

Rismita Sari[1]*, Fauziah[2], Inggit Puji Astuti[1], Ratna Susandarini[3], Irwan Makmur[4]

1) Research Centre for Plant Conservation and Botanic Garden-Indonesian Institute of Sciences (LIPI), Jln. Ir. H. Juanda 13, Bogor 16003, West Java, Indonesia
2) Purwodadi Botanic Garden-Research Centre for Plant Conservation and Botanic Garden (LIPI), Jln. Surabaya-Malang Km 65, Purwodadi-Pasuruan 67163, East Java, Indonesia
3) Faculty of Biology, Gadjah Mada University, Bulak Sumur 55821, Yogyakarta, Indonesia
4) Forest Management Unit (KPH) Ajatappareng, Forestry Service, South Sulawesi Province, Jln. Sultan Hasanuddin No. 95, Barru District, South Sulawesi, Indonesia

Submitted: 28 January 2020; Accepted: 06 April 2020; Published: 15 August 2020

ABSTRACT

An expedition to Lasitae Protected Forest in District of Barru, South Sulawesi was undertaken to collect living plants for Pucak Botanic Garden, South Sulawesi. The aims were to investigate the diversity of the plants and as a dedication to the Expedition of the Republic of Indonesia Corridor Sulawesi. Using an explorative method, we collected the plants and recorded all data in the field following the tracks assisted by the field guide. A total of 179 collection-numbers have been collected from this dry lowland forest including the data for the local names. Many potential and valuable plants occur in the forest, 13 plants were highlighted in this paper for its conservation status, the potentiality for various purposes or its uniqueness: *Diospyros celebica* Bakh. or streak ebony (Ebenaceae), *Arenga pinnata* (Wurmb.) Merr. (Arecaceae), *Phyllanthus lamprophyllus* Mull.Arg. (Phyllanthaceae), *Cycas rumphii* Miq. (Cycadaceae), *Lagerstroemia speciosa* (L.) Pers. (Lythraceae), *Garcinia celebica* L. (Clusiaceae), *Nervilia aragoana* Gaud. (Orchidaceae), *Phalaenopsis amabilis* (L.) Blume (Orchidaceae), *Ophioglossum reticulatum* L. (Ophioglossaceae), *Tetracera scandens* (L.) Merr. (Dilleniaceae), *Derris trifoliata* Lour., *Phytocrene bracteata* Wall. (Icacinaceae), and *Dioscorea hispida* Dennst. *Buchanania arborescens* (Blume) Blume and *Ardisia elliptica* Thunb. can easily be found and widely spread.

Keywords: plant species diversity, protected forest, rare plants

INTRODUCTION

Indonesia is one of the 17 megadiverse countries in the world that has ca. 37,000 plants species, the third rank after Brazil and Colombia (Paknia, Rajaesh & Koh, 2015). Among the five biggest islands in Indonesia, Sulawesi is well-known for its richness on biodiversity and endemicity (Widjaja & Pratama, 2013). In a study in Lore Lindu National Park, Central Sulawesi, it was found that the endemicity reaching ca. 15% of trees (Gradstein *et al.*, 2007). Its position between the Wallace line and Weber line supports its high diversity of flora and fauna among other islands in Indonesia.

One of the protected forests in Sulawesi is Lasitae Protected Forest (*Hutan Lindung Lasitae*) which is located in the District of Barru, South Sulawesi. The District of Barru is situated up north of Makassar city and stretches along the west coast. The Lasitae Protected Forest (PF) is located 10 km from the main city of Barru and covers 49.801 ha km2 areas. (Dinas Kehutanan Kabupaten Barru, 2005).

The flora of Lasitae PF has never been investigated previously, therefore the South Sulawesi Province Government recommended this location to our expedition team. This expedition was carried out to support the collection enhancement for Pucak Botanic Garden (BG) which is located in the District of Maros, South Sulawesi. The Pucak BG is under the management of the South Sulawesi Province Government. The Bogor Botanic Garden has been assisting the establishment of the Pucak BG since 2006. The plants collected in this expedition would

***Corresponding author**
Email: mita_krb@yahoo.com

be submitted as the living collection for the Pucak BG. This expedition was also dedicated to the Expedition of the Republic of Indonesia Corridor Sulawesi (*Ekspedisi NKRI Koridor Sulawesi*) which was organized by the Indonesian Army Special Forces group (Kopassus).

Aims

This expedition aimed to identify the plant species diversity of Lasitae PF, to unveil the unique and potential flora of Lasitae PF and targeted to collect 125 collection numbers for the Pucak BG. The number of the target is the average number that usually can be collected based on the annual expedition of Bogor Botanic Garden.

MATERIALS AND METHODS

Material

The material we collected were the plants in Lasitae PF, Padang Loang Community Based Forest (Hutan Rakyat Padang Loang), Coppo Village & Lipukasi Village, District of Barru.

The equipment we used were the standard expedition equipment package supplied by Bogor Botanic Garden contains small (10x15 cm) and medium (25x30 cm) size black plastic bags, multi sizes clear plastic bags, plastic sacks, yellow tags for living collection, paper tags for herbarium specimens, clip plastic bags for 0,5 kg and 0,25 kg, Rootone-F (PT. Rhone-Poulenc Agrocarb, Surabaya, Indonesia), Dithane M-45 (PT. Dow AgroSciences Indonesia, Medan, Indonesia), alcohol 70%, rolled tissue, used newspaper, shade fabric, polybag, ruler 30 cm, plastic rope, rubber bands, sticky tapes, and cardboard boxes for packing.

Tools we used were altimeter, thermo hygrometer, GPS (Garmin), cutters, scissors, secateurs, machetes, two types of digital camera, field books and stationaries.

Method

The expedition was carried out by an explorative method. The location was determined by the local guides Mr. Irwan Makmur and Mr. Suriadi. We provided a list of the plants we targeted but other plants that we considered were necessary to be collected would also be taken. The timetable for the activities from 27 May-3 June 2013 was made to reach the maximum outcome.

We walked along the track from the Copo Village through Padang Loang Community Based Forest until the border of Lasitae PF for about 400 m. All the plants we collected were documented and labeled while temperature and ecological data were noted. The first day we walked along the track where the villagers used to take their cows to the forest. The last day we walked along the main road that connects the villages.

All the plants collected were sampled for herbarium vouchers and deposited in Bogor Botanic Garden. The herbarium specimens were labeled completed with the name of the species, family, collector's number, the date of collecting and the location. The same label was tagged to the living collection.

RESULTS AND DISCUSSION

Results

A total of 179 collection numbers were successfully collected in this expedition. Each collection number has one or more plants, seeds, or seedlings for the stock if it could not survive in the nursery of Pucak BG. It covered 59 families but only 83 collection numbers have successfully identified (Table 1). The rest were 89 collection numbers have been recognized up to the genus level and 8 collection numbers were only recognized up to family level (Table 2). All plants were collected from four locations as mentioned in the Material section, but the main collection and the most number collections were from Lasitae PF. Some species were collected more than once when we found in different locations as it was considered from different populations that genetic variation might occur.

A few plants were bearing fruits such as *Garcinia celebica* L. (Clusiaceae), *Buchanania arborescens* (Blume) Blume (Anacardiaceae), *Ardisia elliptica* Thunb. (Myrsinaceae), *Acronychia vetandra* (F. Muell.) T.G. Hartley (Rutaceae), *Cycas rumphii* Miq. (Cycadaeae) and *Derris trifoliata* Lour. (Fabaceae). Some other plants were still flowering such as *Dendrobium* sp. (Orchidaceae) and *Tetracera scandens* L. (Dilleniaceae).

The altitude range we explored was from 33-159 m above sea level (asl). The daily temperature during this expedition was between 23-36°C, with relative humidity (RH) ranged between 49-84%. The soil is rocky with pH ranges from 5.7-6.8 and the soil RH 50-100%. The forest type is a typical lowland rain forest.

This forest is the water source for the villagers, supplied by the Padalampe river inside the forest. The river was small with clear water but shallow. The river flows along the rocks giving humidity to the surrounding support the plants with water supplies. There were many plants grew along the riverside. We selected *Dioscorea hispida* Dennst. (Dioscoreaceae), *Tacca palmata* Blume. (Taccaceae), *Tabernaemontana* sp. (Apocynaceae), *Alocasia alba* Schott. (Araceae), *Garcinia xanthochymus* (Roxb), Kurz

(Clusiaceae), *Piper* sp. (Piperaceae), *Daemonorops* sp. (Arecaceae), *Cinnamomum* sp. (Lauraceae) and *Cymbidium finlaysonianum* Lindl. (Orchidaceae), *Pandanus* sp. (Pandanaceae), *Hoya* sp. (Asclepiadaceae) and *Pleomele* sp. (Agavaceae) to be collected.

A bit deeper in the forest, there was the sugar palm, *Arenga pinnata* (Wurmb) Merr. (Arecaceae). People collected *nira* or sweet palm juice from the sugar palm for making palm sugar. There were also streak ebony populations that occur (*Diospyros celebica* Bakh., Ebenaceae) in the forest that was highly protected by the local government as this taxon is a rare endemic tree (World Conservation Monitoring Centre, 1997). There were two populations of streak ebony in this forest, which the bigger population contained 15 mature trees while the other has four mature trees. The seedlings were abundant around the mature trees.

Discussion

The result of this expedition has demonstrated that Padang Loang Community Based Forest and Lasitae PF has a high plant diversity. The 59 families that occur in this forest including the endemic species of streak ebony indicated that this location represents the typical lowland tropical forest of Sulawesi. The plants we collected ranged from ferns to Gymnosperms and Angiosperms have potentiality in different purposes as the previous studies reported. The forest was still dense and mostly in good condition. The total collection numbers we obtained were more than our target and it showed that this forest contains lots of varieties of plants that should be protected from any disturbance.

As this forest is very close from the west coast the weather is hot and the forest tends to be dry with a little source of water. According to the Pemerintah Kabupaten Barru (2013), the soil type in the District of Barru is regosol with medium fertile type. The soil is basically volcanic which is spread all over the district. In the area, we explored the soil is very rocky that makes the soil is relatively hard. In a few sites, the land composed of rocks particularly in Padang Loang Community Based Forest. The rocks in the palm-size were scattered on the ground in a relatively wide area (Figure 1). However, the richness of plant diversity in the forest is remarkably sustained in this type of soil.

Among the plants we collected, there are a few valuable plants based on the economic values, usages or conservation status. We would like to highlight 13 plants in this paper that we considered distinct among other plants. The first one we would like to focus on is *Diospyros celebica* or streak ebony. This plant is one of the most valuable plants in this forest. The massive harvesting of the wood across Sulawesi for years causing the population has been decreasing (Riswan, 2002; Walujo, 2002). This plant is now categorized as Vulnerable (VU) according to the IUCN Red List (World Conservation Monitoring Centre, 1998). The presence of streak ebony in this conservation area was well protected as indicated by the oldest tree still exists (Figure 2).

Figure 1. Padang Loang Community Based Forest (Photograph: Rismita Sari).

The sugar palm *Arenga pinnata* is well-known as a multipurpose palm (Mogea *et al.*, 1991). This palm distributed widely in SE Asia including Indonesia. The main yield of this palm is the palm sugar that processed from the sweet juice collected from the stalk of the flower. The juice is usually collected daily to avoid fermentation. Other usages are the flesh of the fruits known as *kolang-kaling* and the black fibers resulted from the sheath can be used for many household purposes. To obtain the juice or the fruits traditionally people have to climb the tree as the flower and fruit are on the top of the tree (Figure 3). The people in Lasitae PF leave a bamboo ladder on the tree to make them easier to collect the juice that was accumulated in a tin.

The small plants we collected in Padang Loang Community Based Forest, *Phyllanthus lamprophyllus* Mull.Arg. (Phyllanthaceae) (Figure 4) is one of 13 *Phyllanthus* species that have been found in Sulawesi (Bouman *et al.*, 2019). It grows widely in Padang Loang. This plant occurs in Java, Lesser Sunda Island, New Guinea, the Philippines, Queensland (Australia) and Sulawesi (Govaerts, 2000). The plant has not been used by the local people and easy to find in the Padang Loang. The plant itself might be able to be used as an ornamental plant as it has beautiful leaves arrangement and colour.

A Gympospermae plant, *Cycas rumphii*, grows well in the Padang Loang Community Based Forest and Lasitae PF (Figure 5). This cycad has been used

as an ornamental plant and a very common plant in the gardens in SE Asia. In fact, the seed has been known is acutely toxic after a case of Chamorro people who used the flour from the fruits in their cuisine has digestions problems (Cox & Sacks, 2002). According to the IUCN Red List, the category of conservation status of *C. rumphii* is Near Threatened (NT) as the population is decreasing in the wild (Hill, 2010).

Lagerstroemia speciosa (L.) Pers. (Lythraceae) was found at Padang Loang Village. The flowering season has passed leaving dry split fruits on the tree (Figure 6). This plant is a common roadside tree in SE Asian countries. The beautiful flower and the shady canopy have made this tree very popular. Besides as an ornamental tree, people in the Philippines use the plant to cure diabetic and kidney problems (Klein *et al*., 2007). This tree is known contains active chemical compounds that cure some diseases.

There are a few edible fruit plants in the Lasitae PF. At least there are two genera produce fruits, *Garcinia* spp. and *Mangifera* spp. One of the taxa, *G. celebica* L. (Clusiaceae) is known as edible fruits by the locals (Figure 7). The fruits were at a very young stage when we found it, so we were not able to taste the fruits. Compared with the *G. celebica* collection in Bogor Botanic Garden-LIPI, the fruits were smaller. The size of *G. celebica* in the Bogor BG ranges between 3-4 cm in diameter. The *G. celebica* in Lasitae PF was ca. 3 cm in diameter. *Garcinia* has many variations in the wild. The variations range from size, colour, and taste as well.

The orchids in the forest vary from terrestrial to epiphyte while most of them were epiphytes. One of the terrestrial orchids was *Nervilia aragoana* Gaudich. (Orchidaceae) (Figure 8). It was found in the open forest near Lipukasi Village and it is common in the Lasitae forest but a bit rare in other areas in Indonesia. The orchid is unused by the locals, whereas in Taiwan, *N. aragoana* has been used as herbal medicine to heal a few diseases (Kikuchi *et al*., 1981).

The existence of rare orchid *Phalaenopsis amabilis* (L.) Blume (Orchidaceae) was an indication that Lasitae PF is very rich with plant diversity. The orchid *P. amabilis* is one of the very popular orchids and became rare in the wild after excessive harvesting for its beautiful flower including deforestation, commercial trades, illegal logging, land conversion and domestication of native orchids (Semiarti, 2002). It has been well domesticated and cross-pollinated resulting in various hybrids. We only found one individual plant that had an empty peduncle as an indication that the flowering season is over (Figure 9).

The fern *Ophioglossum reticulatum* L. (Ophioglossaceae) is a small fern that occurs in Lasitae PF (Figure 10). The population was found growing in a relatively open area near Lippukasi Village. This fern is also called the adder's-tongue fern that according to IUCN Red List the conservation status is LC (Least Concern) (Irudayaraj, 2011). The genus of this single leaf fern contains 25-30 species that widely distributed in the temperate and the tropics area. This fern has been known to have antimicrobial compounds (Mukherjee *et al*., 2017).

A woody climber, *Tetracera scandens* was in full bloom when we found it at the roadside of the main road to the Lasitae PF (Figure 11a). This plant grows widely in India, China, Indonesia, Myanmar, Philippines, Thailand, Malaysia, and Vietnam (Thanh *et al*., 2015; Muliyah *et al*., 2017). The trunk can be used as a source of drinkable water if it is unavailable in the forest. The water in the trunk can be accumulated by cutting the trunk and it will pour out from the surface of the cut (Figure 11b). The leaves can be used as a sandpaper to smooth the machete handle as it has coarse surface as shown by our field guide.

Another plant that contains drinkable water is *Derris trifoliata*. This woody climber can be recognized in the wild from the fruits (Figure 12a & b). It is widely spread in the world particularly in the tropics (Roskov *et al*., 2020). In Lasitae PF, *D. trifoliata* grows near the small river near the Lipukasi Village. The trunk climbs the other trees across the river. Despite *D. trifoliata* contains various chemical substances in the parts of the plant (Wenjie *et al*., 2009; Mamoon & Azam, 2012) our field guide, Mr. Suriadi showed it is safe to drink the water from the trunk (Figure 12c).

Phytocrene bracteata Wall. (Icacinaceae) is a woody climber that occurs in Lasitae PF. We found the flowers at the early stage (Figure 13). The *P. bracteata* in Sulawesi has been previously reported found in the karst hills valley in the south of Sulawesi (Saiful & Burhan, 2017). The most interesting thing about this plant is the fruits. Despite it is not edible, but the shape is distinct.

Dioscorea hispida is one of the plants that produce carbohydrates and has been used as a food source after treated using some techniques to remove cyanides and other poisonous chemical substances (Kumoro *et al*., 2011). The starchy yam can be made into chips or other food like biscuits. We found a tuber of *D. hispida* on the location from one plant. The size of the tuber is ca. 15 cm in diameter with 8 cm thick (Figure 14). There was only one population of *D. hispida* along the river. The trunk has rare leaves indicating it is an old plant.

Dioscorea is a climber and usually grows easily. The small population of this plant might be influenced by the climate or soil condition that has less support for the plant to regenerate.

There are many more plants in this forest that can be explored for its potentiality. This lowland forest has shown its diversity and many potential plants grow there. It is recommended to explore further into the other parts of the forest to find more information about the plants in this protective area.

Figure 2. The oldest streak ebony (*Diospyros celebica Bakh.*) in Lasitae PF with Mr. Suriadi (Photograph: Rismita Sari).

Figure 3. *Arenga pinnata* (Wurmb) Merr. (Photograph: Rismita Sari)

Figure 4. *Phyllanthus lamprophyllus* Mull.Arg. (Photograph: Rismita Sari).

Figure 5. *Cycas rumphii* Miq. (Photograph: Rismita Sari).

Figure 6. Dry fruits of *Lagerstroemia speciosa (*L.) Pers. (Photograph: Rismita Sari).

Figure 7. Young fruit of *G. celebica* L. (Photograph: Rismita Sari).

Figure 8. *Nervilia aragoana* Gaudich. (Photograph: Rismita Sari).

Figure 9. *Phalaenopsis amabilis* (L.) Blume (Photograph: Rismita Sari).

Figure 10. *Ophioglossum reticulatum* L. (Photograph: Rismita Sari)

(11a)

(11b)

Figure 11. Flower of *Tetracera scandens* (L.) Gilg & Werderm. (a). The water from the trunk of *T. scandens* (b) (Photographs: Rismita Sari).

(12a)

(12b)

(12c)

Figure 12. Dry fruits of *Derris trifoliata* Lour. (a); Young fruits of *D. trifoliata* (b); Mr. Suriadi is showing how to drink the fresh water from the trunk of *D. trifoliata* (c). (Photographs: Rismita Sari)

Figure 13. The flower buds of *Phytocrene bracteata* Wall. (Photograph: Rismita Sari)

Figure 14. The tuber of *Dioscorea hispida* Dennst. is held by Mr. Suriadi. (Photograph: Rismita Sari)

CONCLUSION

The Lasitae PF and the surrounding area is an important forest as it plays an important role in supporting the locals' life such as supplies water source, food, wild fruits, ornamental plants, and other usages including rare plants. It is very important to keep the forest protected, as it contains many useful plants for various purposes. Moreover, there are many plants in this forest that contains phytochemical substances that might be developed in the future. The sustainability of this forest should be maintained for the advantages of the people around the forest and to benefit people in the future.

Table 1. The species that have been identified.

No.	Species	Family	Local names
1.	*Acronychia vetandra* (F. Muell.) T.G. Hartley	Rutaceae	matte-matte komba'
2.	*Aerides odorata* Lour.	Orchidaceae	-
3.	*A. odorata*	Orchidaceae	-
4.	*A. odorata*	Orchidaceae	-
5.	*A. odorata*	Orchidaceae	-
6.	*Aglaonema modestum* Schott ex Engler	Araceae	-
7.	*Alocasia alba* Schott.	Araceae	lawira
8.	*A. alba*	Araceae	-
9.	*Amorphophallus paeoniifolius* (Dennst.) Nicolson	Araceae	bote
10.	*Ardisia elliptica* Thunb.	Myrsinaceae	bineppu
11.	*Arenga pinnata*(Wurmb.) Merr.	Arecaceae	aren
12.	*A. pinnata*	Arecaceae	-
13.	*Brucea javanica* (l.) Merr.	Simarubaceae	tampak
14.	*Buchanania arborescens* Blume	Anacardiaceae	pawale'
15.	*Bulbophyllum lepidum* (Blume) J.J.Smith	Orchidaceae	-
16.	*Cananga odorata* (Lam.) Hook.f.&Thomson	Annonaceae	kenanga
17.	*C. odorata*	Annonaceae	kananga
18.	*Cephaelis ipecachuanha* (Brot.) A.Rich.	Rubiaceae	-
19.	*Clausena indica* (Dalzell.) Oliv.	Rutaceae	-
20.	*Clerodendrum minahassae* Teisjm. & Binn	Boraginaceae	lalik-lalik manuk

Table 1. Contd.

No.	Species	Family	Local names
21.	*Crinum asiaticum* L.	Amaryllidaceae	peno'-peno'
22.	*Cyathea latebrosa* (Wall. ex Hook.) Copel.	Cyatheaceae	-
23.	*Cyathula prostrata* (I.) Blume	Amaranthaceae	pale' anyarang, tapal kuda
24.	*Cycas rumphii* Miq.	Cycadaceae	pattoku
25.	*Cymbidium finlaysonianum* Lindl.	Orchidaceae	-
26.	*Cynometra ramiflora* L.	Fabaceae	lambe-lambe
27.	*Dendrobium anosmum* Lindl.	Orchidaceae	-
28.	*Dendrobium juncifolium* Schltr.	Orchidaceae	
29.	*D. juncifolium*	Orchidaceae	-
30.	*Derris trifoliata* Lour.	Fabaceae	lacciran
31.	*Dillenia ochreata* (Miq.) Teisjm. & Binn. ex Mart.	Dilleniaceae	lakonra
32.	*Dioscorea hispida* Dennst.	Dioscoreaceae	siapa
33.	*Diospyros celebica* Bakh.	Ebenaceae	amara
34.	*D. celebica*	Ebenaceae	amara
35.	*Donax canniformis* (G. Forst.) K.Schum	Marantaceae	bampeng bunga
36.	*D. canniformis*	Marantaceae	bampeng
37.	*Ehretia timoriensis* Decne	Boraginaceae	-
38.	*Elephantopus spicatus* Aubl.	Asteraceae	kasalla'
39.	*Garcinia celebica* L.	Clusiaceae	tire
40.	*G. celebica*	Clusiaceae	tire
41.	*G. dulcis* (Roxb.) Kurz	Clusiaceae	tire
42.	*G. xanthochymus* Hook.f. ex T.Anderson	Clusiaceae	-
43.	*Hoya revoluta* Wight.	Asclepiadaceae	doi'-doi'
44.	*Hypobathrum multibracteatum* Elmer	Rubiaceae	-
45.	*Knema celebica* (Poir.) Warb. var. *cinerea*	Myristicaceae	kuerempang
46.	*Lagerstroemia speciosa* (L.) Pers.	Lytheraceae	allouting
47.	*Leea asiatica* (L.) Ridsdale	Leeaceae	arunganga
48.	*L. rubra* Blume ex Spreng	Leeaceae	mali-mali
49.	*Lepisanthes rubiginosa* (Roxb.) Leenh.	Sapindaceae	jampu nono
50.	*Luisia taurina* J.J.Sm.	Orchidaceae	-
51.	*Lygodium circinnatum* (Burm.f.) Sw.	Schizaeaceae	cawe
52.	*Magnolia candollii* (Blume) H. King var. *candollii*	Annonaceae	sarikajaale
53.	*Malaxis blumei* Bakh.f.	Orchidaceae	pije'-pije'
54.	*Mallotus floribundus* (Blume) Mull.Arg.	Malvaceae	waru-waru
55.	*Mangifera indica* L.	Anacardiaceae	pao cucok
56.	*M. indica*	Anacardiaceae	pao kurisa'
57.	*Mucuna pruriens* L.	Papilionaceae	awiyu
58.	*Nephrolepis cordifolia* (L.) Pr.	Davalliaceae	-
59.	*Nervilia aragoana* Gaudich.	Orchidaceae	-
60.	*N. aragoana*	Orchidaceae	-
61.	*N. aragoana*	Orchidaceae	-
62.	*Ophioglossum reticulatum* L.	Ophioglossaceae	tungke'-tungke'
63.	*Pandanus tectorius* Parkinson ex Du Roi	Pandanaceae	-
64.	*Phalaenopsis amabilis* (L.) Blume	Orchidaceae	-
65.	*Phytocrene bracteata* Wall.	Icacinaceae	-
66.	*Piper amboinense* C.DC.	Piperaceae	ganjing tedong
67.	*P. caninum* Blume	Piperaceae	-
68.	*P. flavimarginatum* C.DC.	Piperaceae	-
69.	*Planchonia valida* (Blume) Blume	Lecythidaceae	alakkan
70.	*Psychotria celebica* L.	Rubiaceae	kopi ale'

Table 1. Contd.

No.	Species	Family	Local names
71.	*Pterospermum celebicum* Miq.	Sterculiaceae	wajo'
72.	*Pyllanthus lamprophyllus* Mull.Arg.	Euphorbiaceae	cempa-cempa
73.	*Sandoricum koetjape* (Burm.f.) Merr.	Meliaceae	-
74.	*Sesuvium portulacastrum* (L.) L.	Aizoaceae	-
75.	*Stemona moluccana* (Blume) C.H. Wright	Stemonaceae	-
76.	*Sterculia foetida* L.	Sterculiaceae	bimpi
77.	*Syzigium aqueum* (Burm.f.) Alston	Myrtaceae	jampu-jampu salo'
78.	*Tabernaemontana celebica* Miq.	Apocynaceae	lambuto
79.	*Tacca palmata* Blume	Taccaceae	-
80.	*Tectaria shahidaniana* Rusea	Aspidiaceae	warang parang
81.	*Tetracera scandens* (L.) Merr.	Dilleniaceae	apelle'
82.	*Thrixspermum mus* S. Rao	Orchidaceae	-
83.	*Vittaria flexuosa* Fee	Aspidiaceae	-

Table 2. The plant families, unidentified species collected and local names.

No	Family	Species & Local names
1.	Acanthaceae	*Acanthus* sp.
2.	Actinidiaceae	*Saurauia* sp.
3.	Adiantaceae	*Adiantum* sp.
4.	Agavaceae	*Pleomele* sp. (jalojo)
5.	Annonaceae	*Desmos* sp.1 (loka-loka), *Desmos* sp.2*, Polyalthia* sp.1 (kayu awayu), *Polyalthia* sp.2, *Polyalthia* sp. 3*, Trigonostemon* sp. (tabo)
6.	Apocynaceae	*Alstonia* sp. (tiro tarik), *Tabernaemontana* sp.*, Stropanthus* sp.*,* Apocynaceae (damak-damak)
7.	Araceae	*Alocasia* sp.1 (lawira), *Alocasia* sp.2 (aladi), *Alocasia* sp.4*, Colocasia* sp.1, *Colocasia* sp.2, *Phylodendron* sp.*, Pothos* sp.1 (banga-banga), *Pothos* sp.2
8.	Araliaceae	*Aralia* sp. (pallong-pallong)
9.	Arecaceae	*Daemonorops* sp.1 (rokan), *Daemonorops* sp.2 (anak rokan), *Licuala* sp. (talitta)
10.	Asclepiadaceae	*Dischidia* sp. (sikeppo), *Hoya* sp.1 (sikeppo)
11.	Burseraceae	*Canarium* sp. (lao bawi')
12.	Celastraceae	*Maitenus* sp.
13.	Clusiaceae	*Calophyllum* sp. (betao), *Cratoxylum* sp. (geleng keleng), *Garcinia* sp.1 (tire), *Garcinia* sp.2 (pakkeci anak lolo), *Garcinia* sp.3 (pakkeci anak lolo), *Garcinia* sp.4 (pakkeci anak lolo)
14.	Ebenaceae	*Diospyros* sp. (amara coppo)
15.	Euphorbiaceae	Euphorbiaceae (kaluku-kaluku)
16.	Fabaceae	*Abrus* sp.*, Desmodium* sp.*, Hymnaea* sp. (kayu bayang), *Peltophorum* sp.*, Pterocarpus* sp.1*,* (*calaipi*), *Pterocarpus* sp.2 (cenrana), Fabaceae1 (warneng), Fabaceae2 (kayu langi)
17.	Flacourtiaceae	*Flacourtia* sp.
18.	Lauraceae	*Cinnamomum* sp. (alinie'), *Litsea* sp.1 (kayu kunyik-kunyik), *Litsea* sp.2 (rela-rela)
19.	Lecythidaceae	*Cydaenanthus* sp.
20.	Lytheraceae	*Lagerstroemia* sp.1 (geleng keleng), *Lagerstroemia* sp.2
21.	Marantaceae	*Donax* sp.1 (bampeng)
22.	Meliaceae	*Dysoxylum* sp. (dare'-dare'), *Aglaia* sp.
23.	Moraceae	*Artocarpus* sp.1 (terou), *Artocarpus* sp.2 (kalompe), *Artocarpus* sp.3 (ampalang), *Ficus* sp.
24.	Myristicaceae	*Knema* sp.1 (kelam pelam, pala hutan), *Myristica* sp. (kayu buang)
25.	Myrtaceae	*Acmena* sp.*, Syzigium* sp.1 (jampu-jampu), *Syzigium* sp.2 (jampu-jampu), *Syzigium* sp.3*, Syzigium* sp.4 (mana'-mana'), *Syzigium* sp.5 (jampu-jampu), *Syzigium* sp.6 (tajulo)
26.	Orchidaceae	*Calanthe* sp. (pije'-pije'), *Dendrobium* sp.*, Habenaria* sp.*, Trichoglottis* sp.
27.	Pandanaceae	*Pandanus* sp. (banga)
28.	Piperaceae	*Piper* sp.1 (ganjing), *Piper* sp.2*, Piper* sp.3, *Piper* sp.4
29.	Rhamnaceae	*Ziziphus* sp. (carrakak panning)
30.	Rubiaceae	*Psychotria* sp.2, Rubiaceae

Table 2. Contd.

No	Family	Species & Local names
31.	Rutaceae	*Citrus* sp., *Melicope* sp.1 (matte-matte hutan), *Melicope* sp.2 (amara siapa), Rutaceae1 (matte-matte), Rutaceae2 (tana-tana)
32.	Sapindaceae	*Euphoria* sp. (jampu nono), Sapindaceae1 (lotong-lotong), Sapindaceae2, Sapindaceae3 (kayu ori), Sapindaceae4
33.	Schizaeaceae	*Lygodium* sp. (cawe)
34.	Smilacaceae	*Smilax* sp. (bana')
35.	Sterculiaceae	*Sterculia* sp.1, *Sterculia* sp.2 (sime'), *Sterculia* sp.3, *Sterculia* sp.4 (kuerempang)
36.	Taccaceae	*Tacca* sp.
37.	Theaceae	Theaceae
38.	Tiliaceae	Tiliaceae (gose-gose)
39.	Verbenaceae	*Vitex* sp.
40.	Zingiberaceae	*Ammomum* sp. (kacimpang)

ACKNOWLEDGMENTS

We would like to express our gratitude to the Research Centre for Plant Conservation and Botanic Garden-LIPI (Bogor Botanic Garden, West Java), Purwodadi Botanic Garden-LIPI (East Java), South Sulawesi Provincial Government, Forest Management Unit (KPH) Ajatappareng, Forestry Service (South Sulawesi), Pucak Botanic Garden (South Sulawesi), Indonesian Army Special Forces, Gadjah Mada University, The Flora Fauna Research Group of *Sub Korwil VII*/Gowa-*Ekspedisi NKRI Koridor Sulawesi*, Mr. Ruspandi for identification and all people who kindly helped and contributed in the expedition.

REFERENCES

Bouman, R.W., Kesler, P.J.A., & van Welzen, P. 2019. Lectotypification and amended description of *Phyllanthus* (Phyllanthaceae) species described by Koorders from Sulawesi, Indonesia. Reinwardtia 18(2):97-103

Cox, P.A. & Sacks, O.W. 2002. Cycad neurotoxins, consumption of flying foxes, and ALS-PDC disease in Guam. Medical Hypothesis. AAN Enterprises Inc.

Dinas Kehutanan Kabupaten Barru. 2005. Rencana Pembangunan Jangka Panjang Kabupaten Barru. Dinas Kehutanan Kabupaten Barru.

Gradstein S.R., Kessler M., Pitopang R. 2007. Tree species diversity relative to human land uses in tropical rain forest margins in Central Sulawesi. In: Tscharntke T., Leuschner C., Zeller M., Guhardja E., Bidin A. (eds) Stability of Tropical Rainforest Margins. Environmental Science and Engineering (Environmental Science). Springer, Berlin, Heidelberg

Govaerts, R. (ed.). 2000. In: Species 2000 & IT IS Catalogue of Life, [2020-01-10] Beta (Roskov, Y; Ower, G; Orrel, T.; Nicolson, D.; Bailly, N.; Kirk, P.M.; Bourgoin, T.; DeWalt, R.E.; Decock, W.; Nieukerken, E. van; Penev, L; eds.). Digital resource at www.catalogueoflife.org/col. Species 2000: Naturalis, Leiden, the Netherlands. ISSN 2405-8858

Hill, K.D. 2010. *Cycas rumphii*. The IUCN Red List of Threatened Species 2010: e.T42081A10623127. https://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T42081A10623127.en.

Irudayaraj, V. 2011. *Ophioglossum reticulatum*. *The IUCN Red List of Threatened Species* 2011: e.T194160A8885705. https://dx.doi.org/10.2305/IUCN.UK.2011-1.RLTS.T194160A8885705.en. Downloaded on 03 February 2020.

Kikuchi, T., Kadota, S., Hanagaki, S., Suehara, H., Namba, T, Lin, C, & K. W. 1981. Studies on the Constituents of Orchidaceous Plants. I. Constituents of *Nervilia purpurea* Schlechter and *Nervilia aragoana* Gaud. Chem. Pharm. Bull. 29(7):2073-3078

Klein, G., Kim, J., Himmeldirk, K., Cao, Y., & Chen, X. 2007. Antidiabetes and Anti-obesity Activity of *Lagerstroemia speciosa*. eCAM 2007 4 (4): 401–407

Kumoro, A.C., Retnowati, D.S. & Budiyati, C.S. 2011. Removal of cyanides from gadung (*Dioscorea hispida* Dennst.) tuber chips using leaching and steaming techniques. J.of App. Sci. Research 7(12):2140-2146

Mamoon, S.A., & Azam, M.G. 2012. Preliminary phytochemical screening and antidiarrhoeal activity of *Derris trifoliata* Lour. Int. J. of Phar. Sci. 3(1): 97-100

Mogea, J., Seibert, B., & Smits, W. 1991. Multipurpose palms: the sugar palm (*Arenga pinnata* (Wurmb) Merr.). Agroforestry Systems 13:111-129

Mukherjee, A., Das, R., Pal, A., & Paul, A.K. 2017. Antibacterial activity of endophytic Bacillus safensis isolated from *Ophioglossum reticulatum* L. Micr. Res. Journ. Int. 18(5): 1-12

Muliyah, E., Sulistijorini, Sulistiyaningsih, Y.C., Rafi, M. 2017. *Tetracera scandens* as a medicinal plant: Secretory structures, histochemistry, and antibacterial activity. The Jour. Of Trop. Life. Sci. 8(1): 68-74

Paknia, O., Rajaei Sh., H & Koch, A. 2015. Lack of well-maintained natural history collections and taxonomists in megadiverse developing countries hampers global biodiversity exploration. Org Divers Evol. 15:619-629. https://doi-org.elibrary.jcu.edu.au/10.1007/s13127-015-0202-1

Pemerintah Kabupaten Barru. 2013. Rencana Program Investasi Infrastruktur Jangka Menengah (RPI2JM) Kabupaten Barru. Barru.

Riswan, S. 2002. Kajian Biologi Eboni (*Diospyros celebica* Bakh.). Berita Biologi 6(2): 211-218

Roskov, Y., Zarucchi, J, Novoselova, M & Bisby, F. (eds.) 2020. ILDIS: ILDIS World Database of Legumes (version 12, May 2014). In: Species 2000 & IT IS Catalogue of Life, 2020-02-24 (Roskov, Y., Ower, G., Orrel, T, Nicolson, D, Bailly, N., Kirk, P.M., Bourgouin, T., DeWalt, R.E., Decock, W., Nieukerken, E. van, Penev, L.). Digital resource at www.catalogueoflife.org/col. Species 2000: Naturalis, Leiden, the Netherlands. ISSN 2405-8858

Saiful, A.M. & Burhan, B. 2017. Lukisan fauna, pola sebaran dan lanskap budaya di kawasan kars Sulawesi bagian Selatan. Jurnal Walennae 15 (2): 75-88

Semiarti, E. 2002. Biotechnology for Indonesian orchid conservation and industry. Proc. Of Inventing Prosperous Future through Biological Research and Tropical Biodiversity Management. AIP. Conf. https://doi.org/10.1063/1.5050118

Thanh, T.B., Thanh, H.N., Minh, P.T., Le-Thi-Thu, H., Ly, H.D.T., & Duc, L.V. 2015. Protective effect of *Tetracera scandens* L. leaf extract against CCl_4-induced acute liver injury in rats. Asian Pac. J. Trop. Biomed 5(3): 221-227

Walujo, E.B. 2002. Gatra Etnobotani Eboni (*Diospyros celebica* Bakh.). Berita Biologi 6(2): 251-254

Wenjie, J., Yuchun, F., Chunji, G., Yunhui, W., & Jie, P. 2009. Extraction and purification of deguelin from *Derris trifoliata* Lour. root. Int. J. Agric. & Biol. Eng. 2(4): 98-103

Widjaja, E. & Pratama, B. 2013. Flora Diversity Loss in Bioregion Sulawesi. Proceeding. International Conference on Forest and Biodiversity. Manado Forestry Research Institute.

World Conservation Monitoring Centre. 1997. Conservation and Management of Trees. Report of Regional Workshop. The Army Hotel, Hanoi, Vietnam.

World Conservation Monitoring Centre. 1998. *Diospyros celebica*. The IUCN Red List of Threatened Species 1998. e.T33203A9765120. https://dx.doi.org/10.2305/IUCN.UK.1998.RLTS.T33203A9765120.en.

Journal of Tropical Biodiversity and Biotechnology
Volume 05, Issue 02 (2020): 100 — 107
DOI: 10.22146/jtbb.53714

Research Article

Physicochemical Characters of Mosquitoes Natural Breeding Habitats: First Record in High Dengue Hemorrhagic Fever Cases Area, East Java, Indonesia

Rosmanida[1,2]*, Shifa Fauziyah[1,3], Adi Pranoto[4]

1) Laboratory of Entomology, Institute of Tropical Disease, Universitas Airlangga, Mulyorejo, 60115, Surabaya, East Java, Indonesia
2) Biology Department, Faculty of Science and Technology, Universitas Airlangga, Mulyorejo, 60115, Surabaya, East Java, Indonesia
3) Master Program of Tropical Medicine Department, Faculty of Medicine, Universitas Airlangga, Tambaksari, 60132, Surabaya, East Java, Indonesia
4) Master of Sport Health Science, Faculty of Medicine, Universitas Airlangga, Tambaksari, 60132, Surabaya, East Java, Indonesia

Submitted: 23 January 2020; Accepted: 29 March 2020 Published: 15 August 2020

ABSTRACT

This research aims to identify physicochemical characteristics in natural breeding habitats/ phytotelmata of dengue vector—including *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) albopictus*. The research was conducted during rainy season and pre-dry season (from January to June 2017) in the region with the high cases of Dengue Hemorrhagic Fever (DHF). The entomological survey was carried out by stratified random sampling in urban and rural areas in order to find potential breeding habitats, every natural breeding habitats in sampling location were checked for the presence of *Aedes* larvae. Physicochemical characters that consist of temperature, turbidity, carbon dioxide, ammonia, nitrate, sulphate, pH and dissolved oxygen were recorded. Larval species were taken and then identifying activities were conducted in the Laboratory of Entomology. Data were analysed using the Chi-square test. Results showed that only dissolved oxygen that significantly associated with larval abundance (p=0.039). while others are not significantly associated. Whereas, other characters are associated with each other, carbon dioxide associated with the ammonia and sulphate (p=0.001; p=0.028). Turbidity associated with the dissolved oxygen (p=0.022) and pH associated with nitrate (p=0.001).

Keywords: *Aedes aegypti*, *Aedes albopictus*, dengue, East Java, natural breeding habitat, phytotelmata

INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is a public health problem in tropical and sub-tropical countries. World Health Organization (WHO) estimated that 50 million people around the world were infected, half of whom need hospitalization. The highest proportion of DHF patients is children with the age of less than five years old (WHO SEARO, 2011). DHF is caused by dengue virus that transmitted by mosquito bites so that its classified as mosquito-borne diseases. DENV has four serotypes namely DENV-1, DENV-2, DENV-3 and DENV-4 (Gubler, 2002). *Aedes (Stegomyia) aegypti* is known as a primary vector of DHF, while *Aedes (Stegomyia) albopictus* is a secondary vector in South East Asia and Western Pacific (World Health Organization, 2008).

East Java Province is one of the endemic provinces that still have high cases in DHF. Based on the data from (Ministry of Health Republic of Indonesia, 2018), the number of DHF cases in East Java Province in 2016 was 24005 cases, with the incidence rate of 61.43 every 100.000 people. DHF prevention methods have been implemented by East Java government such as health promotion, surveillance, periodically larval controlling, public health behavior (drain, buried and close also called 3M) (Ministry of Health Republic of Indonesia, 2018).

Transmission of DHF depends on season

***Corresponding author**
Tel.: +62 812-3565-798
Email: rosma1954@yahoo.com

conditions and availability of its vector which is very sensitive to temperature and moisture (Thai and Anders, 2011). Dengue vaccine still on the developing phase prior to finding high efficacy against all serotypes (Wilder-Smith *et al.*, 2016). Hence, the most effective controlling program of DHF is vector control.

Understanding bionomic and characteristic of vector is crucial in controlling the program. Distribution of *Aedes aegypti* and *Aedes albopictus* is strongly correlated with the global climate (Ravikumar *et al.*, 2013). In case, the developmental cycle of *Aedes aegypti* depends on some environmental factors like rainfall, temperature, relative humidity (Eisen *et al.*, 2014).

Aedes mosquitoes spread widely and have two breeding habitats, indoor and outdoor. Meanwhile, based on their formation, breeding habitats of *Aedes* mosquitoes are divided into two kinds, that are natural and artificial breeding habitats. Artificial breeding habitat is a breeding habitat formed because of human/animal activity such as tank, bathtub, crock, vast, tank, cans and tyres. Natural breeding habitats is a breeding habitat formed without human/animal activity such as pool, tree hole, leaf axils, fruit shells, fallen leaf, fallen spathe and so on (Ali *et al.*, 2014; Chadee *et al.*, 1998).

Research on natural breeding habitats is still limited. Some countries, such as USA and Africa, have been doing research on potential natural breeding sites that might be an account of DHF vector, such as rock holes, tree hole, leaf axils, bamboo joints, coconut shells, and also Bromelia (Chadee *et al.*, 1998). Some Bromeliad species become mosquitoes breeding habitats (Shultis, 2009). Research from (Simard *et al.*, 2006) showed that the highest natural breeding site in Cameroon Africa is a tree hole. Based on this problem, they did research on various kinds of phytotelmata that were found in areas with high DHF cases in East Java Province, Indonesia.

Although intensively control program was conducted, only one research on natural breeding site in Indonesia already published in the past ten years (Rosa *et al.*, 2017). Based on this understanding, this research aims to identify the physicochemical characters that support growth and development of Aedes larvae, specifically in natural breeding habitats.

MATERIALS AND METHODS

Materials

The instruments used in this research were vial bottles (100 mL and 10 mL), tray, plastic pipette, universal pH paper (Merck & Co, NewYork), thermometer (Thermo, Indonesia), DO meter (Lutron DO meter 5510), sling psychrometer TS90, object-glass, cover glass, labels, and plastic glass, gauze, rubber, identification key using larval and adult identification key of *Aedes* in Java, Indonesia.

Methods

The entomological survey was conducted during rainy season and pre-dry season (January to June 2017) in an area that represented as high dengue risk in East Java Province that is Pacitan, Sidoarjo, Malang, Jombang, Banyuwangi, and Bangkalan. Study areas were shown in Figure 1 with legend (A: Pacitan Residence; B: Malang Residence; C:

Figure 1. Research area of entomological survey were signed by red circle (EpiInfo, 2019).

Banyuwangi Residence; D: Sidoarjo Residence; E: Bangkalan Residence; F: Jombang Residence). Study areas were chosen based on unpublished data from East Java Province Health Office, with detail on number of population and number of cases below (Table 1).

Table 1. Number of population and number of cases in each study area.

Legend	Study Area	Population Number	Number of DHF cases
A.	Pacitan	552.307	1149
B.	Malang	2.560.675	950
C.	Banyuwangi	1.599.811	1319
D.	Sidoarjo	2.150.482	1455
E.	Bangkalan	962.771	801
F.	Jombang	1.247.303	741

Based on (Kitching, 1971) phytotelmata can be categorized as six groups namely leaf axils, tree holes, tree stumps, fallen spathe, fruit shells and fallen leaf. Every phytotelma found during research was checked by using a flashlight in order to confirm the presence of *Aedes* larvae. The simple way to differentiate between *Aedes* larvae and other larvae was by checking their siphon and their movement pattern. A total of 53 water samples were collected from various phytotelmata (nine phytotelmata from study site A, five from B, ten from C, fourteen from D, seven from E, and seven from F). By using plastic pipettes, every larva found was collected into 10 mL vial bottle, while 100 mL water sample was added into vial bottle. Every sample was labeled based on the date of collection, location of phytotelmata, phytotelma categories and the name of species. Water samples then packed in cold boxes and were transported to the laboratory for further analysis. Some parameters were measured directly on the spot, such as temperature, dissolved oxygen, and pH using thermometer (Thermo, Indonesia), Lutron DO meter 5510, and universal pH paper (Merck, New York) whereas other parameters measurement were done by analysis service in Balai Besar Teknik Kesehatan Lingkungan Surabaya. Another physicochemical factors (turbidity, carbon dioxide, ammonia, sulphate, and nitrate) were analyse used spectrophotometric based on the guideline of American Public Health Association (American Public Health Association, 1998) In order to identify the species of larvae, fourth instar stage of larvae were killed by 4% formalin, while pupae were maintained until they become adult mosquitoes. Data were analyzed using SPSS for Chi -square test, with the normalized test was done before.

RESULTS AND DISCUSSION

Distribution of phytotelma and physicochemical factors in each study area

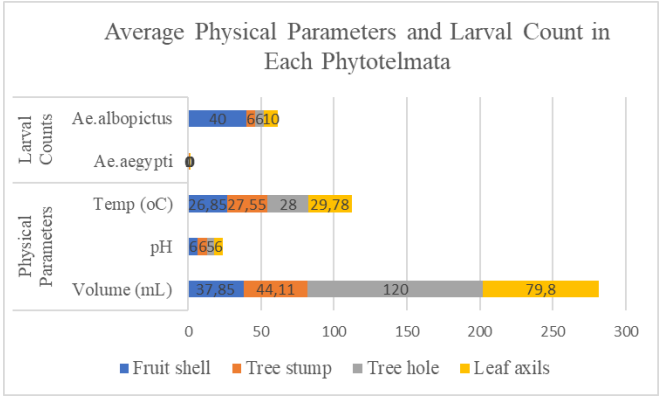
Distribution of each phytotelma that contains *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) albopictus* larvae was shown in Table 2. The number of *Aedes* larvae counted on phytotelmata and physical parameters were shown in Graphic 1. Bivariate correlations between physicochemical factors and larval abundance were shown in Table 3.

Figures of phytotelmata

There were 4 types of phytotelmata found in this research namely leaf axils, tree stumps, fruit shells, and tree holes (Figure 2).

Figure 2. Different types of phytotelmata that were found.

Physical Parameters and Larval Counts in each kind of phytotelma



Graphic 1. Average physical parameters and larval count in each phytotelma.

Table 2. List of phytotelmata that were found during entomological survey.

No.	Plant Species	Plant Family	Leaf Axils	Tree Stumps	Fruit Shells	Tree Holes
1.	*Neoregelia carolinae*	Bromeliaceae	√			
2.	*Neoregelia* 'Royal Burgundy'	Bromeliaceae	√			
3.	*Musa paradisiaca*	Musaceae	√			
4.	*Alcantarea imperialis*	Bromeliaceae	√			
5.	*Dracaena fragrans*	Asparagaceae	√			
6.	*Bambusa balcooa*	Poaceae		√		
7.	*Colocasia esculenta*	Araceae	√			
8.	*Hibiscus macrophyllus*	Malvaceae				√
9.	*Xanthosoma sagittifolium*	Araceae	√			
10.	*Guzmania lingulate*	Bromeliaceae	√			
11.	*Portea petropolitana*	Bromeliaceae	√			
12.	*Agave Americana* var.Marginata	Bromeliaceae	√			
13.	*Neoregelia hybrid*	Bromeliaceae	√			
14.	*Cocos nucifera*	Arecaceae			√	

Table 3. Bivariate Correlations between Physicochemical Factors and Larval Abundance.

	Temperature	Turbidity	Carbon dioxide	Ammonia	Nitrate	Sulphate	pH	DO
Temperature		0,763	0,787	0,853	0,367	0,321	0,474	0,225
Turbidity	0,763		0,908	0,755	0,493	0,234	0,978	0,022*
Carbon dioxide	0,787	0,908		0,001*	0,092	0,028*	0,779	0,556
Ammonia	0,853	0,755	0,001*	-	0	0	0,196	0,399
Nitrate	0,367	0,493	0,092	0	-	0	0,001*	0,371
Sulphate	0,321	0,234	0,028*	0	0	-	0	0,281
pH	0,474	0,978	0,779	0,196	0,001*	0	-	0,996
DO	0,225	0,022*	0,556	0,399	0,371	0,281	0,996	
Larval abundance	0,054	0,977	0,38	0,874	0,601	0,545	0,628	0,039*

Discussion

The occurrence of mosquito larval in natural breeding sites/ phytotelmata related to the oviposition site selection. The mosquito activity can be influenced by physicochemical factors that can play as an attractant or a deterrent (Thangamathi *et al*., 2014). Rainfall, relative humidity, temperature, and wind speed are known as environmental factors that can affect mosquitoes laying eggs, whereas visual, olfactory and tactile responses are biological factors related to metabolism in mosquito's body. Current research found four kinds of phytotelmata, which are leaf axils, tree stumps, fruit shells and tree holes. Phytotelmata in this research were classified into 7 families, which are family of Bromeliaceae, Musaceae, Asparagaceae, Poaceae, Araceae, Malvaceae, and Arecaceae. Leaf axils were the most breeding site that was found followed by tree stumps, fruit shells, and tree holes.

A total of 714 larvae were collected, consisting of 691 larvae *Aedes (Stegomyia) albopictus* and 23 larvae *Aedes (Stegomyia) aegypti*. Some water parameters are important in public health and used as an indicator of drinking-quality (World Health Organization, 2008). Studying physical and chemical factors is significant in order to understand the bionomics of *Aedes*. Some studies found that physicochemical factors are associated significantly with the larval species. (Ghanbari *et al*., 2019) mentioned that 7 physicochemical factors correlate to *Anopheles* species namely pH, total hardness, nitrate, phosphate, calcium, electrical conductivity, and sulphate. This research found that the temperature range of larval breeding sites is 27-32^{o} C; suitable with (Hanafi-Bojd *et al*., 2012) result shows that the temperature of breeding sites during larval collection is 20-32^{o}C. Temperature is a crucial factor that can affect the growth, development, and survival of mosquito. The more increase in temperature, the more decrease in mosquito body size (Rueda *et al*., 1990).

Leaf axils as the most frequent kind of breeding site in this area consisting of many species based on the bromeliad identification key by (Derek Butcher and Dean Fairchild, 2017) namely *Neoregelia carolinae, Neoregelia* 'Royal Burgundy', *Musa paradisiaca, Alcantarea imperialis, Dracaena fragrans, Colocasia esculenta, Xanthosoma sagittifolium, Guzmania lingulata, Portea petropolitana, Agave Americana* var. Marginata, and *Neoregelia hybrid*. Tree stumps consist of two species that is *Bambusa balcooa* and *Bambusa vulgaris*. Otherwise, tree holes consist of *Hibiscus macrophyllus*. Bad management of rubbish especially on fruit shells advances to the occurrence of larval breeding sites, especially the fruit shells of *Cocos nucifera*. This finding corresponded to the research of (Thangamathi *et al*., 2014). In contrast, larval counts on *Cocos nucifera* were mostly *Aedes (Stegomyia) albopictus*, which was different from the result of (Thangamathi *et al.,* 2014) *Cocos nucifera* is mostly found on shady area which can provide ideal condition for mosquito's oviposition.

Reports of breeding site especially natural breeding sites in Indonesia is still limited, only one article that has been published (Rosa *et al*., 2017). Meanwhile, thus research showed more various kinds of phytotelmata: tree holes, tree stumps, fallen spathe, fruit shells, and fallen leaves. However, *Aedes* species found are different from current research that are *Aedes (Stegomyia) albopictus* and *Aedes (Stegomyia) crysolineatus*. The turbidity value in current research is in a range between 0.04-2.42 NTU. The highest turbidity was found in Pacitan, while the lowest was found in Bangkalan. However, there was still no exact values of physicochemical factors related to the abundance of larval mosquito-like both mentioned by Ghanbari *et al.* (2019) and (Hanafi-Bojd *et al*., 2012). Turbidity is one of the physical factors that have little difference in both *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia)* albopictus. Turbidity value related to the accumulation of eroded soils after rain or other materials from stagnant water. A study shows that turbid water attracts more female *Anopheles*, but at some points will affect mortality (Mwangangi *et al*., 2010; Sehgal & Pillai, 1962). However, some studies reveal that turbid water has an importance which provides a suitable place for the development of first till third larval instar and pupal stage. Other than that, turbid water can suppress the development of the fourth larval instar (Sehgal & Pillai, 1962). This controversial still need to be supported with massive research, so that vector control program related to the turbidity of water can be conduct based on currently finding.

Dissolved oxygen shows a significant association with the larval counts. The range value of dissolved oxygen in this research was between 3.4 and 5.7 ppm. Dissolved oxygen contained in water related with the carbon dioxide tension. Association between physicochemical factors and larval abundance was proven. Variable that shows a positive association with the larval abundance in this research was dissolved oxygen ($p=0.039$). These results are in agreement with other research reported (Muturi *et al*., 2008). Dissolved oxygen is an important factor that affected survival and mosquito breeding site (Oyewole *et al*., 2009)

Another physicochemical factor shows negative correlation with the larval abundance: temperature ($p=0.054$); turbidity ($p=0.977$); carbon dioxide ($p=0.38$); ammonia ($p=0.874$); nitrate

(p=0.601); sulphate (p=0.545); pH (p=0.628). Chi-square test was used in the analysis, and the result was shown in Table 3. Whereas, characters are associated with each other, carbon dioxide associated with the ammonia and sulphate (p=0.001; p=0.028). Turbidity associated with the dissolved oxygen (p=0.022) and pH associated with nitrate (p=0.001). Our findings show that temperature didn't have significant association (p=0.054) with the larval abundance. Even though, some studies reveal the influence of temperature on the abundance of mosquito larvae, regarding the classification of mosquitoes as a poikilothermic animal. The more increase in water temperature, the faster mosquito larval will develop. Other than that, adult size of mosquito will decrease, and at the highest temperature, mortality will be increased (Bayoh and Lindsay, 2003; White, 1974). Interaction between temperature, pH, and ammonia can give various impacts. Ideal pH for mosquitoes ranged from 8 to 8.8, high pH above the range will disturb the survival of mosquito related with the increase of ammonia (Kwasi *et al*., 2012).

Bivariate correlation between nitrate and pH value shows a significant association (p=0.001). Mosquito larval were known can tolerate various level of nitrate, and nitrate value related with other organism activities. A study reported that increasing nitrate can increase the density of mosquito larvae (Sunish and Reuben, 2002). Meanwhile, there is also a report that shows decreasing nitrogen can affect increasing of larval densities (Mala and Irungu, 2011).

Water volume that holds in every phytotelma was also measured as additional parameters. Then, it was classified into each kind of phytotelma. Based on Graphic 1, tree hole is a kind of breeding site that can hold high water volume (120 mL), followed by leaf axils (79.8 mL), water-hold in tree stumps and fruit shell does not differ significantly that is 44.11 and 37.85 mL respectively. Stratified random sampling in urban and rural area tributes to the several breeding sites that were found. Bromeliads are mostly found in urban area, whereas the majority of breeding sites in this research in rural area was fruit shells. Each of breeding sites consists of various stages of larval; some of them have been in pupal stage. It can be an indicator that breeding site is suitable for the development of larval, regarding to the optimum value of every physicochemical factor.

Current investigations found some containers contain both larval of *Aedes aegypti* and *Aedes albopictus* in one container. Survivorship of both species in one container cannot be separated from interspecific competition supported by environmental factors. Nutritional sources for mosquito larvae come from physicochemical factors, even though in excessive ratio will give a negative impact. Sulfate is a natural matter that composed of sulfur and oxygen, as a result of plant and animal that decayed. Water in phytotelmata contained sulphate may be due to the position under canopy. The higher sulphate, the more larval abundance will increase (Liu *et al*., 2012). Physical variables can take a role in this case and directly affect the competitive interactions, but how the mechanism and prevalence of it are still unclear (Costanzo *et al*., 2005; Dunson and Travis, 1991). Some studies show that variety of environmental factors affect the spread of *Aedes albopictus* (Alto and Juliano, 2009). Detritus as a well-known factor affects competition between both species when holding in one container (Yee *et al*., 2007). Different types of detritus lead to creating different quantities of microorganisms (Yee *et al*., 2007) that can provide a difference in both quantity and quality of food available for larvae.

Our observation shows that water appearance in each breeding site is different; some breeding sites consist of many detritus in the form of fall leaves, while another does not contain it. Both *Aedes aegypti* and *Aedes albopictus* are vectors of mosquito-borne diseases such as yellow fever, dengue, and West Nile Virus, so it makes an interesting thing when basic bionomics of them are understood (Yuill, 1986). Therefore, research about physicochemical characters, especially under laboratory conditions, still needs to be conducted, regarding the different geographical area can lead to different environmental conditions.

CONCLUSION

Dissolved oxygen shows significantly associated with larval abundance (p=0.039) while others are not significantly associated. Whereas, other characters are associated with each other, carbon dioxide associated with the ammonia and sulphate (p=0.001; p=0.028). Turbidity associated with the dissolved oxygen (p=0.022) and pH associated with nitrate (p=0.001). Research about physicochemical characters especially under laboratory conditions still needs to be conducted, regarding the different geographical area can lead to different environmental conditions and useful for making integrated vector management policy.

ACKNOWLEDGMENTS

We would like to thank the Ministry of Education and Technology for the support through research grant (106/PMK.02/2016).

REFERENCES

Ali, K.M., Asha, A. V, Aneesh, E.M., 2014. Bioecology and Vectorial Capacity of Aedes mosquitoes (Diptera : Culicidae) in Irinjalakuda Municipality , Kerala , India in Relation to Disease Transmission. Int. J. Curr. Res. Acad. Rev. 2, 43–49.

Alto, B.W., Juliano, S.A., 2009. Temperature Effects on the Dynamics of Aedes albopictus (Diptera: Culicidae) Populations in the Laboratory . J. Med. Entomol. 38, 548–556.

American Public Health Association, 1998. Standard methods for the examine of water and freshwater. American Public Health Association (APHA), Washington, D.C.

Bayoh, M.N., Lindsay, S.W., 2003. Effect of temperature on the development of the aquatic stages of Anopheles gambiae sensu stricto (Diptera: Culicidae) . Bull. Entomol. Res. 93, 375–381.

Chadee, D.D., Ward, R.A., Novak, R.J., 1998. Natural habitats of Aedes Aegypti in the Caribbean--a review. J. Am. Mosq. Control Assoc. 14, 5–11.

Costanzo, K.S., Kesavaraju, B., Juliano, S.A., 2005. Condition-specific competition in container mosquitoes: The role of noncompeting life-history stages. Ecology 86, 3289–3295.

Derek Butcher and Dean Fairchild, 2017. Bromeliad Key for Dummies.

Dunson, W.A., Travis, J., 1991. The role of abiotic factors in community organization. Am. Nat. 138, 1067–1091.

Eisen, L., Monaghan, A.J., Lozano-Fuentes, S., Steinhoff, D.F., Hayden, M.H., Bieringer, P.E., 2014. The Impact of Temperature on the Bionomics of Aedes (Stegomyia) aegypti , With Special Reference to the Cool Geographic Range Margins . J. Med. Entomol. 51, 496–516.

Ghanbari, M.R., Khourshid, R.A., Salehi, A., Hasanzehi, A., 2019. THE STUDY OF PHYSICAL AND CHEMICAL FACTORS AFFECTING BREEDING PLACES OF ANOPHELES IN IRANSHAHR. Zahedan J. Res. Med. Sci. 7, 221–227.

Gubler, D.J., 2002. The global emergence/ resurgence of arboviral diseases as public health problems. Arch. Med. Res. 33, 330–342.

Hanafi-Bojd, A.A., Vatandoost, H., Oshaghi, M.A., Charrahy, Z., Haghdoost, A.A., Sedaghat, M.M., Abedi, F., Soltani, M., Raeisi, A., 2012. Larval habitats and biodiversity of anopheline mosquitoes (Diptera: Culicidae) in a malarious area of southern Iran. J. Vector Borne Dis. 49, 91–100.

Kitching, R.L., 1971. An Ecological Study of Water-Filled Tree-Holes and their Position in the Woodland Ecosystem. J. Anim. Ecol. 40, 281.

Kwasi, B., Biology, A., Kumasi, T.-, 2012. Physico-chemical Assessment of Mosquito Breeding Sites from Selected Mining Communities at the Obuasi Municipality in Ghana . 2, 123–130.

Liu, X.B., Liu, Q.Y., Guo, Y.H., Jiang, J.Y., Ren, D.S., Zhou, G.C., Zheng, C.J., Liu, J.L., Chen, Y., Li, H.S., Li, H.Z., Li, Q., 2012. Random repeated cross sectional study on breeding site characterization of Anopheles sinensis larvae in distinct villages of Yongcheng City, People's Republic of China. Parasites and Vectors 5, 1–12.

Mala, A.O., Irungu, L.W., 2011. Factors influencing differential larval habitat productivity of Anopheles gambiae complex mosquitoes in a western Kenyan village. J. Vector Borne Dis. 48, 52–57.

Ministry of Health Republic of Indonesia, 2018. Indonesian Health Profile Year 2017. Minist. Heal. Indones. 107–108.

Muturi, E.J., Mwangangi, J., Shililu, J., Jacob, B.G., Mbogo, C., Githure, J., Novak, R.J., 2008. Environmental factors associated with the distribution of Anopheles arabiensis and Culex quinquefasciatus in a rice agro-ecosystem in Mwea, Kenya. J. Vector Ecol. 33, 56–63.

Mwangangi, J.M., Shililu, J., Muturi, E.J., Muriu, S., Jacob, B., Kabiru, E.W., Mbogo, C.M., Githure, J., Novak, R.J., 2010. Anopheles larval abundance and diversity in three rice agro-village complexes Mwea irrigation scheme, central Kenya. Malar. J. 9, 1–10.

Oyewole, I.O., Momoh, O.O., Anyasor, G.N., Ogunnowo, A.A., Ibidapo, C.A., Oduola, O.A., Obansa, J.B., Awolola, T.S., 2009. Physico-chemical characteristics of Anopheles breeding sites: Impact on fecundity and progeny development. African J. Environ. Sci. Technol. 3, 447–452.

Ravikumar, R., Reegan, A.D., Chandrasekar, P., Kumar, C.S., 2013. Distribution of Dengue Vectors during Pre- and Post-Monsoon Seasons in Higher Attitudes of Nilgiri Hills of Western Ghats, India. J. Insects 2013, 1–5.

Rosa, E., Kanedi, M., Okatviani, P.M., Ningsih, W.N., 2017. Phytotelmata Might Account for the High Prevalence of Dengue Hemorrhagic Fever in Lampung , Indonesia. Adv. Life Sci. 7, 15–20.

Rueda, L.M., Patel, K.J., Axtell, R.C., Stinner, R.E., 1990. Temperature-dependent development and survival rates of Culex quinquefasciatus and Aedes aegypti (Diptera: Culicidae). J. Med. Entomol. 27, 892–898.

Sehgal & Pillai, 1962. Preliminary Studies on the Chemical Nature of Mosquito-Breeding Waters in Delhi. Stanford Med. Bull. 20, 156–162.

Shultis, E.B., 2009. Bromeliads as a breeding site for the Dengue vector Aedes aegypti. ISP Collect. 616.

Simard, F., Nchoutpouen, E., Toto, J.C., Fontenille, D., 2006. Geographic Distribution and Breeding Site Preference of <I>Aedes albopictus</I> and <I>Aedes aegypti</I> (Diptera: Culicidae) in Cameroon, Central Africa. J. Med. Entomol. 42, 726–731.

Sunish, I.P., Reuben, R., 2002. Factors influencing the abundance of Japanese encephalitis vectors in ricefields in India - II. Biotic. Med. Vet. Entomol. 16, 1–9.

Thai, K.T.D., Anders, K.L., 2011. the Role of Climate Variability N Change in the Transmission of Dengue. Exp. Biol. Med. 236, 944–954.

Thangamathi, P., Ananth, S., Kala, Maheshwari, R., Gnanasoundrai, A., Nagamani, N., 2014. Seasonal Variations and Physicochemical Characteristics of the Habitats in Relation To the Density of Dengue Vector Aedes Aegypti in Thanjavur, Tamil Nadu, India 5, 271–276.

White, G.B., 1974. Anopheles gambiae complex and disease transmission in Africa. Trans. R. Soc. Trop. Med. Hyg. 68, 278–298.

WHO, 2008. WHO guidelines for drinking-water quality. WHO Chron. 38, 104–8.

WHO SEARO, 2011. Summary for Policymakers. In: Intergovernmental Panel on Climate Change (Ed.), Climate Change 2013 - The Physical Science Basis. Cambridge University Press, Cambridge, pp. 1–30.

Wilder-Smith, A., Vannice, K.S., Hombach, J., Farrar, J., Nolan, T., 2016. Population Perspectives and World Health Organization Recommendations for CYD-TDV Dengue Vaccine. J. Infect. Dis. 214, 1796–1799.

Yee, D.A., KAUFMAN, M.G., JULIANO, S.A., 2007. The significance of ratios of detritus types and micro-organism productivity to competitive interactions between aquatic insect detritivores. J. Anim. Ecol. 76, 1105–1115.

Yuill, T.M., 1986. The Ecology of Tropical Arthropod-Borne Viruses Author (s): Thomas M. Yuill Source : Annual Review of Ecology and Systematics , Vol . 17 (1986), pp . 189-219 Published by : Annual Reviews Stable URL : https://www.jstor.org/stable/2096994 REFERENCES L.

Journal of Tropical Biodiversity and Biotechnology
Volume 05, Issue 02 (2020): 115 — 123
DOI: 10.22146/jtbb.43381

Research Article

Estimation of Above Ground Carbon Sequestration in Trembesi (*Albizia saman*) and Johar (*Senna siamea*) at PT Multi Harapan Utama, East Kalimantan

Widya Fajariani[1]*, Medi Hendra[1], Dwi Susanto[1]
1) Department of Biology, Faculty of Mathematics and Natural Sciences, Mulawarman University, Jl Barong Tongkok, Gunung Kelua, Samarinda Ulu, East Kalimantan, Indonesia 75123

Submitted: 05 February 2019; Accepted: 07 June 2020; Published: 15 August 2020

ABSTRACT
The open-pit mining method has a very large ecological impact. It causes the loss of forest vegetation which decreases CO_2 absorption. Measuring the amount of carbon stored in plant biomass can represent the amount of CO_2 that can be absorbed in the atmosphere. The objective of this research is to determine the carbon sequestration of *Albizia saman* and *Senna siamea* in different age classes at PT MHU Busang Jonggon Block, Kukar, East Kalimantan. Estimation of carbon sequestration in the stands of *A. saman* and *S. siamea* was carried out by non-destructive methods using biomass allometric equations while in understorey and litter using the destruction sampling. The results showed that the highest carbon absorption value of *A. saman* was 314.28 tons/ha which appear at six years old stands and the lowest value was 193.31 tons/ha at three years old stands while the highest carbon absorption value of *S. siamea* was 113.65 tons/ha which appear at nine years old stands and the lowest value was 24.64 tons/ha at three years old stands. *A. saman* could be more promising plant species than *S. siamea* according to its higher level of carbon sequestration and their high adaptation level. All data from this study could suggest several information for increasing carbon sequestration level in forest ecosystem as well as achieving forest rehabilitation purpose.

Keywords: *Albizia saman, Senna siamea,* biomass, carbon sequestration and mining activity

INTRODUCTION
Climate change is a change of the atmospheric composition and climate variability over a period of time (Regulation of the Minister of Environment and Forestry, 2017). It is caused by the rising temperatures of the earth or known as global warming due to an increase in greenhouse gases (GHG) in the atmosphere. Greenhouse gases have formed by carbon dioxide (CO_2), carbon monoxide (CO), NOx nitrogen oxides, chlorofluorocarbons (CFC), fluoride sulphate, methane, hydrocarbon, water vapour, and others (Bhattacharjee, 2010).

As the terrestrial carbon absorbers, forest plays a vital role in the carbon cycle and able to minimize greenhouse gases in the air. It is because plants can absorb carbon dioxide in the process of photosynthesis (Canadell and Raupach, 2008). Indonesia is one of the countries with a large forest area, which is around 109,961 million hectares consisting of 29,037 million hectares of protected forest, 23,214 million hectares of natural reserves, and the remaining 57.7 million hectares as production forests (Forestry Minister Regulation, 2011). This land cover condition has the potential to store large amounts of carbon stocks.

Forest vegetation in Indonesia can conserve more than 14 billion tons of biomass with total carbon storage of 3.3 billion tons (Nandika, 2005). However, over time the rate of deforestation and forest degradation in Indonesia has increased, thereby reducing the effectiveness of forests in reducing carbon emissions in the air. One of the causes of deforestation and forest degradation in Indonesia is the activity of conversion of forest land used as mining businesses (Masripatin *et al.*, 2010b).

***Corresponding author**
Tel.: +6282157133902
Email: wijayawidya8@gmail.com

Reclamation and revegetation activities of post -mining land are very important in reducing greenhouse gas emissions. According to Hairiah *et al.* (2011), one of the conditions in implementing a reduction in carbon emissions through a REDD+ scheme (Reducing Emissions from Deforestation and Degradation) using the MRV system (Measurable, Reportable and Verifiable). It requires substantial research to strengthen the measurement and estimation of biomass and carbon transparently which can support data as an effort to conserve and increase carbon stocks in the converted forest areas, especially on post-mining revegetation land.

The company that succeeds in implementing revegetation efforts to organize the area disrupted by the mining activities is PT Multi Harapan Utama (MHU). The dominant species planted at MHU are *Falcataria moluccana, Acacia mangium, Acacia auriculiformis, Macaranga gigantea, Vitex pinnata, Peronnema canescens, Gliricidia moculatam, Albizia saman,* and *Senna siamea*. The results of the study prove that the pioneer plants over five years old are dominated the reclamation area (Maharani *et al*., 2010). Based on factual data, *Senna siamea* and *Albizia saman* have the complete age ranges than the other type.

Another example of revegetation effort at Sorowako, South Celebes by local mining company was implemented using the following species, such as johar (*S. siamea*), bitti (*Vitex cofassus*), kayu angin (*Casuarina* sp.) and sengon buto (*Enterolobium macrocarpum*) (Setiadi and Adinda, 2012). *Cassia siamea* Lamk. was measured its growth and carbon stocks at a coal mining area after revegetation (Ilyas, 2013). Moreover, *A. mangium* and *F. moluccana* were calculated their carbon stocks in three consecutive years at PT. Jorong Barutama Greston, South Kalimantan (Hilwan and Nurjannah, 2014). Carbon sequestration for supporting the revegetation process was estimated on pine stands of a post-mining area at PT. Holcim Indonesia Tbk. (Fahmi and Rusdiana, 2016). Johar (*S. siamea*), along with Laban (*Vitex* sp.) and sengon (*F. moluccana*) was planted on modified media at a post-mining area in KHDTK Labanan, Berau District, East Kalimantan (Cahyani and Hardjana, 2017).

The results of the Ilyas (2013) research on *S. siamea* stands in the revegetation area with a range of ages three to seven years showed a value of biomass deposits of 69.95-108.56 tons/ha whereas in non-mining areas with an age of seven years biomass deposits were obtained in stands of 136.39 tons/ha.

The objective of this research is to determine the carbon sequestration *of A. saman* and *S. siamea* in different age classes at PT MHU, Kukar, East Kalimantan.

MATERIALS AND METHODS

This research was conducted from March to June 2018 in a revegetation land of PT Multi Harapan Utama, Kutai Kartanegara, East Kalimantan. Based on Figure 1, the studied area was conducted in Loa Kulu sub-district, Kutai Kartanegara, East Kalimantan geographically located at 116° 29'– 117° 03 'EL and 0°26'– 0°54'SL.

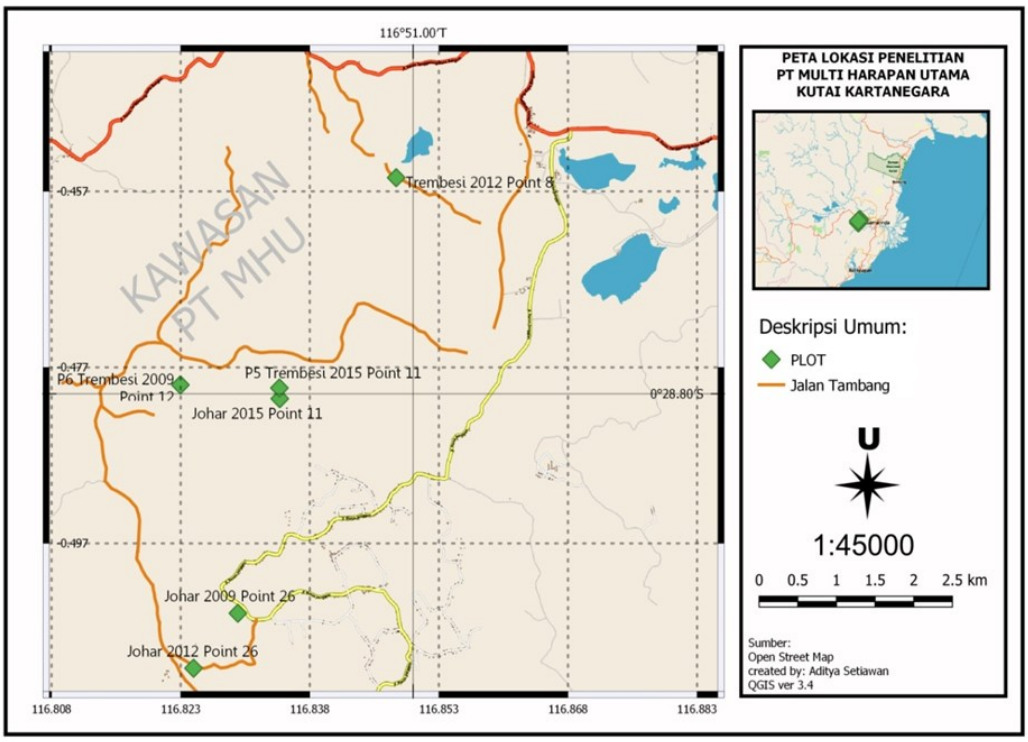


Figure 1. Map Location of Revegetation Area in PT Multi Harapan Utama, Busang Jonggon Block, Kutai Kartanegara, East Kalimantan.

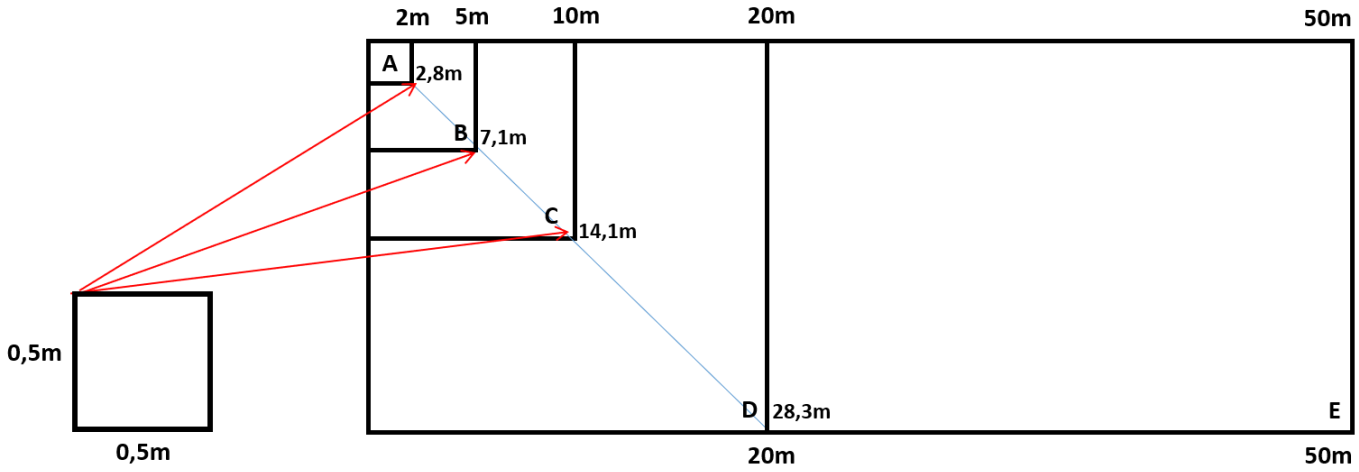


Figure 2. Placement of a 20 x 50 m rectangular plot and subplot size of 0.5 x 0.5 m for understorey and litter biomass sampling.

Determination of Points and Making Plots

The starting point for making a plot is determined by choosing the condition of the vegetation that can represent all the types of land cover in that location (Hairiah *et al.*, 2011). Figure 2 showed that the data retrieval was done by making a rectangular plot measuring 20 x 50m in which there are subplots of size 0.5 x 0.5 m for measuring understorey biomass and litter (Rusolono *et al.*, 2015).

Measuring Stand Biomass, Lower Plants and Litter, Necromass

Measuring tree stand biomass is done by using non-destructive methods by measuring the diameter of the stem at a DBH (Diameter at Breast Height) or about 1.3 m from the ground surface and the total height of the tree (Hairiah *et al.*, 2011).

Measurements of understorey biomass were carried out using destructive methods in which all live plants in the form of seedlings of <5cm, herbs, and shrubs that were in plots of 0.5 x 0.5m and separated between woody plant species and not woody weighed around 100-300g to find out the wet weight. Drying was done using an oven with a temperature of 80-100°C for 48 hours and weighed to determine the dry weight (Hairiah *et al.*, 2011).

Estimation of above-ground biomass was carried out using destructive method, which alive plants (or seedlings) ± 5 cm height, either herbs or shrubs were collected from previous subplot, were determined between woody or non-woody plants, and were weighed about 100 – 300 g to obtain its fresh weight. Drying was conducted using an oven at a temperature of 80 – 100 °C for 48 h, then re-weight to obtain its dry weight (Hairiah *et al.*, 2011).

Necromass measurements were carried out on the main plots with data collected from each plot, namely the diameter, height, or length of woody necromass and necromass specific gravity (Rusolono *et al.*, 2015).

Data Analysis

Calculation of stand biomass

Calculation of stand biomass was carried out using allometric equations of species that has been developed by the accordance with ecosystem types (Krisnawati *et al.*, 2012). The allometric equation estimates the stand biomass and volume as follows:

A. saman (Hairiah *et al.*, 2011):

$$DW = \pi * exp(-1.499 + 2.148\ ln(D) + 0.207\ (ln(D))2 - 0.0281\ (ln(D))3)$$

S. siamea (Ilyas, 2013):

$$AGB = 0.3699 * D^{1.9374}$$

Where, AGB is Above Ground Biomass; DW is dry weight (kg); π is Trembesi specific gravity of 0.6 g / cm³ (IPCC, 2006); and D is stem diameter at breast height (cm).

Calculation of Lower Plant Biomass and Litter

Calculation of understorey above ground biomass and litter was carried out by calculating the total dry weight (Hairiah *et al.*, 2011) by the formula:

$$TDW = SDW / SWW \times TWW$$

Where TDW is the total dry weight (kg); SDW is sample dry weight (kg); SWW is sample wet weight (kg), and TWW is the total wet weight (kg).

Calculation of Necromass Trees, Poles, and Dead Piles Potentials

The calculation of tree, pole and stake deadness is carried out using tree biomass values multiplied by the level of tree integrity (Figure 3) with reference to the formula obtained from National Standardization Agency (2011) as follows:

$$Ni = Bi \times f$$

Where, Ni is necromass (kg); Bi is stand biomass (kg), and f is the integrity of dead trees.

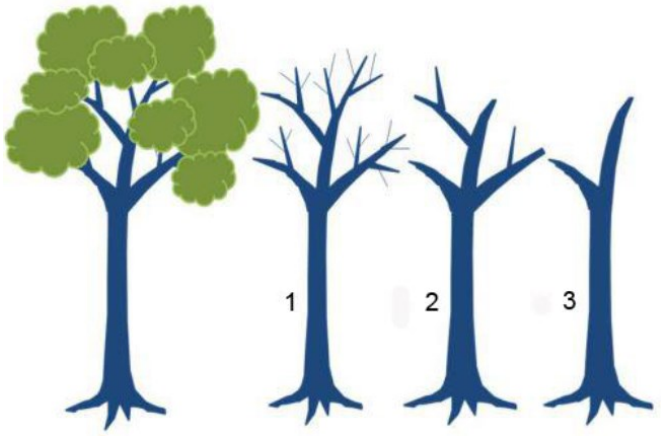


Figure 3. The integrity of Trees, Poles, and Stakes. 1. The level of dead trees integrity without leaves with a correction factor of 0.9; 2. the level of tree integrity without leaves and twigs with a correction factor of 0.8; 3. The level of tree integrity without leaves, twigs, and branches with a correction factor of 0.7 (Rusolono *et al.*, 2015).

Calculation of Carbon Reserves in Stands, Lower Plants and Litter, Necromass

Calculation of carbon from biomass uses the following formula:

$$C = B \times \% C$$
$$C = TDW \times \% C$$
$$C = N \times \% C$$

Where, C is carbon content from biomass (kg); B is total biomass (kg); TDW is dry weight of understorey kilns and litter (kg); Nis total necromass, expressed in kilograms (kg); %C is the percentage value of carbon content, which is 0.47 which is a conversion factor of international standards for estimating carbon or using the percent carbon value obtained from measurements in the laboratory.

Calculation of Carbon Absorption (C-sequestration)

Carbon absorption value (C-sequestration) is obtained by the conversion factor of C atoms in CO_2 compounds which is equal to 3.67 (Sutaryo, 2009). This value is multiplied by the value of carbon stocks by the formula:

$$CO_2 = C \times 3.67$$

Where, CO_2 is the amount of CO_2 absorption; C is carbon stocks stored, and 3.67 is the conversion factor of C atoms in CO_2 compounds.

Calculation of carbon per hectare for above-ground biomass

The accumulation of the area per hectare is obtained by the calculation results. The formula used (National Standardization Agency, 2011) is:

$$Cn = (Cx / 1000) \times (10000 / A)$$

Where, Cn is carbon content per hectare in each carbon pool in each plot (ton/ha); Cx is carbon content in each carbon pool in each plot (kg), and A is the plot area in each pool (m^2).

Statistical Analysis Two Way ANOVA using IBM SPSS 22

Analysis of Two Way ANOVA with replication was conducted to interpret the differences between carbon sequestration of both species *A. saman* and *S. siamea* and the interaction of the plant species and the age class on carbon sequestration ability.

RESULTS AND DISCUSSION

Deposits of Above Ground Biomass

The location of PT MHU has a wet tropical climate with relatively high average rainfall. According to Koppen classification, this region categorized as an Af or tropical region which indicates the coldest average temperature of more than 18ºC with an average level of precipitation not less than 60 mm per month. According to statistical data of BPS (2018), the rainfall average was 169 mm with the highest rainfall in April was 303 mm.

Based on Table 1, the highest total biomass deposits from various components found in six-year -old *A. saman* stands planted in 2012 which were 177.12 tons/ha, and the lowest was the three-year-old *S. siamea* stand planted in 2015 which was 13, 31 tons/ha. Concerning the several components, the highest biomass deposits found in stands dominated

Table 1. Soil Biomass Deposits in *A. saman* and *S. siamea* stands at PT Multi Harapan Utama (ton/ha).

No	Component	Biomass (ton/ha)					
		Albizia saman			*Senna siamea*		
		2009	2012	2015	2009	2012	2015
1	Stands	113,23±0,25	176,01±0,16	106,79±0,17	100,56±0,13	60,00±0,02	13,25±0,004
2	Understorey	0,011±0,001	0,010±0,001	0,01±0,001	0,002±0,001	0,007±0,001	-
3	Litter	0,032±0,002	0,019±0,001	0,016	0,025±0,002	0,015±0,001	0,006±0,001
4	Necromass	1,35±0,002	1,08±0,02	0,60±0,003	0,99±0,001	1,97±0,01	0,05 ±0,001
	Total	**114,62**	**177,12**	**107,42**	**101,58**	**61,99**	**13,31**

Sources: Hairiah *et al.* (2011) and Ilyas (2013).

by categories of stakes with the diameter ranges about 4-28 cm. Biomass deposits in *A. saman* are not directly proportional to the addition of plant age due to differences in the location of planting which causes the differences in physical and chemical conditions, especially on the soil (Hairiah *et al.*, 2011).

The component of soil organic matter is very influential in storing plant biomass because it is interrelated with the growth in diameter and height of plants (Hairiah *et al.*, 2011). Generally, reclaimed land has lower levels of organic matter compared to natural forests (Arsyad, 2010). The lowest value of biomass deposits in *S. siamea* stands in 2015 was caused by the soil conditions which dominated by the overburden. According to Sofyan (2013), the form of topsoil mixed with overburden can decrease plant growth so that it has the potential to reduce the value of biomass deposits.

Along with the increase in plant life, the component of soil organic matter will increase caused by an increase in the amount of weathered litter produced by plants in the form of leaves, stems or twigs (Arsyad, 2010) and the total biomass deposit will tend to be greater on older age revegetated land. However, from the table, it can be seen that the value of biomass deposits in *A. saman*, which is nine years old, was decreased compared to six-year-old plants. This is due to an increase in the type of plant at the site of the nine-year-old *A. saman* research plot which resulted in a large number of other species found in the plot through reducing the number of *A. saman* stands that can be measured compared to other observation points which tend to be dominated by one particular species.

The factor that affects biomass deposits is the diversity of conditions, which results in differences in yield between stand conditions that grow in each area. According to Krisnawati *et al.* (2012), particularly variations in plant diameter and height are influenced by the differences in growing conditions including slope, place quality, and silvicultural treatment applied to the area.

Understorey biomass and litter are the smallest fractions of total carbon in most forests (Brown, 2002). In this study, understorey biomass was inversely proportional to stand biomass due to the increasing age of the plant. Whereas the litter biomass is directly proportional to stand biomass because of the higher age of the plant causing the complexity of the plants inside. So, it increases the amount of litter that can be counted.

The largest necromass deposit found in the six -years-old *S. siamea* stands which was 1.97 tons/ha with the largest proportion from dead poles without leaves. The number of stands measured in the plots is higher than the other locations. Based on the condition of the dead poles and canopy size, there is competition in the absorption of nutrients and sunlight which causes an effect of the stands growth. The smallest necromass deposit found in the three-years-old *S. siamea* stands which was 0.05 tons/ha. The youngest stand was dominated by saplings so that the stand mortality rate is low. Some dead stands were characterized by the loss of young leaves stands, stagnant stand conditions due to inundation, and topsoil conditions still dominated by the overburdened soil types. According to Adinda (2012), water torrent results in compaction of the soil that can damage the water system (water percolation) which occurs in the inhibition of water absorption into the soil through obstructing the development and circulation of air inside the roots.

Deposits of Above Ground Carbon Stock

Based on Table 2, the value of total carbon deposits on PT MHU revegetated land is directly proportional to the total biomass value obtained. The highest total carbon stock value of various components found in *A. saman* stands which were six years old at 83.42 tons/ha, and the lowest was three years old of *S. siamea* stand which was 6.28 tons/ha because the calculation of total carbon deposits has obtained by the sum of biomass from several components. It was measured and converted by the percentage value of carbon content of 0.47 which means that 47% of the biomass is composed

Tabel 2. The Total Deposits of Above Ground Carbon Stock in *A. saman* and *S. siamea* Stands at PT MHU.

No	Component	Carbon stock (ton/ha)					
		A. saman			*S. siamea*		
		2009	**2012**	**2015**	**2009**	**2012**	**2015**
1	Stands	53,22	82,73	50,19	47,26	28,20	6,23
2	Understorey	0,07	0,06	0,06	0,01	0,04	-
3	Litter	0,20	0,12	0,10	0,16	0,10	0,03
4	Necromass	0,63	0,51	0,28	0,47	0,93	0,02
	Total	**54,12**	**83,42**	**50,63**	**47,9**	**29,27**	**6,28**

of carbon elements (National Standardization Agency, 2011) an extension in biomass content has followed by an increase in stored carbon stocks because these two components have a positive correlation (Chanan, 2012).

The Total Absorption of CO_2

Carbon absorption values can describe the amount of CO_2 in the atmosphere that can be absorbed by plants. Based on Chanan (2012), the biomass content will also affect carbon sequestration. The factors that cause an increase in carbon potential are thinning which causes competition between trees that will increase the quality of tree growth and stand dimensions, age class of tree will increase the amount of carbon sequestration because the more age increases the stand size increases so that carbon potential increases. Estimation of the potential of CO_2 uptake in revegetation, understorey, litter and necromass stands in the post-mining area of PT Multi Harapan Utama was presented in the graphs below.

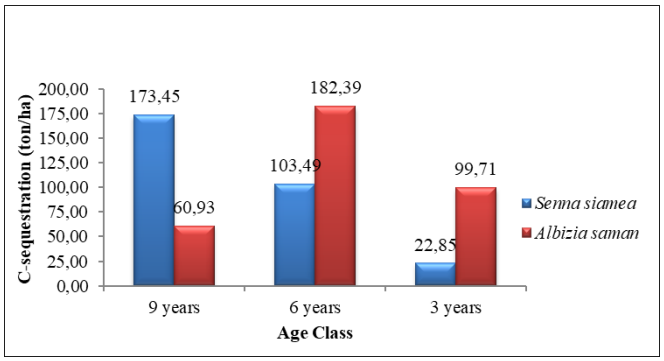


Figure 4. The Potential of C-sequestration values of the stands at PT Multi Harapan Utama.

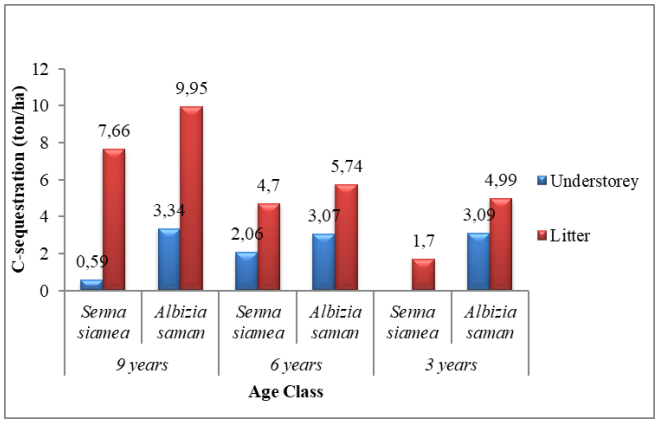


Figure 5. The potential of C-sequestration values of Understorey and Litter at PT Multi Harapan Utama.

Based on figures 4, 5 and 6, the lowest carbon sequestration value of various components was found in the three years old *S. siamea* stand of 2015 planting year. Due to the factual conditions in the field showed stagnation in the young *A. saman* plant which caused a significant difference in the size of the diameter and height of the plant compared to the six years old *A. saman*. The *A. saman* planting location in 2015 is directly around the former mining pond with very dry soil conditions and has a relatively low soil productivity rate (MHU, 2016).

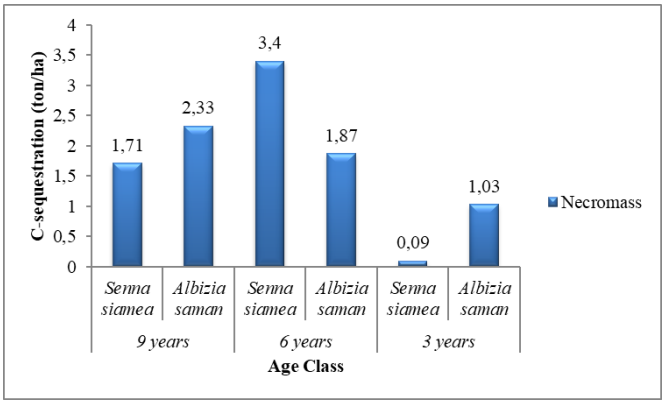


Figure 6. The potential C-sequestration values of Necromass at PT Multi Harapan Utama.

Based on Table 3, the highest potential value of carbon absorption (C-sequestration) in PT MHU, i.e six years old *A. saman* of the planting year of 2012, which was 314.28 tons/ha and the lowest value was found in the three years of *S. siamea* amounting to 24.64 tons/ha. However, when viewed from the ability of carbon sequestration per year by dividing the total carbon sequestration with plant age, the highest potential value of carbon absorption, precisely three years *A. saman* which was 64.44 tons/ha.

Tabel 3. The Potential total carbon sequestration value in *A. saman* and *S. siamea* stands at PT Multi Harapan Utama.

Age Class (year)	CO_2 Absorption (ton/ha)		CO_2 Absorption (ton/ha/year)	
	A. saman	***S. siamea***	***A. saman***	***S. siamea***
9	210.93	183.41	23.44	20.38
6	314.28	113.65	52.38	18.94
3	193.31	24.64	64.44	8.21

The statistical analysis using Two-way ANOVA with replication shows the P-Value of the variable plant species is 0.056917 (P-value ≤ 0,05) which means the variable type of plant has significant differences in the carbon absorption. The P-Value value of the variable plant age plant is 0.000495 (P-value ≤ 0,05) meaning that there is a substantial difference in the variation of plant age on carbon absorption. Besides that, the P-value of plant species is 0.056917 (P-value ≤ 0,05), it means that

the interaction of plant type and plant age also shows the significant differences in the carbon absorption.

Based on the results of the field measurements in both species, *A. saman* has the ability to store higher carbon stocks than *S. siamea*. Due to the morphological advantages through the growth stage. It can grow up to 15-25 meters with a maximum DBH reaching 2 meters. Using these characteristics, *A. saman* is able to store greater biomass compared to other species.

According to Cahyani and Hardjana (2017), *S. siamea* has a relatively low percentage of life in the first ten months compared to two other species of fast-growing plants i.e *Falcataria moluccana* and *Vitex* sp. of 62.63% because the young plant is generally susceptible to several health problems in plants such as stagnation, chlorosis, necrosis, loss to black spots on leaves, through reducing adaptability to extreme conditions on mining land (Adinda, 2012).

This type of plant is highly influential on the ability to absorb carbon in the atmosphere. *A. saman* (trembesi) are alternative plants that used for revegetation of ex-mining land. This plant is a type of fast-growing species that spread in tropical and sub-tropical countries. The litter produced from this plant can increase the soil nitrogen content more than other N-fixing legumes and reduce the concentration of aluminium in the soil. In addition, this plant also can adapt to soil types with high pH (6.0-7.4) and tolerant to pH 8.5 and a minimum pH of 4.7. *A. saman* is a tree species that has the ability to absorb carbon dioxide from large air which is able to absorb 28,488.39 kg CO_2/tree every year Bashri *et al.* (2014).

Plant growth rates have a large influence on stand biomass and carbon stock conditions. According to Pamoengkas and Randana (2013), the decrease in crop increment was caused by an increase in plants. The increasing photosynthetic energy used to support metabolic processes such as respiration, translocation, and absorption of mineral nutrients. According to Chanan (2012), an increase in carbon content of plant life is due to an increase of photosynthesis to increase plant size.

The difference in the number of carbon deposits is quite large due to differences in climate, soil quality and also silvicultural treatment given at each location. Post-mining land generally has the land quality which tends to be lower when compared to forest land (Hilwan and Nurjannah, 2014).

The carbon uptake values obtained in this study were higher, namely at three to nine years old *S. saman* at 193.31-314.28 tons/ha and *S. siamea* from three to nine years old at 24.64-183.31 tons/ha when compared to carbon uptake in the revegetation stand planted with eight to ten years old of *A. mangium* and *F. moluccana* in South Kalimantan at 90.10-147.09 tons/ha (Hilwan and Nurjannah, 2014). However, when compared with natural forests, the value of carbon stocks obtained in this study was lower, which amounted to 6.28-83.42 tons/ha for stands aged three to nine years. According to Samsoedin *et al.* (2009), dipterocarp natural forest in Malinau, East Kalimantan, which is dominated by stands with a diameter of 7-70 cm, stores carbon stocks of 204.92-264.70 tons/ha. In primary lowland natural forests in the same area with stands of 7-70 cm in diameter stored carbon stocks, which amounted to 230.10-264.70 tons/ha. The study in Sungai Wain protected the forest, East Kalimantan which was dominated by stands with DBH 5-40 cm, the value of stored carbon reserves was 211.86 tons/ha. In addition, the measurement of carbon stocks in logged-over secondary forests in the Malinau Research Forest, East Kalimantan, which is dominated by stands with DBH 7-70 cm in logged-over age after five to thirty years stores carbon stocks of 171.8-249.1 ton/ha (Masripatin *et al.*, 2010a).

More intensive conservation of post-mining land must be done, especially in improving soil quality which has a substantial impact on plant growth. In addition, it should be evaluated to determine the percentage of plant life regularly and discover the most relevant plant conservation techniques. The use of appropriate silvicultural techniques has a generous impact on the amount of carbon stored in the plant.

Data and information regarding carbon stocks in various types of forest and plant ecosystems are very important to be used as a reference in efforts to maintain forest areas, especially for companies with activities that affect the structure and function of forest land. So that with this data all parties can consider the most optimal rehabilitation techniques in improving the land to restore the function of the forest as usual. Rehabilitation of forest ecosystems is carried out to accelerate the natural succession process and biological productivity, increase soil fertility, and increase biotic control of biogeochemistry flows in plant-covered ecosystems. An increase in the amount of carbon sequestration in an ecosystem can illustrate the success in ecosystem rehabilitation efforts (Setiawan, 2003).

CONCLUSION

Carbon sequestrations content was different among age classes. *A. saman* has the highest number in carbon sequestration about 314.28 tons/ha at 6 years old stands, while the lowest number was 193.31 tons/ha at 3 years old stands. On the other hand, *S. siamea* has the highest number of about 113.65 tons/

ha at 9 years old stands, while the lowest number was 24.64 tons/ha at 3 years old stands. *A. saman* has a higher number in carbon sequestrations than *S. siamea* because of its fast-growing ability, including various soil type and soil pH (even tolerate from 4.7 – 8.5). Meanwhile, *S. siamea* has lower carbon sequestration presumably because, since a young age, the seedlings must cope with various health problems and maintain its adaptability during the harsh condition in the mining area. Lastly, all data from this study could be useful for using as a reference to improve the carbon sequestration level in the forest ecosystem through achieve forest rehabilitation purposes.

ACKNOWLEDGMENTS

The author conveyed to PT Multi Harapan Utama Busang Jonggon Block, Kutai Kartanegara, East Kalimantan especially Mr. Salim Basir, Panji Setyadi and the entire family of the Department of Reclamation and Rehabilitation for the opportunity to conduct research at that location.

REFERENCES

Adinda. 2012. Evaluation of Tree Growth in Post Vale Mining Land Revegetation Locations. Sorowako, South Sulawesi, Institut Pertanian Bogor, Bogor.

Arsyad, S. 2010. Soil and Water Conservation. Second ed. Institut Pertanian Bogor Press, Bogor.

Bashri, A., B. Utami, and P. R. Primandiri. 2014. Growth of Trembesi Seedlings (Samanea saman) with Arbuskula Mycorrhizal Fungal Inoculation in the Media of Final Disposal Site (TPA) Klotok Kediri. Seminar Nasional Pendidikan Biologi FKIP UNS 11: 5.

Bhattacharjee, P. K. 2010. Global Warming Impact on the Earth. International Journal of Environmental Science and Development 1 (3).

Brown, S. 2002. Measuring carbon in forests: current status and future challenges. Journal of Environmental Pollution 116: 363-372.

Cahyani, R. W., and A. K. Hardjana. 2017. Treatment of crop type and crop media on post-mine C mine at Labanan KHDTK, Berau Regency, East Kalimantan. Prosiding Seminar Nasional Biodiversity Indonesia.

Canadell, J. G., and M. R. Raupach. 2008. Managing Forests for Climate Change Mitigation. American Association for the Advancement of Science (AAAS) 320: 1456-1457.

Central Statistics Agency of Kutai Kartanegara. 2018. Loa Kulu in Figures. East Kalimantan: BPS.

Chanan, M. 2012. Estimation of Carbon Reserves (C) Stored Above Ground on Vegetation of Teak Forest Plant (Tectona grandis Linn.F) in Sengguruh BKPH RP Sengguruh KPH Malang Perum Perhutani II East Java. Jurnal Gamma 7(2): 61-73.

Forestry Minister Regulation No. P.4 / Menhut-II / 2011 concerning Guidelines for Reclamation of Former Mining in Forest Areas.

Hairiah, K., A. Ekadinata, R. R. Sari, and S. Rahayu. 2011. Measurement of Carbon Reserves from Land Level to Landscape. World Agroforestry Center, Bogor.

Hilwan, I., and A. S. Nurjannah. 2014. Potential Carbon Deposits in Post-Mining Land Revegetation in PT Jorong Barutama Greston, South Kalimantan. Jurnal Silvikultur Tropika 5: 188-195.

Ilyas, S. 2013. Carbon Sequestration and Growth of Stands of Cassia siamea Lamk. in Coal Mining Reforestation Area. Indian Journal of Science and Technology 6: 5405-5410.

IPCC (Interngovernmental Panel on Climate Change). 2006. Good Practice Guidance for Land Use, Land Use Change and Forestry. Institute for Global Environmental Strategy, Hayama (Japan).

Krisnawati, H., W. C. Adinugroho, dan R. Imanuddin. 2012. Monograph of Allometric Models for Estimating Tree Biomass in Various Forest Ecosystem Types in Indonesia. Research and Development Center for Conservation and Rehabilitation, Forestry Research and Development Agency, Bogor.

Maharani, R., A. Susilo, S. Sugiharto, and A. Fernandes. 2010. Status of Post Coal Mining Reclamation Research. In: R. Setiyono (ed.). Dipterokarpa Research Center, Samarinda.

Masripatin, N., K. Ginoga, G. Pari, W. S. Dharmawan, and C. A. Siregar. 2010a. Carbon Reserves in Various Forest Types and Plant Types in Indonesia. Center for Research and Development on Climate Change and Policy, Bogor.

Masripatin, N., K. Ginoga, A. Wibowo, W. S. Dharmawan, and C. A. Siregar. 2010b. Carbon Measurement Guidelines to Support the Implementation of REDD + in Indonesia. Center for Research and Development on Climate Change and Policy, Bogor.

Nandika, D. 2005. Forests for National Resilience. Muhammadiyah University Press, Surakarta.

National Standardization Agency. 2011. Measurement and Calculation of Carbon Reserves - Field Measurement for Estimating Forest Carbon Reserves (Ground Based Forest Carbon Accounting). Jakarta (ID): BSN.

Pamoengkas, P and Randana, F. 2013. Response of Red Meranti Growth to Planting Path Width and Sunlight Intensity in the TPTJ Silviculture System. *Jurnal Silvikultur Tropika* Vol 4(1):51-56

Regulation of the Minister of Environment and Forestry of the Republic of Indonesia No. P73/MENLHK/SETJEN/KUM.1/12/2017 Regarding Guidelines for the Implementation and Reporting of the National Greenhouse Gas Inventory

Rusolono, T., T. Tiryana, and J. Purwanto. 2015. Guide to Surveying Carbon Reserves and Biodiversity in South Sumatra. German International Cooperation (GIZ), Jakarta.

Samsoedin, I., I. W. S. Dharmawan dan C. A. Siregar. 2009. Potential carbon biomass in natural forests and logged-over forests after 30 years in the Malinau Research Forest, East Kalimantan. *Jurnal Penelitian Hutan* 6, 2009. Center for Forest Research and Nature Conservation. Bogor.

Setiawan, I. 2003. Evaluation of the success rate of revegetation on the former PT. KOBA TIN, Koba, Bangka-Belitung, Bogor Agricultural Institute, Bogor.

Journal of Tropical Biodiversity and Biotechnology
Volume 05, Issue 02 (2020): 124 — 131
DOI: 10.22146/jtbb.53677

Research Article

Induction of Microspore Embryogenesis of Eggplant (*Solanum melongena* L.) 'Gelatik'

Devi Bunga Pagalla[1], Ari Indrianto[1], Maryani[1], Endang Semiarti[1]*

1) Faculty of Biology, Universitas Gadjah Mada, Jl. Teknika Selatan, Sekip Utara, Bulaksumur, Yogyakarta 55281, Indonesia. Tel/ Fax. +62-274-580839

Submitted: 22 January 2020; Accepted: 18 June 2020; Published: 15 August 2020

ABSTRACT

The haploid or double haploid plant of eggplants could be produced from microspore culture (embryogenesis of microspores). In the breeding programs, microspore can be developed into an embryo directly after exposure to stress treatment during cultured. Stress (temperature and starvation medium) is an important factor in the induction of embryogenesis microspore. This study aims to induced embryogenic microspores from eggplant CV. Gelatik. The stage late-uninucleate microspore (*Vacuolate Microspore*/VM) and early binucleate (*Young Bicellular Pollen*/YBP) are the suitable stages to induce multinucleate structure. There are 3 methods used in this research; 1) Determination of the stage development of microspore based on flower buds length and anther length. 2) Induction of embryogenic microspore on the pre-treatment and starvation medium. 3) After giving pre-treatment for 4 days, micropores were transferred to culture medium A2 at 28°C in dark conditions to induce the multicellular structures. This study reported that 50-68.51% of the VM+YBP stage obtained in the range of flower bud lengths of 10-17 mm, and 5.0-6.9 mm, the range of anther length containing VM+YBP of 50-77.48%. The pre-treatment heat shock at 33°C in the medium B for 2 days, produced embryogenic microspores with a high percentage, that is about 50.19%, while microspores at 25°C and 4°C respectively 46.17% and 49.28%. Pre-treatment for 4 days at 4 °C, 25 °C, and 33°C with the percentage of embryogenic microspores apiece 32.87%, 27.45%, and 37.34%. The multicellular (*starlike*) structure begins forming on the fifth day of incubation in culture medium (A2) after pre-treatment in B medium at 33°C.

Keywords: Eggplant, flower bud, microspore, stress treatment, embryogenic microspore

INTRODUCTION

Eggplant (*Solanum melongena* L.) is one of the essential vegetables in tropical and subtropical regions around the world. It is the fifth most economically crucial solanaceous plant after potatoes, tomatoes, pepper, and tobacco (FAO, 2014; Taher *et al.*, 2017). Improvement on eggplant production carried out through biotechnology and hybridization approaches (Kalloo, 1993; Kashyap *et al.*, 2003; Magioli and Mansur, 2005; Bal *et al.*, 2009) such as regeneration of haploid or double haploid plants, through anther or microspore culture (*Microspore Embryogenesis*). Microspore embryogenesis represents a unique system which can produce homozygous lines in a relatively short period and does not require much effort and cost compared to conventional crossing methods which require a lot of crossing over time (Snape, 1989; Maluszynski *et al.*, 2003; Adhikari and Kang, 2017). Usually, microspores will develop into mature pollen (*pollen grain*) and ready to fertilize an egg. Through in vitro culture, the normal development of microspores can be transformed into the development of the sporophytic phase that will form embryos directly. The ability of male gametophytes to change their developmental fate from pollen to embryonic development can occur when exposure to stress treatments during culture. This process referred to as microspore embryogenesis (Soriano *et al.*, 2013). Stress treatment has an essential role in this process, encouraging microspore differentiation and conditioning the androgenic responses (Munoz-Amatriain *et al.*,

***Corresponding author**
Email: endsemi@ugm.ac.id

2009).

In microspore embryogenesis, free microspores isolated from anther are cultured in vitro and after pre-treatment stress, microspores will develop sporophytically (Shariatpanahi *et al.*, 2006; Bal *et al.*, 2009). Some stress treatments have been known to induce embryogenic microspores, including cold shock, heat shock, carbon starvation, and nitrogen in growing medium and colchicine treatment (Moraes *et al.*, 2004). During the embryogenesis induction, identification of microspores that have embryogenic abilities and the mechanism of transformation of microspores into embryogenic cells are carried out. Although the initial stage of development of microspores for the induction of embryogenesis differs between species, usually microspores are chosen in the vacuolated stage. Vacuolated microspore stage is the development stage that allows reprogramming of microspores in most species (Olmedilla, 2010). Salas *et al.* (2012) revealed microspores at the stage of young microspore (YM) and mid-microspore (MM) in eggplant did not show a growth response after being transferred to embryogenesis medium. The optimal stage for embryogenesis induction is not the transition stage from vacuolate microspore (VM) to the young bicellular pollen (YBP) stage. When VM and YBP were cultured in a liquid medium, both showed a growth response towards embryogenic microspores. In this study, we determine the stage of microspore development through the selection of flower buds as the first step in microspore culture. To observe the anatomy of the anther, we analyze the correlation between the stages of microspore development and the stage of anther development. Furthermore, induction of embryogenic microspores by pre-treatment of temperature stress (4°C, 25 °C, and 33°C) with a combination of medium starvation (B) for 4 days will follow, the protocol of the microspore culture of eggplant cv. Bambino (Bal *et al.*, 2009).

MATERIALS AND METHODS

Plant Material

The seeds of eggplant cv. Gelatik were sown in a plug-tray and transferred to a pot in a greenhouse of Laboratory of Biotechnology, Faculty of Biology, Universitas Gadjah Mada. The seedling, one and a half months of age or has 4 leaves transferred to a pot measuring 30x27x17 cm^3 and filled with planting media consisting of soil, manure, and compost. The plants watered daily, and the soil was supplemented with Growmore fertilizer NPK (15:15:15) and for leaves.. The plants were maintained and pruned by bud shoot (*dominancy apical*) to produce many branches. Flower buds then picked as the primary materials of this study.

Determination of Microspore Developmental Stage

The flower buds of various sizes were collected and grouped according to the size of the flower bud length and anther length (shown in Table 1). The buds were measured using Nankai digital calipers 0-150 mm. The length measured from the base of the calyx to the tips. After the measurement, the anther removed from the flower bud. Anther from each flower bud was crushed in medium B to observe the development of microspores by Microscope (Nikon Diaphot 300 Fluorescence; Japan) and images were taken by OptiLab face 2.2 Miconos. Determination of the percentage of each microspore stage in the same anther was counted from 10 randomly selected images from five display fields. The number of each microspore is calculated by dividing the number of microspores at a certain stage by the total number of microspores based on the calculation of 10 images.

Anatomic Preparation of Anthers

An anther of different size flower buds was put in a vial bottle. It is then fixed in FAA fixative solution (formald ehyde: absolute alcohol: glacial acetic acid: aquadest 2:10:1:7) for 24 hours, followed by staining with safranin 1% orange in 70% alcohol for 24 hours, washing and dehydration: 70% alcohol, 80%, 95%, 100% twice, each for 30 minutes, dealcoholization: the ratio of alcohol: xylol 3: 1; 1: 1; 1: 3, and pure xylol twice, each for 30 minutes. The last step was washing by the xylol: paraffin ratio of 1:9 at 57 °C for 24 hours twice. The stage of planting or making a blocking was after incubation for 1 hour in pure paraffin. Paraffin was cut using a Rotary Microtome with a thickness of 15 µm. The last step was washing and staining using safranin 1% in 70% alcohol, and fast green 1%. Washing materials were xylol twice, alcohol: xylol (1: 3, 1: 1 and 3: 1), alcohol 100% twice, 95%, 80%, and 70% each for 3 minutes, Safranin 1% for 1 hour. After safranin, the slides were washed in aquadest, 70% alcohol, fast green staining, 80% alcohol, 95%, 100% twice, alcohol: xylol (3: 1.1: 1, and 1: 3), and lastly with xylol each for a minute. The Slide was mounted by Balsam Canada and covered with glass cover. Anatomy of anther was observed using a Boeco Binocular Microscope (BM-180 SP Germany).

Induction of Microspore Embryogenic with Temperature Stress and Starvation Medium

Anther from flower buds contained high-percentage VM + YBP microspores were used as microspores donors for the induction of embryogenic

microspores by stress treatment. Microspores were cultured in B Medium (Indrianto *et al.*, 2014) containing macronutrients: KCl 1.490 mg/L, $MgSO_4.7H_2O$ 250 mg / L, $CaCl_2.2H_2O$ 110 mg / L, KH_2PO_4 136 mg / L, Mannitol 54.630 mg / L with pH 7. The cultures incubated at 33°C, 25 °C, and 4 °C for 4 days. The observations were presented as a percentage of embryogenic microspores (VM, YBP, and Multinucleates) and non-embryogenic (YM, MM, and lysis microspores).

Development of Embryogenic Microspore

The binucleate and multinucleate microspores from the best pre-treatment combination of temperature stress and starvation medium were transferred to A2 medium (Touraev *et al.*, 1996) containing macronutrients: KNO_3 2.800 mg/L, $(NH_4)_2SO_4$ 460 mg/L, KH_2PO_4 400 mg/L, $CaCl_2.2H_2O$ 166 mg/L, $Mg.SO_4.7H_2O$ 185 mg/L, Iron 1 mL, micronutrients 1 mL, Vitamine (B5) 1 mL, MES (Morfolino Ethanol Sulfonic Acid) 1.950 mg/L, Glutamine 500 mg/L, and Maltose 90.000 mg/L, with pH 6.2. Embryogenic microspores were cultured at 25°C in dark conditions. This development observed for three weeks.

Statistical Analysis

The quantitative data consists of the number of embryogenic microspores and the increased diameter of microspores at different temperature treatments were analyzed using Microsoft Excel 2019. The data were obtained from three replications and presented as mean ± standard deviation in the tables.

RESULTS AND DISCUSSION

Developmental Stage of Microspore of Eggplant cv. Gelatik

The suitable stage of the flower bud (Figure 1) is a critical determinant of the success of microspores culture. The morphological size of flower buds, the length of flower buds, and the length of anther correlate with the stage of microspore development and the stage of anther development. In some studies, bud length and anther length are also used as benchmarks for the stages of microspore development such as in anther culture of tomato (Segui-Simarro and Nuez, 2005), eggplant (Salas *et al.*, 2012), and correlation with the development of pollen in anther culture of apple (Zhang *et al.*, 2013).

In this study, the microspore development stages were identified based on the length of flower

Table 1. Stage development of microspore in eggplant cv. Gelatik, based on the length of flower bud and length of anther.

Length of a Flower bud (mm)	Stage of Microspore (%±sd)					
	Tetrad/ Meiocyte	Uninucleate			Early Binucleate (Yong bicellular pollen)	Mature Pollen
		Early (Young microspore)	Mid (Mid microspore)	Late (Vacuolate microspore)		
6-7	100±0	-	-	-	-	-
8-9	-	*26.46±4.00*	*40.08±2.37*	33.46±3.88	-	-
10-11	-	10.67±6.52	19.03±6.05	**41.46±4.66**	**17.61±5.62**	11.23±7.02
12-13	-	5.42±4.38	17.42±8.39	**40.22±9.04**	**28.29±4.96**	8.65±4.43
14-15	-	*19.80±5.51*	*23.09±6.41*	**46.63±7.40**	**7.37±3.82**	3.11±1.85
16-17	-	*19.38±3.04*	16.47±4.37	**50.01±7.64**	**6.02±2.22**	8.12±2.52
18-19	-	-	-	11.01±1.17	**21.09±1.26**	67.90±5.08
20-21	-	-	-	-	-	100±0
>22	-	-	-	-	-	100±0
Length of Anther (mm)	Tetrad Meiocyte	Uninucleate			Early Binucleate (Yong bicellular pollen)	Mature Pollen
		Early (Young microspore)	Mid (Mid microspore)	Late (Vacuolate microspore)		
3.0-3.9	100±00	-	-	-	-	-
4.0-4.9	-	*38.93± 3.02*	*27.09±4.14*	**33.98±8.18**	-	-
5.0-5.9	-	16.40±2.04	*26.27±6.17*	**52.84±7.75**	3.11±1.27	1.38±1.08
6.0-6.9	-	-	12.07±4.70	**45.29±6.45**	*32.19±8.42*	10.45±1.23
7.0-7.9	-	-	-	-	*39.33±5.53*	60.67±4.66
8.0-8.9	-	-	-	-	-	100±0
>9.0	-	-	-	-	-	100±0

The result is expressed as a percentage of each stage ± sd
Bold values are flower bud lengths with a high percentage of VM+YBP, whereas *Italicized* values are bud lengths with a high percentage of Young Microspore (YM) and Mid Microspore (MM).

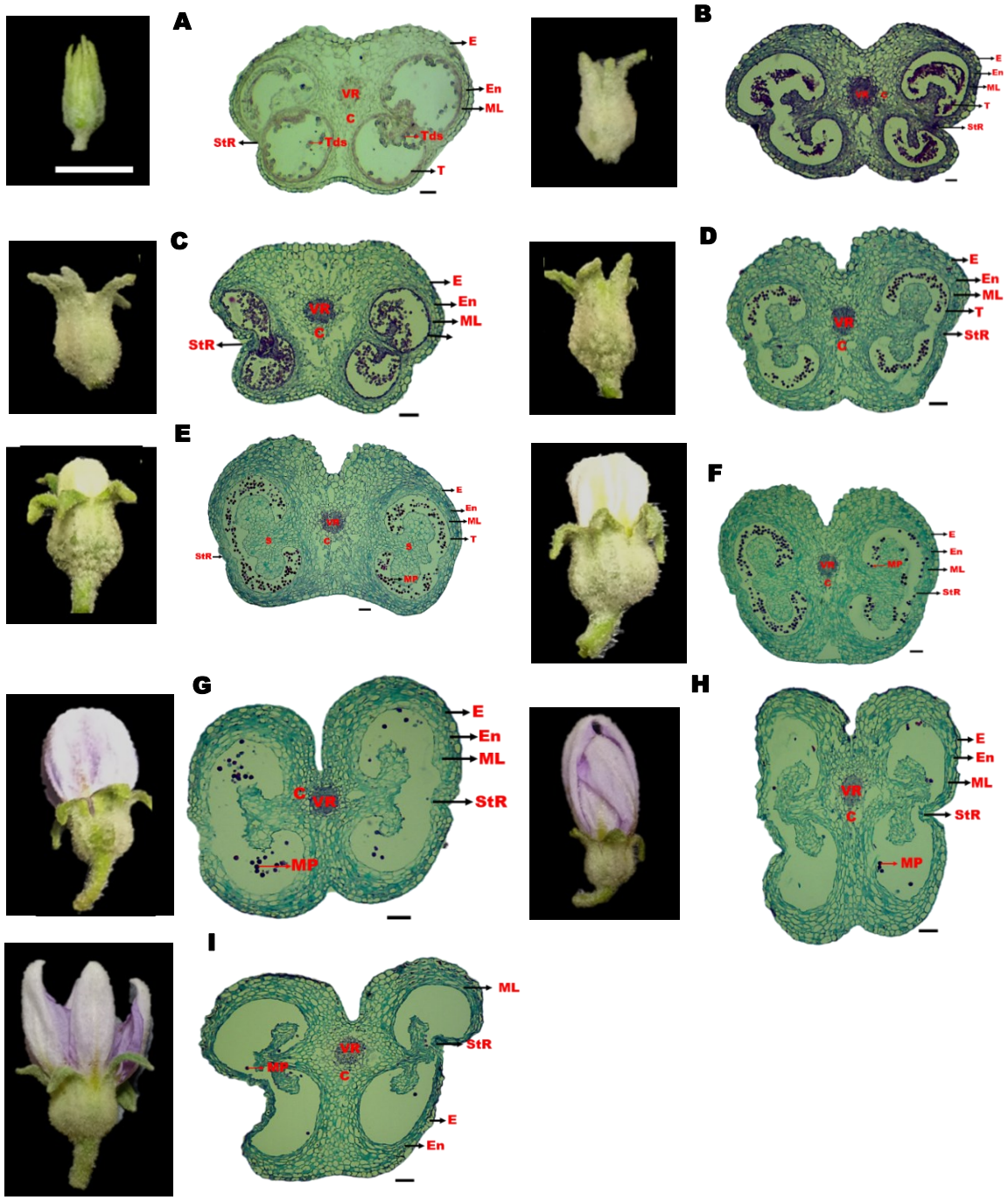


Figure 1. Morphology of the flower bud and transverse section of eggplant cv. Gelatik anther. The associated anther length of the flower bud with the developmental stage of the anther. A=3 mm; B= 4.5 mm; C=5.0 mm; D=5.5 mm; E=6.5 mm; F= 7 mm; G= 7.2 mm; H= 8 mm; I= 8.2 mm (Bar 10 mm). Transverse sections of eggplant anthers. The sample is anther of flower buds (1-9). StR, Stomium region; T, Tapetum; ML, Middle layer; E, Epidermis; En, Endothecium;VR, Vascular region; C, Connective; S, Septume; Tds, Tetrad; MP, Mature pollen (pollen grains). Scale bar =100 μm. (Personal documentation, taken on 19 October 2019, by OptiLab advance 2.2 (Miconos-Indonesia).

buds and the length of anther of eggplant cv. Gelatik. Table 1 shows that the VM + YBP stage of 50-68.51% is in the range of flower bud length of 10 -17 mm, and 5.0-6.9 mm range of anther length containing VM + YBP of 50-77.48%. The highest percentage of YM + MM was observed in the range of flower bud length and anther length of 8.0-9.0 mm with a percentage of about 66.54% and 4.0-4.9 mm at 66.02%. The length of anthers was suitable for cultured in the starvation medium at 5.0 – 5.9 mm, not for all measures. In this study, the same flower bud length has a different anther length. Therefore the percentage of mature pollen in the range of flower bud length 14-15 mm is lower than 16-17 mm or 12-13 mm. The YM and MM after pre-treatment at 33^{o}C, 25^{o}C, and 4^{o}C, then subcultured into A2 medium did not show a growth response, even some microspores experienced shrinkage. Salas *et al.* (2012) also stated that in the microspore culture of eggplant cv. Bandera. In the study of

morphological markers for the development of microspores in maize, Moraes *et al.* (2008) stated that anther length was the most reflected cytological stage of microsporogenesis. In wheat, it is also found that the length of the anther is considered an appropriate morphological marker for the assessment of the specific development of microspores (Immonean and Antila, 1998). However, it is essential to recognize that the measurements obtained for these parameters in each microspore developmental stage varied according to the genotype and the cultivation place.

Developmental Stage of Eggplant Anther

Stamens consist of two different structures, namely anther and filament. The length of the anther indicates the stage of anther development and the stage of microspores in the anther locus. Microspore development stages were identified based on cellular characteristics such as cell shape, cell number, nucleus type, and position in the cell, and state of the chromosomes (Adhikari and Kang, 2017). According to Browne *et al.* (2018) the length of the wheat anther is an accurate parameter measure to determine the stage of anther development and can be applied among wheat cultivars. Each stamen contains filaments and anthers with four lobes connected to the filament by connective tissues (Zhang and Wilson, 2009). In this study, the stage of anther development of wheat (Browne *et al.*, 2018), used as a reference for determining the developmental stage of the anther in eggplant cv. Gelatik.

In this research, the observation of anther development is starting from stage 8 (Figure 1A), namely the *Programmed Cell Death* (PCD) tapetum stage and the last tetrad stage (before the microspore tetrad is released as young microspore). Stage 9 anther (Figure 1B), which is the stage in which tetrad microspores are released as young microspores. Stage 10 (Figures 1C, 1D, 1E) is the vacuolated microspore stage. Stage 11 (Figure 1F), the anther contains the early binucleate microspores. Stage 12 (Figure 1G), the tapetum portion is completely degraded. Stage 13 (Figure 1H), the septum between the upper and lower locus begins to degrade. Stage 14 (Figure 1H), initial dehiscence, and stomium are degraded. The last stage of anther development is stage 15 (Figure 1I), where pollen released from the anther locus.

Induction of Embryogenic Microspore

The range of flower bud lengths 10-11, 12-13, 14-15 and 16-17 mm each contains VM + YBP 59.07%, 68.51%, 54%, and 56.03. The size of the flower bud used 12-13 mm for embryogenic microspores. Another main factor besides the phase of flower buds is the stress treatment because this is the main factor to determine the success of microspore embryogenesis induction. Microspores were cultured in 2 ml of liquid B medium for 4 days and incubated at three different temperatures (4°C, 25°C, and 33°C). The parameters observed were the percentage of embryogenic microspores (late uninucleate, binucleate and multinucleate microspores), and non-embryogenic microspores including young microspores (YM), mid- uninucleate (MM) and lysis or shrinking microspores. Microspores at this stage, if exposed to stress treatments (such as heat shock, cold shock, and starvation medium) will develop into an embryo. A high percentage of embryogenic microspores is also a determinant success of the stress treatment used. The results showed (Table 2) that, embryogenic microspores were observed during pre-treatment at 33 °C (heat shock), 25°C and 4°C (cold shock) for 2 days of culture and liquid B medium, respectively 50.19%, 46.17%, and 49.28%, after 4 days of incubation obtained embryogenic microspores of 32.87%, 27.45% and 37.34% at each incubation temperature. After 4 days of incubation, several microspores were lysis and shrinking. Incubation microspore of eggplant cv. Gelatik at 33°C for 2 days, enough to induce embryogenic microspore. This is different from broccoli when the embryo production is significantly increased in almost all genotypes of broccoli, which is incubated in a medium of ½ NLN-13 at 32°C for 1 day, compare to if it is incubated at a standard temperature of 30°C for 2 days. Bal *et al.* (2009) revealed, the combined effects of heat pre-treatment

Table 2. Percentage of embryogenic microspore and non-embryogenic.

Length of a flower bud (mm)	Temperature stress (°C)	Time of Incubation (days)	Percentage of microspore±sd	
			Embryogenic	Non-embryogenic
12-13	33	2	**50.19±2.45**	49.81±3.53
		4	32.87±2.76	67.13±5.49
	25	2	**46.17±2.16**	53.83±3.02
		4	27.45±4.04	72.55±4.94
	4	2	**49.28±1.58**	50.72±4.14
		4	37.34±2.86	62.66±1.87

Table 3. The increase of diameter late uninucleate and binucleate microspores.

Temperature stress (°C)	Time of Incubation (day)	Average of microspore diameter (µm) ±sd	
		Late uninucleate	Binucleate
33	0	**17,240±1,072**	**20,030±0.874**
	2	19,121±1,276	23,090±1,381
	4	**19,999±1,270**	**23,775±1,629**
25	0	**17,534±1,115**	**20,985±1,760**
	2	18,787±0,963	21,852±1,022
	4	**19,134±1,263**	**23,270±1,661**
4	0	**17,469±0,954**	**20,237±0,818**
	2	17,378±0,874	20,336±1,296
	4	**17,890±0,909**	**21,362±1,595**

and starvation were the same as Miyoshi's (1996) study, which cultured eggplant microspores in mannitol starvation medium and incubated at 35°C for 3 days.

The incubation for 48h at 32°C obtained unoptimized results for embryo production. That research reported that broccoli is sensitive to high temperatures (Carlos and Dias, 2001). In eggplant cv. Bambino, microspores treated with cold shock rapidly will lose viability after being subcultured into an AT3 medium (Bal *et al.*, 2009). The same problem was also found in this study, the pre-treatment incubation of microspores at 4°C for 4 days after subculture to A2 medium were found many microspores underwent lysis. Microspores that underwent nucleus symmetrical division and form multinucleate structures were obtained at low frequency after the microspores were transferred from heat shock and starvation to the AT3 medium containing 0.25 M maltose. The frequency of multinucleate structures were 17.3% and 19.4% after one week of incubated in an AT3 medium (Bal *et al.*, 2009).

Table 3. showed, the late uninucleate and early binucleate microspores were cultured in liquid B medium and incubated at 33°C for 4 days. There is an increase in the diameter size of microspores, respectively about 2.759 µm and 3.745 µm. Late uninucleate and binucleate culture of the microspores at 25 °C for 4 days was 1.6 µm and 2.285 µm respectively, whereas microspores were at 4 °C for 4 days each at 0.421 µm and 1.125 µm. Microspores incubated at 4°C developed very slowly. The increase in the diameter size of the microspore of both the late uninucleate and binucleate is unlike if the microspores incubated at temperatures 25°C and 33°C. According to Bal *et al.* (2009), heat shock was successfully used as a stress factor for microspore embryogenesis in eggplants. However, starvation pre-treatment, combined with heat shock, namely the microspore embryogenesis protocol in tobacco, is more effective. Stress treatments such as heat shock also provide optimal results for the induction of embryogenic microspores in eggplant cv. Gelatik. In addition to temperature stress, incubation time is also important in the induction of embryogenic microspores in eggplant.

Development of Microspore Embryogenic in Culture Medium

Embryogenic microspores have been treated with heat and cold shock temperature stress for 4 days, then subcultured into embryogenesis medium A2 to release microspores from stress to develop microspores towards embryo formation. Embryogenic microspores from B medium at 33°C (Figure 2) after one week of incubation in a new medium (medium A2), swoll very quickly, while in embryogenic microspores from 25°C to 25°C, many microspores were lysed. Thus microspores from 4°C temperature were more lysed with very slow development and the number of embryogenic and *starlike* microspores was less than the others.

Embryogenic microspores formed after stress treatment were characterized by several physiological and morphological changes (Figure 2). In tobacco and wheat, embryogenic microspores form a nucleus in the middle surrounded by structures like vacuoles. Microspores with cytoplasm form *starlike* structures, commonly found in microspores of wheat, barley, and tobacco. Swollen in *Brassica* is marked as embryogenic microspores (Bal *et al.*, 2012). In this study, several embryogenic microspores were observed, swollen, increased diameter of the microspores, the cell nucleus continuously divided to form multinucleate structures. The induction of microspores embryogenesis in eggplants in A2 medium (Figure 2), after 3 weeks of incubation produced no globular embryo, only microspore enlargement or bulging into swollen microspore, and

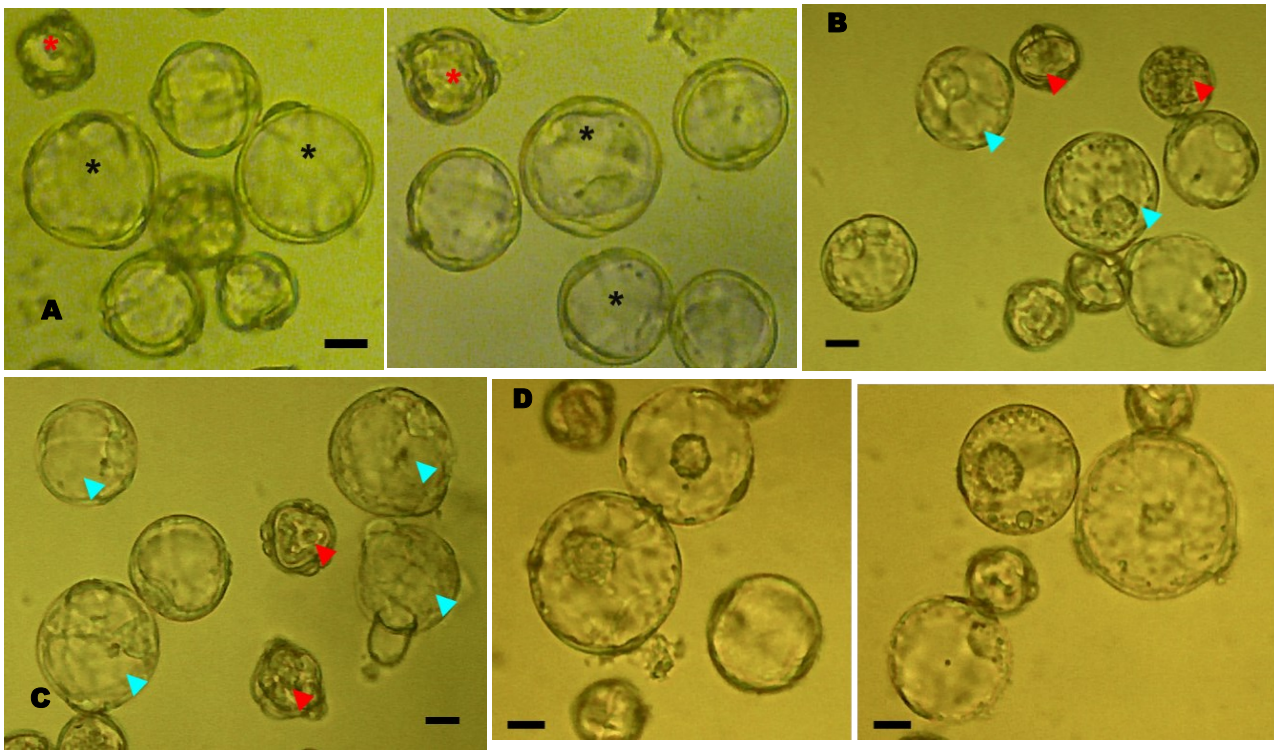


Figure 2. Development of embryogenic microspores in A2 medium at 25°C for 3 weeks. A) Embryogenic microspores from B medium at 33 oC for 4 days Incubation, B,C) Microspores resemble star-like structures (blue arrows: star-like structures, red arrows: microscopic lysis (1 week incubation) D) Star-like microspores, with the diameter of the microspores increases and swollen occurs (3 weeks incubation). Scale = 10 μm.

only formed a *star-like* structure. This shows that the A2 medium is less suitable for eggplant embryogenesis. According to Sumarmi *et al.* (2014) A2 medium is more suitable for the microspore culture of monocot plants. Medium A2 succeeded in spurring the growth of embryogenic microspores, resulting in symmetrical division and multinucleate structures formed in the culture of palm microspores (Indrianto *et al.*, 2014). Besides the A2 medium, some embryogenesis medium often used for induction of embryogenesis is AT3 medium in the culture of eggplant cv. Bambino (Bal *et al.*, 2009), and B5 medium with 9% maltose in wheat microspore culture (Zheng, 2003).

CONCLUSION

The incubation of microspores at 33°C in the medium for 4 days is effective to induce embryogenic microspores in eggplants. In addition, the early binucleate stage is preferably chosen for the induction of embryogenic microspores.

ACKNOWLEDGMENTS

This project was supported by Research Grant from the Ministry of Research Technology, Higher Education Republic Indonesia with a scheme of PMDSU 2018-2019 to Ari Indrianto as the PI and Devi Bunga Pagalla as the member of the researcher.

REFERENCES

Adhikari, P. B., and Kang, W.H. 2017. Association of Floral Bud and Anther Size with Microspore Developmental Stage in Campari Tomato. *Horticultural Science and Technology.* 35 (5):608-617.

Bal, U., Ellialtioglu, S., Abak, K. 2009. Induction of symmetrical nucleus division and multinucleate structures in microspores of eggplant (*Solanum melongena* L.) cultured in vitro. *Sci Agric.* 66:Pp.535–539.

Browne, R.G., Lacuone, S., Li, F.S., Dolferus, R., and Parish, R.W. 2018. Anther Morphological Development and Stage Determination in *Triticum aestivum Front. Plant Sci.* 9:228.

Carlos, J., and Dias, S. 2001. Effect of incubation temperature regimes and culture medium on broccoli microspore culture embryogenesis. *Euphytica.* 119:389–394.

FAO. 2014. *FAOSTAT Production Databases.* Available online at: http://faostat.fao.org. Viewed December 20, 2018.

Kalloo, G.1993. Eggplant. In Kalloo, G., Bergh, B.O. (Ed) Genetic Improvement of vegetable crops. *Oxford:Pergamon Press*: 587-604.

Kashyap, V., Kumar, S.V., Collonnier, C., Fusari, F., Haicour, R., Rotino, G.L., Sihachakr, D., and Rajam, M. 2003. Biotechnology of Eggplant. *Scientia Horticulturae.* 97:1-25.

Magioli, C., and Mansur, E. 2005. Eggplant (*Solanum melongen*a L.):tissue culture, genetic transformation and use as an alternative model plant. *Acta Botanica Brasilica*.19:139-148.

Maluszynski M, Kasha, K.J., and Szarejko, I. 2003. Published doubled haploid protocols in plant species. In Maluszynski,M., Kasha, K.J., Forster,B.P., and Szarejko, I. eds, Doubled haploid production in crop plants: A manual. *Springer,* Dordrecht, The Netherlands:309-335.

Miyoshi, K. 1996. Callus induction and plantlet formation through culture of isolated microspores of eggplant (*Solanum melongena* L). *Plant Cell Rep* 15:391–395.

Moraes, A.P.de., Bonadese-Zanettini, M.H., Callegari -Jacques, S.M., and Kaltchuk-Santos, E. 2004. Effect of Temperature Shock on Soybean Microspore Embryogenesis. *J.Brazilia Archieves of Biol and Tech*. 47 (4):537-544.

Moraes, A.P.de., Bered, F., Carvalho, F.I.F.de., and Kaltchuk-Santos, E. 2008. Morphological markers for microspore developmental satge in maize. *Braz. arch. biol. technol.* 51 (5):911-916.

Munoz-Amatriain, M., Svensson, J.T., Castillo, A.M., Close, T.J., and Valles, M.P. 2009. Microspore embryogenesis: assignment of genes to embryo formation and green vs. albino plant production. *Funct Integr Genomics*. 9:311–323.

Olmedilla, A. 2010. *Plant developmental biology-biotechnical perspective*: volume 2. Chapter 2 Microspore embryogenesis. Springer-Verlag Berlin Heidelberg.

Salas, P., Rivas-Sendra, A., Prohens, J., and Seguı´-Simarro, J.M. 2012. Influence of the stage for anther excision and heterostyly in embryogenesis induction from eggplant anther cultures. *Euphytica*. 184:Pp.235–250.

Segui-Simarro, J.M., and Nuez, F. 2005. Meiotic metaphase I to telophase II as the most responsive stage during microspore development for callus induction in tomato (*Solanum lycopersicum*) anther cultures. *Acta Physiologiae Plantarum* Vol.27: 675-685.

Shariatpanahi, M.E., Bal, U., Heberle-Bors, E., and Touraev, A. 2006. A Stress applied for the reprogramming of plant microspores towards *in vitro* embryogenesis. *Physiologia Plantarum*. 127:519-534.

Snape, J. 1989. Doubled haploid breeding: theoretical basis and practical applications. In Review of advances in plant biotechnology. 2nd Int. Symposium Genetic Manipulation in Crops. International Maize and Wheat Improvement Center and International Rice Research Institute (CIMMYT and IRRI), Manila, The Philippines:19-30.

Soriano, M., Li, H., and Boutilier, K. 2013. Microspore embryogenesis: establishment of embryo identity and pattern in culture. *Plant Reprod*. 26 :181–196.

Sumarmi, Daryono, B.S., Rachmawati,D., and Indrianto, A. 2014. Determination of Soybean (*Glycine max* L. Merrill) Microspores Development Stage Based on The Length of Flower Buds. *Journal of Biological Researches* 20: 6 -11.

Taher, D., Svein, S., Jaime, P., Yu-yu, C., Mohammed, R., and Tien-hor, W., 2017. World Vegetable Center Eggplant Collection: Origin, Composition,Seed Dissemination and Utilization inBreeding. *Frontiers in Plant Science*. 8:1484.

Zhang, C., Tsukuni, T., Ikeda, M., Sato, M., Okada, H., Ohashi, Y., Matsuno, H., Yamamoto, T., Wada, M., *et al.* 2013. Effects of the microspore development stage and cold pre-treatment of flower buds on embryo induction in apple (*Malus domestica* Borkh.) anther culture. *J Jpn Soc Hortic Sci* 82:114-124.

Zhang, DB., and Wilson, Z.A. 2009. Stamen specification and anther development in rice. *Chinese Sci Bull*. 54:2342–2353.

Zheng, M.Y. 2003. Microspore Culture in Wheat (*Triticum aestivum*) Double Haploid Production via Induced Embryogenesis. Review of Plant Biotechnology and Applied Genetics. *Plant Cell Tissue and Organ Culture* 73:213-230.

Journal of Tropical Biodiversity and Biotechnology
Volume 05, Issue 02 (2020): 132 — 142
DOI: 10.22146/jtbb.54011

Research Article

Plants Flowering and Fruiting Behaviour in Alas Purwo National Park, Banyuwangi, East Java

Dewi Ayu Lestari[1]*, Agung Sri Darmayanti[1]
1) Purwodadi Botanic Garden, Research Center for Plant Conservation and Botanic Garden, Indonesian Institute of Sciences (LIPI), Jl. Surabaya-Malang Km. 65, Purwodadi, Pasuruan, East Java 67163

Submitted: 03 February 2020; Accepted: 27 June 2020; Published: 15 August 2020

ABSTRACT
Alas Purwo National Park (APNP) is a conservation area with lowland forest type. The adaptation of plants conserved is strongly influenced by environmental factors and the behaviour of flowering and fruiting. The aims of this research were to find the number, species, dominance, and abundance of flowering and fruiting plants, comparison of flowering and fruiting species, and environmental factors affecting the flowering and fruiting time in APNP observation tracks. This study used purposive random sampling in each observation track where flowering and fruiting plants were found. Environmental factors (temperature, humidity, light intensity, soil pH, elevation, and coordinates) in each observation track were measured. Data analysis was conducted using Microsoft Excel and PAST 4.0. statistic program. The behaviour of flowering and fruiting plants species in APNP was unique. There were 90 species of flowering and fruiting plants in APNP from 45 families. Most species often found flowering and fruiting were *Orophea enneandra, Polyalthia littoralis* and *Leea angulata* which were scattered in Moto Lele, Patirtan Mas, and Sadengan Savanna. Fruiting plants species were more often found than flowering ones. Temperature and light intensity became the two most affecting environmental factors on flowering and fruiting plants behaviour. The study of flowering and fruiting behaviour is very important for genetic resources conservation and conservation areas management.

Keywords: Alas Purwo National Park, behaviour, conservation, flowering, fruiting

INTRODUCTION
According to the Regulation of the Indonesian Minister of Environment and Forestry No. 46/2016 article 1 paragraph 2, a national park is a natural conservation area with an original ecosystem, managed with a zone system utilized for research, science, education, tourism, recreation and supporting cultivation. Based on this regulation, native plants of each national park area must be maintained since it is the icon of this area. The types of most national parks in eastern Java are lowland forests, including Alas Purwo National Park (APNP). Lowland rainforest becomes the dominant area of APNP with mangrove and coastal forest as additional formations on an altitude of 0-322 m asl. According to Tisnawati *et al.* (2012), more than 700 plant species were identified from 123 families within this area. Lowland rainforests were dominated by bamboo vegetation, in which the highest numbers of species were within the family of Verbenaceae and Poaceae (Hidayat, 2008). The huge number of rare plants having potential as medicine in APNP causes it to be vulnerable to exploitation (Hidayat, 2008) and the existence of native plants could be threatened.

Information concerning the adaptation of plants to their environment, especially to climate change, is available through the observation of the behaviour of flowering and fruiting since it is one of the biological activities affected by the ecological factors of a plant (Nanda *et al.*, 2017) and microclimate factors (Lestari & Sofiah, 2015). Changes in plant behaviour factors when flowering and fruiting affect the efforts for its conservation. Each plant has different flowering and fruiting behaviour (phenological characters) because it is

***Corresponding author**
Tel.: +62 341 426046
Email: chunyang_dee@yahoo.co.id

influenced by genetic and environmental character (Goldsworthy & Fisher, 1992; Milla *et al.*, 2006).

Plants flowering and fruiting behaviour comprises the pattern and period of flowering and fruiting. It greatly affects the conservation efforts of these species in nature since the information of the pattern and period of flowering and fruiting leads to be understanding of plants response to their environment. In addition, it is also a very important issue in the successful management of forest genetic resources (Khanduri *et al.*, 2013; Micheloud *et al.*, 2018). For APNP, basic knowledge about the behaviour of the in-situ conserved flowering and fruiting plants would have a positive impact on wildlife in APNP considering the feed of various wild animals in APNP is very dependent on the presence of fruits in their habitat. In return, animals in APNP also helps the natural pollination of plants conserved.

The aims of this research were to find out the number, species, dominance, and abundance of flowering and fruiting plants in each APNP observation track, to figure out the comparison of flowering and fruiting plants in APNP and environmental factors affecting the flowering and fruiting plant periods in APNP. This study was expected to be the basic for plant management in supporting in-situ conservation in APNP.

MATERIALS AND METHODS

Materials

This study was conducted in Alas Purwo National Park, Tegaldlimo, Banyuwangi, East Java Indonesia, on 15 – 27 April 2019. Observation tracks of flowering and fruiting plants behaviour were forest plantation in Rowobendo, Trianggulasi beach, Birdwatching Track (JPB), Sadengan savanna, Parang Ireng beach, Pancur beach, Istana cave, Patirtan Mas, Moto Lele, Curah Kembang and Semar Moyo (Figure 1).

Methods

The method used was purposive random sampling in each observation track. Flowering and fruiting plants in each observation track were inventoried, documented and percentage scored by Arisoesilaningsih & Soejono (2001), Hatta & Darnaedi (2005), Anderson *et al.* (2005) and Handayani (2016). Environmental factors such as temperature, humidity, light intensity, soil pH, elevation, and coordinates in each observation track were measured. Temperature and humidity were determined by using a thermohygrometer, light intensity by using a luxmeter, soil pH by using a pH meter, while elevation and coordinates by using a Garmin GPS. The observed parameters were the number and species of flowering and fruiting plants, the dominant number and plants in each observation track, the abundance of flowering and fruiting plants, comparison of flowering and fruiting plants, and the environmental factors affecting the flowering and fruiting behaviour. Species of flowering and fruiting plants in observation tracks were noted the number and its species. The dominant species number was counted in each observation track. The abundance of flowering and fruiting plants was counted by scoring method

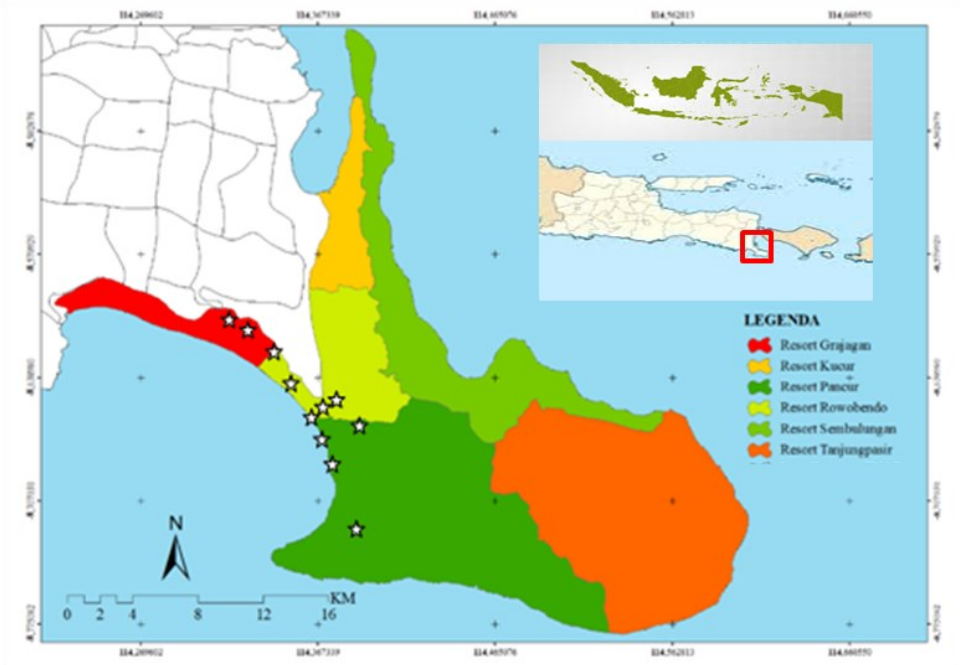


Figure 1. Research location in APNP, Banyuwangi, East Java, Indonesia (white star = observation track; ordinate: S 08° 35'23.1" E 114°20'55.9" to S 08°43'26,1" E 114°22'50.2").

(Anderson *et al.*, 2005; Handayani, 2016; Lestari, 2019). Total from each flowering plant species compared with fruiting plant species in each observation track. The effect of environmental factors on flowering and fruiting behaviour was measured by analyzation of environmental factors data in each observation track using Principal Component Analysis (PCA) method.

Data were analyzed descriptively using Microsoft Excel and PAST 4.0. statistic program. PCA method was used to figure out the environmental factors affecting the plant flowering and fruiting behaviour in APNP.

RESULTS AND DISCUSSION

The behaviour of flowering and fruiting plants in each APNP observation track showed a unique characteristic. In several observation tracks, flowering and fruiting plants were dominated by one species, whereas in other tracks, those plants varied.

Number and species of flowering and fruiting plants in APNP

There were 90 flowering and fruiting plants within 45 families in APNP (Table 1). Flowering and fruiting plants varied in each observation track. *Orophea enneandra, Polyalthia littoralis* and *Leea angulata* were the most plants found flowering and fruiting in the study sites. The most flowering and fruiting plants found in the observation tracks were Euphorbiaceae.

Several plant species were found flowering and fruiting in each observation track. Flowering and fruiting plants found in more than 3 observation tracks were *Chydenanthus excelcus, Corypha utan, Donax canniformis, Dysoxylum cyrtobotryum, Ficus hispida, Ficus montana, Harrisonia perforata, Leea angulata, Leea chinensis, Memecylon floribundum, Polyalthia littoralis, Spondias pinnata, Tacca palmata, Tetracera scandens*, and *Uvaria grandiflora*. It indicated that those plants could easily adapt to their environment and had flowering and fruiting patterns throughout the year so that they were easy to find. Plants having the same pattern including *Donax canniformis* (Brink & Escobin, 2003), *Ficus hispida*, and *Ficus montana* (Backer & van den Brink, 1968), *Orophea enneandra* (Lestari, 2019), and *Tetracera scandens* (van Valkenburg & Bunyapraphatsara, 2002). Some plants have an uncertain flowering season and it is mostly unaffected by climate such as *Tacca palmata* (Lemmens & Bunyapraphatsara, 2003). Flowering season can also be influenced by pollinators, pollination types, and predators. Because flowering and fruiting behaviour were associated with biotic and climatic factors interaction (da Maia *et al.*, 2013; Mohandass *et al.*, 2018). Meanwhile, there are plants flowering and fruiting once or twice a year and the time is exactly the same as this research activity. For example, *Dysoxylum cyrtobotryum* in India flowers from February to April, while the fruits ripe from June to July (Kumar, 2009). As a result of climate differences in Indonesia, there may be a slight shift for fruit

Table 1. Flowering and fruiting plants in APNP.

No	Species	Observation track									
		RWB	TRB	JPB	SS	PIB	PCB	IC	PM	ML	CK	SM
1	*Aglaonema simplex* (Blume) Blume			+								
2	*Aleurites moluccanus* (L.) Willd.								+			
3	*Allophyllus cobbe* (L.) Raeusch.	+				+						
4	*Alocasia gigantean* (Schott) G.Don		+									
5	*Alstonia spectabilis* R.Br.	+										
6	*Antidesma bunius* (L.) Spreng					+						
7	*Antidesma montanum* Blume			+							+	
8	*Archidendron bigeminum* (L.) I.C.Nielsen							+				
9	*Ardisia elliptica* Thunb.									+		
10	*Ardisia humilis* Vahl.					+						
11	*Ardisia* sp.								+			
12	*Arytera serrata*		+									
13	*Bambusa blumeana* Schult.f.									+		
14	*Bischofia javanica* Blume			+					+			
15	*Calophyllum inophyllum* L.	+										

Table 1. Contd.

No	Species	Observation track										
		RWB	**TRB**	**JPB**	**SS**	**PIB**	**PCB**	**IC**	**PM**	**ML**	**CK**	**SM**
16	*Canarium hirsutum* Willd.			+								
17	*Canavalia rosea* (Sw.) DC.	+										
18	*Carapichea ipecacuanha* (Brot.) L. Andersson							+		+		
19	*Casearia grewiifolia* Vent.		+									
20	*Cassia fistula* L.								+			
21	*Randia* sp.									+		
22	*Cerbera odollam* Gaertn.		+									
23	*Cheilocostus speciosus* (J.Koenig) C.D. Specht				+				+			
24	*Chydenanthus excelcus* (Blume) Miers				+				+	+	+	
25	*Cleistanthus collinus* (Roxb.) Benth. ex Hook.f.				+							
26	*Corypha utan* Lam.				+		+	+		+		
27	*Crotalaria juncea* L.									+		
28	*Diospyros cauliflora* Blume								+		+	
29	*Diospyros maritima* Blume					+						
30	*Diospyros vera* (Lour.) A.Chev.				+							
31	*Donax canniformis* (G.Forst.) K.Schum.				+			+	+		+	
32	*Dovyalis caffra* (Hook.f. & Harv.) Sim				+					+		
33	*Dracaena angustifolia* (Medik.) Roxb.					+						
34	*Drypetes serrata* (Maycock) Krug & Urb.									+		
35	*Dysoxylum cyrtobotryum* Miq.		+	+		+						
36	*Embelia ribes* Burm.f.			+								
37	*Ficus racemosa* L.	+					+					
38	*Ficus callophylla* Blume (1)	+									+	
39	*Ficus callophylla* Blume (2)				+							
40	*Ficus callosa* Willd.									+		
41	*Ficus drupacea* Thunb.									+		
42	*Ficus hispida* L.f.	+				+			+	+		
43	*Ficus montana* Burm.f.				+			+	+		+	
44	*Ficus variegata* Blume			+				+				
45	*Gnetum gnemon* L.					+						
46	*Grewia asiatica* L.									+		
47	*Harpullia arborea* (Blanco) Radlk.									+		
48	*Harrisonia perforata* (Blanco) Merr.				+				+	+	+	
49	*Hernandia nymphaeifolia* (J.Presl) Kubitzki	+				+						
50	*Ipomoea pes-caprae* (L.) R. Br.	+										

Table 1. Contd.

No	Species	Observation track										
		RWB	**TRB**	**JPB**	**SS**	**PIB**	**PCB**	**IC**	**PM**	**ML**	**CK**	**SM**
51	*Ixora smeruensis* Bremek.		+									
52	*Ixora* sp.							+				
53	*Knema cinerea* (Poir.) Warb.								+		+	
54	*Lantana camara* L.				+					+		
55	*Leea angulata* Korth. ex Miq.	+	+	+	+				+	+		
56	*Leea chinensis*		+	+	+							
57	*Mallotus dispar* (Blume) Mull.Arg.									+		
58	*Mallotus* sp.					+						
59	*Memecylon floribundum* Blume			+				+		+		
60	*Mimosa pudica* L.					+						
61	*Musa acuminata* Colla								+			
62	*Nauclea* sp.									+		
63	*Nicolaia* sp.									+		
64	*Ochrosia ackeringae* (Teijsm. & Binn.) Miq.		+									
65	*Oplismenus burmanni* (Retz.) P.Beauv.					+						
66	*Orophea enneandra* Blume			+				+	+		+	
67	*Palaquium* sp.										+	
68	*Pangium edule* Reinw.								+			
69	*Pavetta indica* L.									+		
70	*Phaleria capitata* Jack.			+					+	+		
71	*Physalis angulata* L.					+						
72	*Piper cubeba* L.f.	+								+		
73	*Piper retrofractum* Vahl.	+										
74	*Polyalthia littoralis* (Blume) Boerl.			+				+		+		
75	*Sandoricum koetjape* (Burm.f.) Merr.											+
76	*Pseuderanthemum carruthersii* (Seem.) Guillaumin					+				+		
77	*Senna siamea* (Lam.) H.S. Irwin & Barneby	+					+					
78	*Spondias pinnata* (L.f.) Kurz.			+					+		+	
79	*Sterculia foetida* L.		+							+		
80	*Suregada glomerulata* (Blume) Baill.	+										
81	*Tabernaemontana pandacaqui* Lam.	+										
82	*Tabernaemontana sphaerocarpa* Blume										+	
83	*Tabernaemontana* sp.				+							
84	*Tacca leontopetaloides* (L.) Kuntze									+		
85	*Tacca palmata* Blume			+	+			+				

Table 1. Contd.

No	Species	Observation track										
		RWB	TRB	JPB	SS	PIB	PCB	IC	PM	ML	CK	SM
86	*Tetracera scandens* (L.) Merr.			+	+					+		
87	*Uvaria grandiflora* Roxb. ex Hornem			+	+					+		
88	*Voacanga grandifolia* (Miq.) Rolfe	+										
89	*Vitex pinnata* L.				+		+					
90	*Vitis* sp.									+		

Note: RWB = Rowobendo Forest Plantation, TRB = Trianggulasi Beach, JPB = Birdwatching Track, SS = Sadengan Savanna, PIB = Parang Ireng Beach, PCB = Pancur Beach, IC = Istana Cave, PM = Patirtan Mas, ML = Moto Lele, CK = Curah Kembang, SM = Semar Moyo.

ripening i.e. in April. *Polyalthia littoralis* which is ex-situ conserved in Purwodadi Botanic Garden and resulting from plant exploration activity in APNP, have twice a year flowering pattern and once a year fruiting pattern, from January to August (Handayani, 2016; Lestari, 2019). *Uvaria grandiflora* has a fruiting period from January to May (Lestari, 2019) in Purwodadi Botanic Garden. *Spondias pinnata* in southeastern India has a fruiting period from June to November, and probably due to the different seasons, it has an earlier fruiting period in May. Some species of *Spondias* fruit in May or approaching May in the tropics (Mitchell & Daly, 2015). *Corypha utan* has flowering and fruiting period only once i.e. at the end of its life (Heyne, 1987).

The dominant plants and their number in each observation track

The observation track with the most flowering and fruiting plants was Moto Lele (20%), followed by Patirtan Mas and Sadengan savanna (12%) (Figure 2). The dominant flowering and fruiting plants in each observation track varied. The dominant flowering and fruiting plants in Rowobendo Forest Plantation were *Piper cubeba, Piper retrofractum* (fruiting), and *Suregada glomerulata* (flowering and fruiting). *Casearia grewiifolia* was only found fruiting on the edge of Trianggulasi beach. *Orophea enneandra* was found fruiting in Bird Watching Path (JPB), Istana Cave (flowering), Patirtan Mas, and Curah Kembang. Two species were found fruiting in two different locations, namely *Leea angulata* in Sadengan savanna and Moto Lele, and *Polyalthia littoralis* in JPB and Moto Lele. *Ficus hispida* and *Diospyros maritima* were found fruiting along the Parang Ireng beach. On the edge of Pancur beach, many *Corypha utan* were found fruiting. Besides *Orophea enneandra, Tacca palmata* and *Ficus montana* were found fruiting in the Istana cave. *Bambusa blumeana* was also found blooming in Moto Lele, besides *Polyalthia littoralis* and *Leea angulata* that were fruiting (Figure 3). There was no dominant flowering and fruiting plants in Semar Moyo.

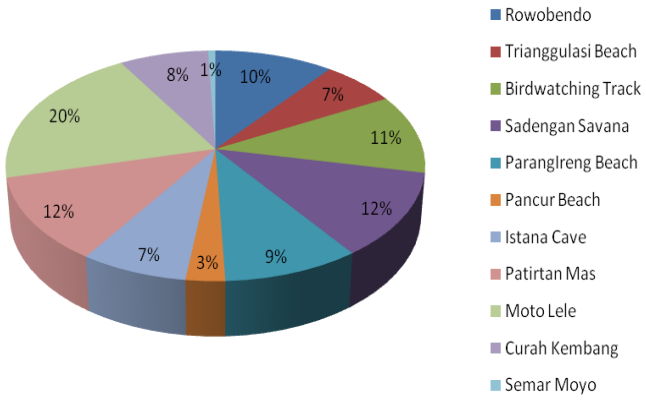


Figure 2. The percentage of flowering and fruiting plants in each observation track.

Flowering and fruiting plants in Moto Lele were more numerous than other observation tracks. It was because it had warm air temperature (32.34° C), medium level of humidity (65.2%), and light intensity (665.28 lux) to support the process of plant metabolism. Therefore, it was more optimal for flower and fruit formation than others. Sufficient light intensity affects the level of photosynthesis as the source of energy for the flowering process. Denser vegetation canopy in other observation tracks causes less light intensity hence the growth and development of fruit would not be optimized. Low light intensity is closely related to PAR (Photosynthetically Active Radiation), where the denser canopy level causes low PAR value and vice versa. Plants experience a double decline in fruit production under low light intensity, which is closely related to the distribution of carbon to the fruit and influenced by the balance between starch and sucrose (Sitompul, 2010). Moto Lele was a type of natural forest with fairly dense vegetation canopy

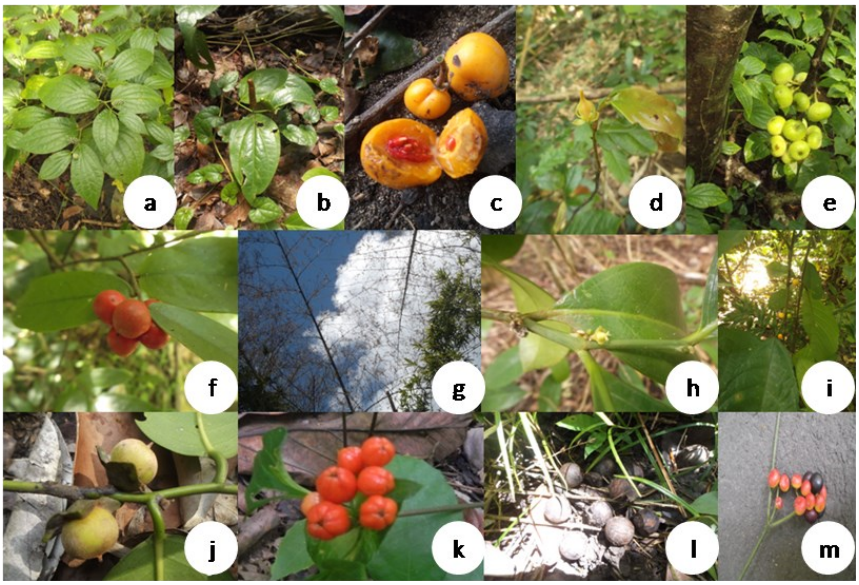


Figure 3. The dominant flowering and fruiting plants in APNP; a. Immature fruit of *Piper cubeba,* b. Ripe fruit of *Piper retrofractum,* c. Ripe fruit of *Casearia grewiifolia,* d. flower of *Orophea enneandra,* e. Immature fruit of *Ficus hispida,* f. Mature fruit of *Polyathia littoralis,* g. Blooming of *Bambusa blumeana*, h. Blooming of *Suregada glomerulata*, i. Mature fruit of *Ficus montana,* j. Immature fruit of *Diospyros maritima,* k. Mature fruit of *Tacca palmata,* l. Mature fruit of *Corypha utan,* m. Mature fruit of *Leea angulata* percentage of flowering and fruiting plants.

and still relatively native (Darmayanti *et al.*, 2019). Thus, it became the preferred habitat for butterflies. The butterflies found in APNP were 39 species and some of them were classified as pollinators (Budiarto, 2014). Besides that, there found vulnerable bird species such as *Leptoptilos javanicus* and *Pavo muticus* in Sadengan savanna as a representation of ideal place to take a rest and to forage (Widodo, 2016). There are 13 bats species with 2 *Near Threatened* species playing role as zoopollinator known to be scattered in the cave area of APNP (Rianti *et al.*, 2009). The number of zoopollinator living in forest areas certainly influences the number of pollinated plants and thus the number of fruiting plants.

The abundance of flowering and fruiting plants

The abundance of flowering and fruiting plants in each observation track was shown in Figure 4. Species with the greatest abundance in each observation track were *Calophyllum inophyllum* in Rowobendo Plantation Forest, *Tacca palmata* in Istana cave, and *Corypha utan* in Moto Lele.

Comparison of flowering and fruiting plants number

Based on Figure 5, it can be inferred that the number of fruiting plants in each observation track

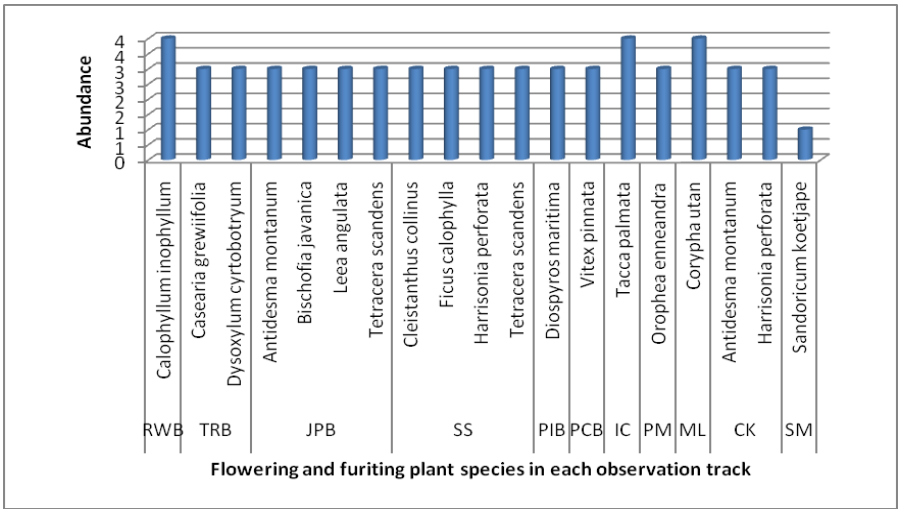


Figure 4. The abundance of flowering and fruiting plants in each observation track; RWB = Forest Plantation of Rowobendo, TRB = Trianggulasi Beach, JPB = Birdwatching Track, SS = Sadengan Savanna, PIB = Parang Ireng Beach, PCB = Pancur Beach, IC = Istana Cave, PM = Patirtan Mas, ML = Moto Lele, CK = Curah Kembang, SM = Semar Moyo.

is more than flowering ones. Even in JPB, Curah Kembang, and Semar Moyo, there were no flowering plants found. Observation tracks where flowering plants were mostly found in Moto Lele, followed by Sadengan savanna and Parang Ireng beach. While fruiting plants were mostly found in Moto Lele, followed by Patirtan Mas and JPB.

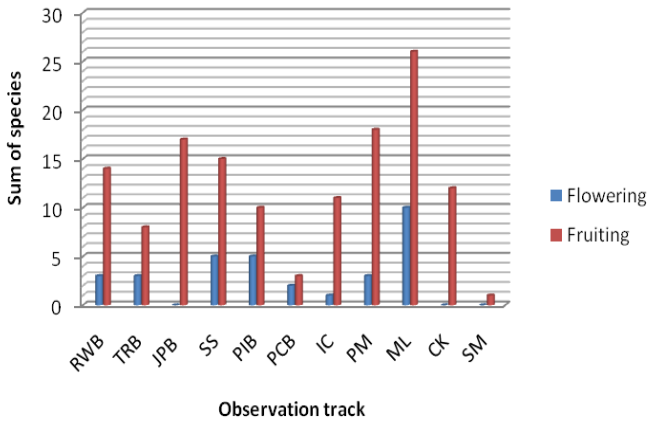


Figure 5. Comparison of flowering and fruiting plants number in each observation track; RWB = Forest Plantation of Rowobendo, TRB = Trianggulasi Beach, JPB = Birdwatching Track, SS = Sadengan Savanna, PIB = Parang Ireng Beach, PCB = Pancur Beach, IC = Istana Cave, PM = Patirtan Mas, ML = Moto Lele, CK = Curah Kembang, SM = Semar Moyo.

The numbers of fruiting plants were more than the flowering ones. According to Anderson *et al.* (2005), the peak of the flowering season in tropics usually in the wet season. Meanwhile, this research was carried out during the dry season, so that the number of flowering plants was less than the fruiting ones. Flowering initiation is influenced by external factors such as environment and also stimulated by endogenous factors such as hormonal factors, flowering time initiation, and an adequate carbon or nitrogen balance (Larcher, 1995). During the dry season, immature fruits are formed immediately after the flowers bloom. Immature fruits take several months to become ripe fruits (Arisoesilaningsih *et al.*, 2001).

Influence of environmental factors on flowering and fruiting behaviour

Based on Table 2, range of general environmental conditions in the observation track were 29.8-32.34° C (temperature), 65.2 – 77.88% (humidity), 21.7-73 m asl (elevation), 6-7 (soil pH), 83.2-2306.75 lux (light intensity) and the coordinates location was between S 08°35'23.1" E 114°20'55.9" to S 08° 43'26,1" E 114°22'50.2". The influence of environmental factors on the number of flowering and fruiting plants was shown in Figure 6.

Temperature and light intensity were the two most influential factors of the flowering and fruiting period in APNP, especially in Rowobendo Plantation Forest, JPB, Sadengan savanna, Parang Ireng beach, Istana cave, and Moto Lele. The numbers of flowering and fruiting plants found in those observation tracks were more compared to other regions.

Environmental factors influencing the plants to flower are humidity, temperature, sunlight, rainfall, and nutrients (Sulistyawati *et al.*, 2012). Based on Figure 6, temperature and light intensity are environmental factors affecting the behaviour of flowering and fruiting plants found in observation tracks. Microclimate is the most important factor and has a significant effect on flowering and fruiting

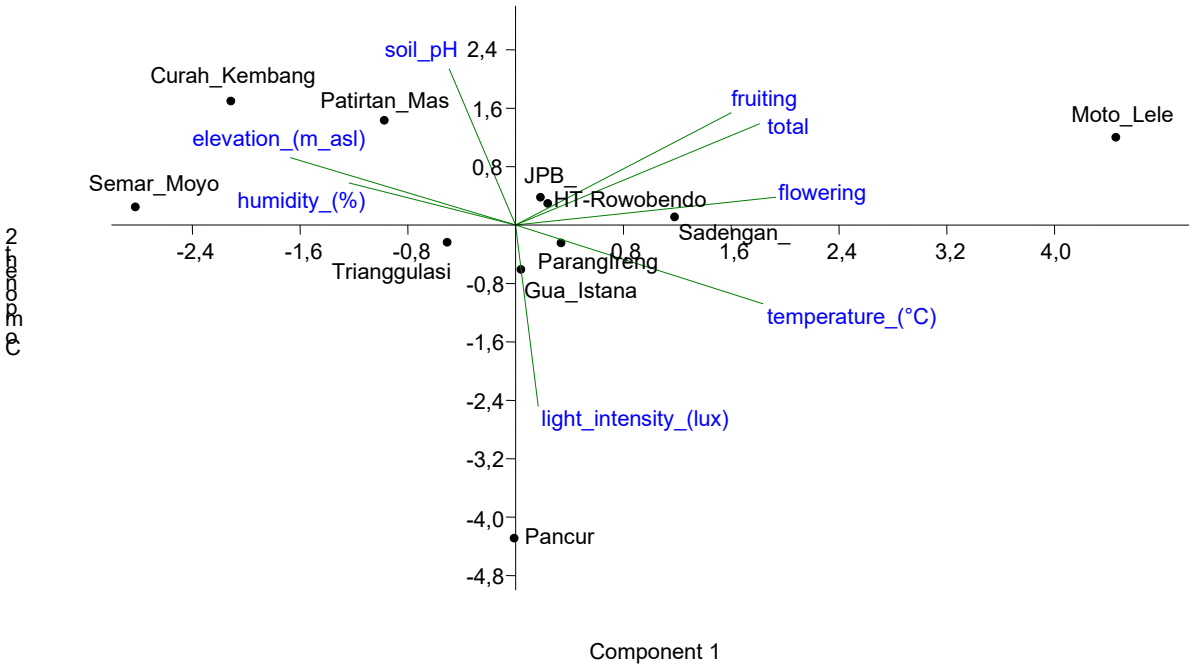


Figure 6. Effect of microclimate factors on flowering and fruiting plants in APNP.

Table 2. Average of microclimate factors in each observation track; RWB = Forest Plantation of Rowobendo, TRB = Trianggulasi Beach, JPB = Birdwatching Track, SS = Sadengan Savanna, PIB = Parang Ireng Beach, PCB = Pancur Beach, IC = Istana Cave, PM = Patirtan Mas, ML = Moto Lele, CK = Curah Kembang, SM = Semar Moyo.

Observation track	Average of microclimate factors						Sum of species		Total
	Temperature (°C)	Humidity (%)	Elevation (m asl)	Soil pH	Light intensity (lux)	Coordinate	Flowering	Fruiting	
RWB	30.75	74.4	29.6	6.67	937.56	S 08°38'24,6" E 114°20'55,9" - S08°38'40,8" E 114°21'23,7"	3	14	16
TRB	30.7	75.5	26.13	6.68	1181.13	S 08°39'00,0" E 114°21'39,5" - S08°39'22,7" E 114°21'43"	3	8	10
JPB	31.32	74.07	33.21	6.59	591.58	S 08°39'11,1" E 114°21'52,6" - S08°39'35,3" E 114°22'03,9"	0	17	17
SS	30.9	69.88	27	6.6	1193.75	S 08°39'05,7" E 114°22'00,8" - S08°39'13,9" E 114°22'18,3"	5	15	18
PIB	30.48	70.5	28.25	6.75	2306.75	S 08°41'05,6" E 114°22'30,3" - S08°41'07,8" E 114°22'31,7"	5	10	14
PCB	31.7	71	30	6	6200	S 08°40'42,3" E 114°22'27,2"	2	3	
IC	31.42	68.4	43.6	6.56	1156.15	S 08°40'17,7" E 114°22'27,2" - S08°40'42,3" E 114°22'46,8"	1	11	11
PM	30.06	77.88	70.88	6.56	544.1	S 08°37'53,4" E 114°22'32,4" - S08°38'02,9" E 114°22'50,2"	3	18	18
ML	32.34	65.2	21.7	6.78	665.28	S 08°42'29,6" E 114°22'14,3" - S08°43'26,1" E 114°22'34,7"	10	26	31
CK	29.8	76	62	7	83.2	S 08°35'23,1" E 114°22'05,0"	0	12	12
SM	30.1	69	73	7	624	S 08°36'39,1" E 114°22'19,8"	0	1	1

periods compared to height and location (Panchen, 2016).

Time or period is an important component in plant reproduction. Individual plants with early flowering would have a limited capacity to produce fruit (Milla *et al*., 2006; Khanduri, 2014). Certain plants produce fruits from flowers in a very short period, but other plants require a longer time. This behaviour is very important and useful in planning the conservation improvement programs and strategies for a plant species. The need to recognize the flowering and fruiting behaviour of a plant species and observe its phenological development is very closely related to its ecological studies (Augspurger, 1983; Abu-Asab *et al*., 2001). In addition, the study of flowering and fruiting plant

behaviour is important from the perspective of genetic resources conservation and conservation areas management (Omondi *et al.*, 2016). Especially for in-situ conservation areas, such as APNP, where fauna becomes one of the important components in its ecosystem. Flowering plants provide many benefits, beside become food sources, they also become places to lay eggs, hiding, and inviting other fauna species in the ecosystem such as pollinators, natural enemies, as well as other ecological functions. Through flowering and fruiting plants, the ecosystem would be more stable, thus the ecosystem components balance could be maintained (Kurniawati & Martono, 2015).

CONCLUSION

There were 90 species of flowering and fruiting plants in APNP from 45 families. Most species often found flowering and fruiting were *Orophea enneandra, Polyalthia littoralis*, and *Leea angulata* which were scattered in Moto Lele, Patirtan Mas, and Sadengan Savanna. Fruiting plants species were more often found than flowering ones. Temperature and light intensity became the two most affecting environmental factors on flowering and fruiting plants behaviour.

ACKNOWLEDGMENTS

This research was financially supported by Indonesian the Institute of Sciences through seed bank research program. We would like to acknowledge the Director of Research Center for Plant Conservation and Botanic Garden, the Director of Purwodadi Botanic Garden for the opportunity to conduct field activities, the Director of Alas Purwo National Park, Alas Purwo National Park team for their assistance in the field, Mr. Achmad Suef for his assistance in the field for plant identification and other technical assistance, and Elok Rifqi Firdiana for her assistance in checking the English spelling and grammar. For author contribution, DAL is the main author.

REFERENCES

Abu-Asab, M.S., Peterson, P.M., Shetler, S.G., & Orli, S.S., 2001, Earlier plant flowering in spring as a response to global warming in the Washington, DC, area, *Biodivers Conserv* 10(4), 597–612.

Anderson, D.P., Nordheim, E.V., Moermond, T.C., Gone Bi, Z.B., & Boesch, C., 2005, Factors influencing tree phenology in Ta'i National Park, *BIOTROPICA* 37(4), 631-640, DOI 10.1111/j.1744-7429.2005.00080.x.

Arisoesilaningsih, E., & Soejono, 2001, Purwodadi Botanic Garden is a dry climate hortus?, Proceeding of National Seminary on Conservation and Utilization of Dry Lowland Plant Diversity, Purwodadi Botanic Garden – Indonesian Institute of Sciences and Brawijaya University, Pasuruan.

Arisoesilaningsih, E., Soejono, Widyati, A., Palupi, I., & Kiswojo, 2001, Reproductive activities of three an endangered tree lowland species in Purwodadi Botanic Garden, Proceeding of National Seminary on Conservation and Utilization of Dry Lowland Plant Diversity, Purwodadi Botanic Garden – Indonesian Institute of Sciences and Brawijaya University, Pasuruan.

Augspurger, C.K., 1983, Phenology, flowering synchrony, and fruit set of six neotropical shrubs, *Biotropica* 15(4), 257–267.

Backer, C.A., & van den Brink, R.C.B., 1968, *Flora of Java, Third Edition,* Noordhoff Groningen, The Netherlands.

Brink, M., & Escobin, R.P., 2003, *Donax canniformis, Plant Resources of South East Asia No 17. Fibre Plants, Prosea Foundation, Bogor.*

Budiarto, E., 2014, Inventory of Butterflies (Lepidoptera: Rhopalocera) in Pancur Resort, Alas Purwo National Park, Banyuwangi, East Java, *Thesis, Department of Biology, Faculty of Mathematic and Natural Sciences, University of Jember, Jember.*

Da Maia, F.R., Malucelli, T.S., & Varassin, I.G., 2013, Ecological factors affecting the fruiting success of a *Tibouchina trichopoda (DC.) Baill. (Melastomataceae) flower, Acta Bot. Bras. 27(1): 142 -146.*

Darmayanti, A.S., Lestari, D.A., & Suef, A., 2019, *Report of Seed Collection Activities in Alas Purwo National Park for the Development of Purwodadi Botanic Garden Seed Bank, Purwodadi Botanic Garden-LIPI, Pasuruan.*

Goldsworthy, P.R., & Fisher, N.M., 1992, *Physiology of Tropical Cultivated Plants*, Gadjah Mada University Press, Yogyakarta.

Handayani, T., 2016, Flowering and fruiting time of Annonaceae species in Bogor Botanic Gardens, *Buletin Kebun Raya* 19(2), 91-104.

Hatta, H., & Darnaedi, D., 2005, *Phenology and Growth Habits of Tropical Trees: Long Term Observations in the Bogor and Cibodas Botanic Gardens, Indonesia*, National Science Museum Tokyo, Japan.

Heyne, K., 1987, *Indonesian Useful Plants I*, Forestry Research and Development, Jakarta.

Hidayat, S., 2008, Structure, composition and status of medicinal plants in the forest area of Alas Purwo National Park, *Jurnal Biologi* 12(1), 9-13.

Khanduri, V.P., Sharma, C.M., Kumar, K.S., & Ghildiyal, S.K., 2013, Annual variation in flowering phenology, pollination, mating system, and pollen yield in two natural populations of *Schima wallichii* (DC.) Korth., *Sci. World J.* 350157, 1-11.

Khanduri, V.P., 2014, Annual variation in floral phenology and pollen production in *Lagerstroemia speciosa*: an entomophilous tropical tree, *J Sci. Tech.* 36(4), 389–396.

Kumar, A.N., 2009, *Exploring the "Bio- Cultural" Heritage in Conservation of 5 Rare, Endemic & Threatened (RET) Tree Species of Western Ghats of Kerala*, Final Report (2008-1009), MS Swaminathan Research Foundation Community Agrobiodiversity Centre, India.

Kurniawati, N., & Martono, E., 2015, The role of flowering plants as a conservation media of arthropod natural enemies, *Jurnal Perlindungan Tanaman Indonesia* 19(2), 53-59.

Larcher, W., 1995, *Physiological Plant Ecology*, Springer Verlag, Berlin.

Lemmens, R.H.M.J., & Bunyapraphatsara, N., 2003, *Tacca palmate, Plant Resources of South East Asia No 12(3). Medicinal and Poisonous Plants 3*, Prosea Foundation, Bogor.

Lestari, D.A., 2019, Evaluation of flowering and fruiting periods of selected Annonaceae species in Purwodadi Botanic Garden, *AIP Conference Proceedings* 2120, 030023, https://doi.org/10.1063/1.5115627.

Lestari, D.A., & Sofiah, S., 2015, Flowering and fruiting times on four species of *Annona* (Annonaceae) in Purwodadi Botanic Garden, *JTLS* 5(1), 45-52.

Micheloud, N.G., Castro, D.C., Buyatti, M.A., Gabriel, P.M., & Gariglio, N.F., 2018, Factors affecting phenology of different *Citrus* varieties under the temperate climate conditions of Santa Fe, Argentina, *Rev. Bras. Frutic.* 40(1), 1-9.

Milla, R., Castro-Díez, P., Maestro-Martínez, M., & Montserrat-Martí, G., 2006, Costs of reproduction as related to the timing of phenological phases in the dioecious shrub *Pistacia lentiscus* L. *Plant Biol.* 8(1), 103–111.

Mitchell, J.D., & Daly, D.C., 2015, A revision of *Spondias* L. (Anacardiaceae) in the Neotropics, *PhytoKeys* 55, 1–92.

Mohandass, D., Campbell, M.J., Chen, X., & Li, Q., 2018, Flowering and fruiting phenology of woody trees in the tropical-seasonal rainforest, Southwestern China, *Current Sci.* 114(11): 2313-2322.

Nanda, A., Suresh, H.S., & Khrisnamurthy, Y.L., 2017, Phenology of tree species in a tropical evergreen forest of southern India, *J Global Ecol. Env.* 6(1), 1-12.

Omondi, S.F., Odee, D.W., Ongamo, G.O., Kanya, J.I., & Khasa, D.P., 2016, Synchrony in leafing, flowering, and fruiting phenology of *Senegalia senegal* within Lake Baringo Woodland, Kenya: Implication for conservation and tree improvement, *IJFR* 6904834, 11, http://dx.doi.org/10.1155/2016/6904834.

Panchen, Z., 2016, Impact of climate change on flowering and fruiting times of Nunavut Arctic Plants, *InfoNorth* 69(4), 444-449.

Rianti, I.P., Santosa, Y., & Sunkar, A., 2009, Pola penggunaan ruang bertengger oleh beberapa jenis kelelawar penghuni gua di TNAP Jawa Timur [The pattern of use of perch space by several types of bats, cave dwellers in TNAP East Java], *Indonesian Cave and Karst Journal* 5(1), 36-47.

Sitompul, S.M., 2010, *Radiation on Agroforestry System*, http://old.worldagroforestry.org/sea/Publications/files/lecturenote/LN0034-04/LN0034-04-5.pdf., Accessed on 8 Oktober 2019.

Sulistyawati, E., Mashita, N., Setiawan, N.N., Choesin, D.N., & Suryana, P., 2012, Flowering and fruiting phenology of tree species in Mount Papandayan Nature Reserve, West Java, Indonesia, *TLSR* 23(2), 81-95.

Tisnawati, V., Mala, Y.A., Utami, J., Yanuarefa, M.F., Pratiwi, L.E., Purwanto, Fahrurozi, R., Haryono, E., & Wijayanti, A., 2012, *Book of Alas Purwo National Park Research Information*, Alas Purwo National Park, Banyuwangi.

Van Valkenburg, J.L.C.H., & Bunyapraphatsara, N., 2002, *Tetracera*. In: van Valkenburg, J.L.C.H., & Bunyapraphatsara, N. (eds), *Plant Resources of South East Asia No 12(2). Medicinal and Poisonous Plants 2,* Prosea Foundation, Bogor.

Widodo, W., 2016, Distribution and diversity of restricted-range bird species in the Alas Purwo National Park, *Proceeding Biology Education Conference* 13(1), 690-700.

Journal of Tropical Biodiversity and Biotechnology
Volume 05, Issue 02 (2020): 143 — 148
DOI: 10.22146/jtbb.53004

Research Article

The Effectiveness of Red Spinach (*Amaranthus tricolor* L.) and Green Spinach (*Amaranthus hybridus* L.) Extracts for *Bacillus thuringiensis* var. *kurstaki* Protectant against UVB Radiation for the Control of Armyworm (*Spodoptera litura* Fab.)

Siti Sumarmi[1], Mifta Arlinda[2], Sukirno Sukirno[1]*

1) Entomology Laboratory, Faculty of Biology, Gadjah Mada University, Jalan Teknika Selatan, Sekip Utara, Sleman, Yogyakarta, Indonesia 55281
2) Undergraduate student, Faculty of Biology, Gadjah Mada University, Jalan Teknika Selatan, Sekip Utara, Sleman, Yogyakarta, Indonesia 55281

Submitted: 30 December 2019; Accepted: 25 June 2020; Published: 15 August 2020

ABSTRACT

Spodoptera litura Fab. is an insect that damage cultivated plants in Indonesia. Efforts to control it can be done by using biological agents for example by using *Bacillus thuringiensis kurstaki (Btk.)*. Unfortunately, the *Btk.* is easily degraded by UV radiation. This research aimed to study the effectiveness of red and green spinach as UVB protection for *Btk.* and to observe the pathogenicity of *Btk.* formulations against armyworm. Furthermore, the sublethal effect of *Btk.* against *S. litura* was investigated. The morphology of the endospore, protein crystal, and bacterium were observed under a contrast phase microscope. The extracts at 2% (w/v) were mixed with *Btk.* suspensions at 5 x 10^4, 5x10^5, and 5x10^6 (spores/ml), respectively. The formulations then exposed under Ultraviolet B (UVB) lights for 3, 6, and 9 hours then tested against the 3rd larval instar of armyworm. The larval mortality was observed daily and the analysis of variance was analyzed by one way anova. The sublethal effects of the treatment to the pupal and adult stages were observed when the moths emerge. The results showed that the larval mortality caused by *Btk.* mixed with red spinach ranged from 11.7 to 26.7%. The sublethal effects of *Btk.* resulted in smaller sizes of pupae and imago, darker pupae, and wings abnormality of the adult stage, compared to any control treatment. The morphological observation of the bacteria showed that extracts gave UV protection against UVB. These results suggested that red and green spinach potentially can be used as a protectant for *Btk.* against UVB.

Keywords: *Bacillus thuringiensis* var. *kurstaki (Btk.)*, Pathogenicity, Spinach, *Spodoptera litura*, UVB

INTRODUCTION

Spodoptera litura Fab. is an insect that causes damage and losses on a cultivated plant in Indonesia. The high losses caused by these insects on cabbage plantations caused efforts to control these pests become a priority. Many control measures were made to control this pest including the use of chemical, herbal, and biological agents. The chemical controls such as using chemical insecticides resulting in resistance and resurgence of the pests (Marwoto & Neering, 1992). Whereas, the use of herbal controls, for example, using frangipani (*Plumeria* sp.) and chickweed (*Ageratum conyzoides* L.). One of the biological control agent commonly used is *Bacillus thuringiensis kurstaki* (*Btk.*) (Feitelson *et al.,* 1992).

Btk. easily degrades when exposed to ultraviolet (UV) lights. It causes the spores to be inactive (Griego & Spence, 1978). Several antioxidants can be used as additives for anti ultraviolets (El-Sharkawey *et al.,* 2009). The use of red and white dragon fruits (Sukirno *et al.*, 2017), aloe vera (Tarigan, 2019), and tea leaves (Ningrum, 2019) have been used for the study of *Btk.* protectants. Spinach contains compounds of vitamin A, vitamin C, vitamin E, flavonoids, and phenol

***Corresponding author**
Email: sukirnobiougm@ugm.ac.id

which can be used as antioxidants (Amin *et al.*, 2006). Therefore, in this research red spinach and green spinach extracts were used as protectants of *Btk*. against UV B to control armyworm. It is expected that the addition of protectants can increase the pathogenicity of *Btk*. against the pest.

MATERIALS AND METHODS

Materials

The materials used in this study include the 3rd instar of *S. litura* larvae, *Btk*. (Dipel WP ®, Abbot Co., IN) as a biological agent, red spinach, and green spinach extract as protectants of *Btk*., brain heart infusion (BHI) (Oxoid, ThermoScientific, UK), and bacteriological agar (BA) (Oxoid, ThermoScientific, UK) for culture media of *Btk*.

Methods

Insect Collection

Armyworm at the larval stage for the initial stock was collected from cabbage farming in Cangkringan, Sleman. It was collected directly by taking the infested cabbage leaves. As many as 60 larvae were used as parental. The collected larvae were maintained in an artificial diet at Entomology Laboratory, Faculty of Biology, Gadjah Mada University.

Artificial Diet

White bean-based artificial diet (Sutanto *et al.*, 2016, Sukirno *et al.*, 2017; 2018) was used for the armyworm mass rearing. As much as 125 g of white bean was soaked overnight in tap water. Then, the bean was boiled until soft. After that, it was grinded in a commercial blender by adding 500 ml of distilled water. After that, the mixture was added with 50 g agar powder, 80 g fermipan, 10 g sodium benzoic, and 750 distilled water, and then boiled. After boiled, the mixture was left at room temperature for 10 minutes until the temperature drops to ± 50°C. After that 5 g of ascorbic acid was then added to the mixture and homogenously. 25 ml of the artificial diet was poured into a 90 ml plastic cup. The cup then kept at room temperature for 45 minutes then stored in a refrigerator at 4 °C until used.

Mass Rearing of Armyworm

Larvae collected from the field were transferred on to an artificial diet (Shorey & Hale, 1965) with some modifications until pupation. The seven days old pupae then collected and surface sterilized in 5% (v/v) chlorox (Bayclin ®, SC Johnson, IN), then air-dried for 30 minutes and transferred into a glass jar (d: 20 cm, h: 40 cm) for the adult emergence. After emerges, the adults were provided with 10% honey solutions for adult feeding and opac paper for egg-laying substrate. The laid eggs were collected daily and transferred into an artificial diet for larval feeding. The F_2 of armyworm were used for the bioassay.

Btk. Culture on BHIA

Btk. from the commercial product (Dipel WP®, Abbot Co., IN) were cultured BHIA medium in a 15 ml test tube. *Btk*. isolate then incubated for five days at room temperature (28°C) until the protein crystals and spores were formed. The formation of these was observed under a contrast phase microscope (Nikon BX -1, JP).

Red and Green Spinach Extraction

As much as 20 g of each of stems and leaves of red and green spinach were weighed and washed in running tap water. Then blended with a commercial blender by adding 180 ml of distilled water. The suspension then filtered using two layers of muslin cloth then stored in a refrigerator (4°C) as a 10% (w/v) stock.

Btk. Formulations Exposure under UVB

Additive extracts at 2% (w/v) were used for making *Btk*. serial concentrations at $5x10^5$, $5x10^6$, and $5x10^7$ (spores/ml). One ml of each of the formula was taken and homogenously dropped into a disposable plastic petri dish (d: 6cm, h: 1.5 cm), then exposed under UVB lights (2x 10 W Phillips tube, IN) for 3, 6, and 9 hours. After exposed, the treated suspensions then collected by adding 9 ml of autoclaved distilled water to get $5x10^4$, $5x10^5$, and $5x10^6$ (spores/ml) final concentration for the bioassay. The effects of UVB to the morphology of vegetative cell, spore, and crystal protein were observed under a phase-contrast microscope.

Btk. Bioassay against the 3rd instar of Armyworm

Each of the above final concentrations of *Btk*. suspension was taken and homogenously added into the surface of an artificial diet provided in a disposable petri dish (d: 6cm, h: 1.5 cm), then air-dried at room temperature for one hour. After that, 20 larvae of the 3rd instar of armyworm were added carefully using a soft brush. The bioassay was carried out using 3 replicates for each treatment. The addition of autoclaved distilled water was used as a control. Mortality parameters were measured at 24, 48, and 72 hours after treatment. Sublethal effects were observed until the 10th day after treatment.

Experimental Design and Statistical Analysis

This study was done using a completely randomized design. The effects of the treatments to the variance of means of larval mortality were done using one-way anova at 95% significance, then followed by LSD if the anova was significant. The pathogenicity prediction of the formula was predicted using LC_{50} and LC_{90} calculation based on probit analysis (Finney, 1949). All the analysis procedures were done by using SPSS ver. 21.

RESULTS AND DISCUSSION

The effects of UV B to *Btk.* Morphology

The observation on the effects of UVB to *Btk.* morphology was done (Figure 1, 2, and 3). On the UVB radiation for 3 hours, spores and protein crystal were clearly can be observed. Whereas, on 6 and 9 hours UVB radiation the protein crystal and spores were degraded, and only they were remained in fewer numbers compared to controls. UV rays resulting in the loss of toxicity and cell pathogenicity. The longer exposure time to UV made the protein crystal and spores degraded (Griego & Spence, 1978) and caused the loss of pathogenicity (Khasdan *et al.*, 2001). Spinach leaves contain vitamin A, vitamin C, vitamin E, flavonoids, and phenols as antioxidants (USDA, 2020). Carotenoids, vitamins, and anthocyanins have important roles to fight free radicals (Anbhudasan, 2014). The number of spores was more visible in the red spinach protectant. This could be caused by the red spinach by which containing higher antioxidant compounds compared to green spinach. By this, it can give more protection to the *Btk.* compared to green spinach.

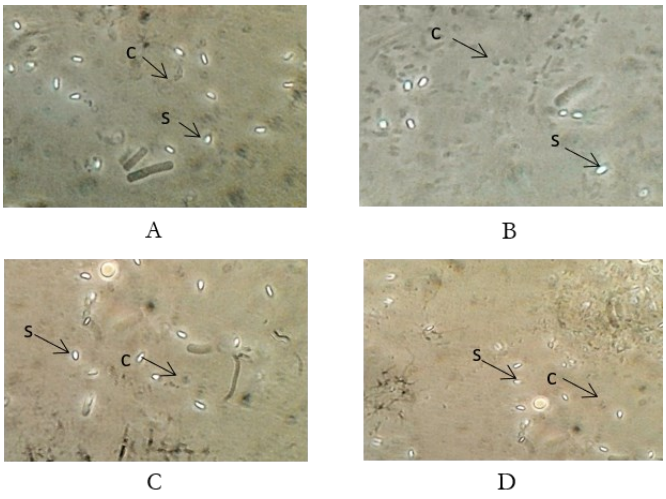


Figure 1. The effects of UVB exposure for 3 hours to *Btk.* spores and protein crystals (1,200 magnification under a contrast phase microscope) under concentrations of $5x10^6$ spores/ml in red and green spinach extracts (A: *Btk.* formulated with red spinach exposed to UVB; B: *Btk.* formulated with green spinach exposed to UVB; C: *Btk.* formulated with red spinach un-exposed to UVB; D: *Btk.* formulated with green spinach un-exposed to UVB; c: protein crystals); s: spore).

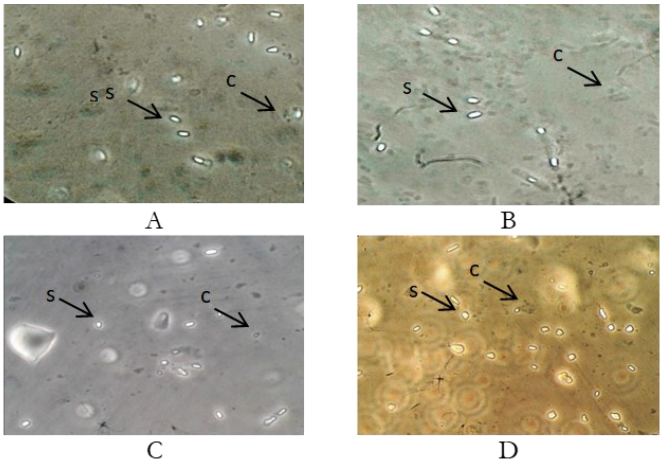


Figure 2. The effects of UVB exposure for 6 hours to *Btk.* spores and protein crystals (1,200 magnification under a contrast phase microscope) under concentrations of $5x10^6$ spores/ml in red and green spinach extracts (A: *Btk.* formulated with red spinach exposed to UVB; B: *Btk.* formulated with green spinach exposed to UVB; C: *Btk.* formulated with red spinach un-exposed to UVB; D: *Btk.* formulated with green spinach un-exposed to UVB; c: protein crystals); s: spore).

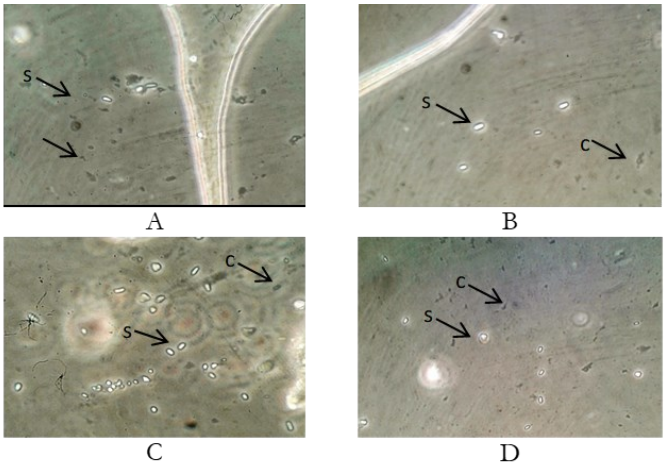


Figure 3. The effects of UVB exposure for 9 hours to *Btk.* spores and protein crystals (1,200 magnification under a contrast phase microscope) under concentrations of $5x10^6$ spores/ml in red and green spinach extracts (A: *Btk.* formulated with red spinach exposed to UVB; B: *Btk.* formulated with green spinach exposed to UVB; C: *Btk.* formulated with red spinach un-exposed to UVB; D: *Btk.* formulated with green spinach un-exposed to UVB; c: protein crystals); s: spore).

The Pathogenicity of *Btk.* Formulations against Armyworm Larvae

Based on the observations of armyworm larval mortality treated with *Btk.* formulations which exposed to different periods of UVB were shown in Figure 4.

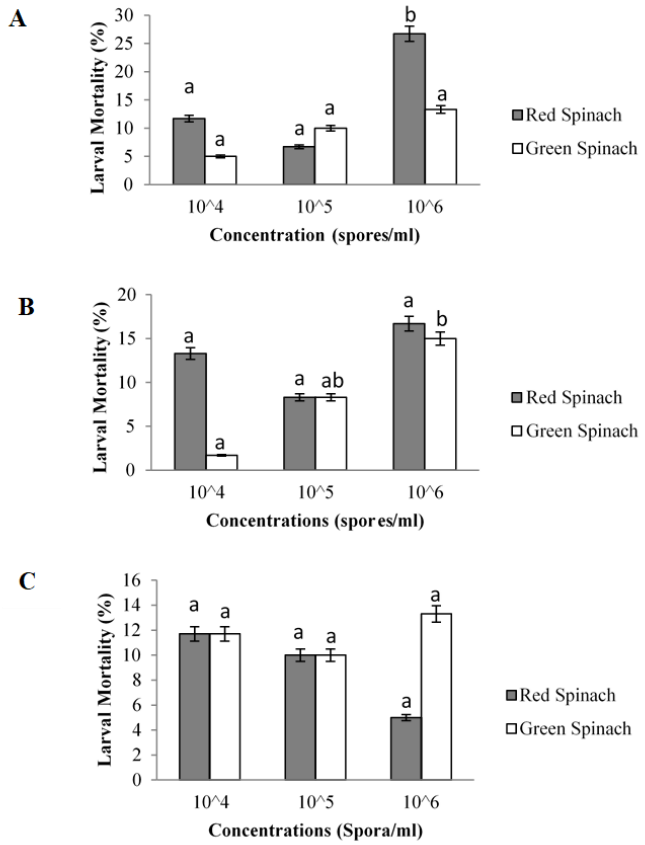


Figure 4. The mortality percentage of 3rd instar larvae of armyworm during after by treated by *Btk*. formulated with red spinach and green spinach extracts which was exposed at UVB for 3 hours (A); UVB for 6 hours (B); UV B for 9 hours (C).

In the UVB treatments at 3 and 6 hours of exposures, *Btk*. with protective red spinach in the concentration of $5x10^6$ spores/ml had a mortality of 26.7 % and 16.7%, respectively. This is because the levels of antioxidants that function to protect *Btk*. from UV light on red spinach are higher than green spinach. The number of spores and protein crystals in red spinach is higher than green spinach, so it is better at killing *S. litura*. Whereas, at 9 hours of UVB exposures, *Btk*. with green spinach extract at the concentration of 10^6 spores/ml had the highest mortality (13.3%), while for concentrations of 10^5 and 10^4 spores have mortality <7% in third instar larvae.

Mortality increases in parallel with periods of the treatments (Bouda *et al*., 2001). The larval mortality in the treatment of green spinach extract at a concentration of 10^6 spores/ml at 3 and 9 hours of UVB exposures was higher compared to the mortality at 6 hours exposure. The percentage of larval mortality in *Btk*. with red spinach protectant was higher than in green spinach. This can be due to the amount of protein crystals and spores in *Btk*. with red spinach were higher than *Btk*. with green spinach, thus it had higher pathogenicity. The protein crystal that enters the body of the insects passed through the insect's digestive tract and was activated by the alkaline conditions in the digestive tract to become δ-endotoxin or protoxin proteins. Protoxin will become a toxin if activated by the insect protease enzyme and is bound specifically to receptors in the digestive tract (Schunemann *et al*., 2014). The symptoms of sub-lethal effects to the larvae caused by *Btk*. are hardened of the body, stiff, blackish in color/ melanization and their size is shorter than the size before treatment (Khetan, 2001). The bacterial toxin infection may damage the digestive system of the larvae and may cause mortality (Schnepf *et al*., 1998).

Table 1. The lethal concentrations (spores/ml) of *Btk*. formulated with red and green spinach treated under UVB lights for 3, 6, and 9 hours against armyworm 3rd larval instar (Arlinda, 2019).

UVB Exposures (h)	Protectants	Lethal Concentrations (spores/ml)	
		LC_{50}	LC_{90}
3	Red spinach	1.5×10^4	1.9×10^5
	Green spinach	2.8×10^4	4.1×10^5
6	Red spinach	5.0×10^8	6.8×10^{14}
	Green spinach	8.5×10^3	3.1×10^4
9	Red spinach	3.8×10^2	1.0×10^3
	Green spinach	3.8×10^{13}	4.1×10^{19}

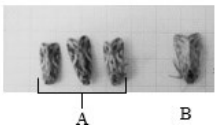
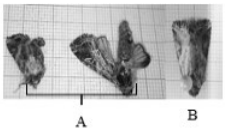
In this study, LC_{50} and LC_{90} values (Table 1) were used as the prediction of the pathogenicity for *Btk*. formulation. At 3 and 6 hours UVB exposures, the red spinach treatment was more pathogenic than the green spinach, with LC_{50} 1.5×10^4 and LC_{90} 1.9×10^5 (spores/ml), respectively. Red spinach gave a better UVB protection compared to green. This is possible because of antioxidant contents in red spinach is higher than green spinach.

Sub- lethal Effects of *Btk*. formulations exposed to UVB against armyworm larvae.

Some of the treated armyworms were survived and developed into pupa and adult stages but posed abnormal morphologies (Table 2). In this study, the survived larvae were able to become pupae but are in smaller sizes, darker in color, and shorter than those control. Adults in *Btk*, treated also had relatively smaller sizes compared to control. Additionally, the pupae that succeeded emerge had an abnormal wing with the characteristics of curly and short wings. A similar result was observed on the studies on the effects of genetically modified maize and *Bt*. genes

on corn borer (*Ostrinia nubilalis*) (Cagan and Barta, 2008). It showed that the growth and development of the larval stage were slower compared to control.

Table 2. The sub- lethal effects of *Btk*. formulated with spinach extracts to the pupal and imago stages of armyworm (Arlinda, 2019).

Parameters	Description
1. Pupae 	The size of pupae in *Btk*. treated were smaller and shorter and the color were darker compared to control
2. Adults size 	The size of adults in *Btk*. treated were smaller than the control
3. Adult's wings 	The wings of the adults in *Btk*. treated were abnormal and had curly wings

Description: A and B respectively correspond to the morphological performance of pupae as the result of *Btk* and control treatment during the larval stage (A: *Btk*. treated; B: control).

CONCLUSION

Based on the research that has been done, it can be concluded that *Btk*. with red spinach extract gave more protection to *Btk*. when exposed under UVB compared to green spinach. The sub-lethal effects of *Btk*, formulations include the lesser of the growth and development of larvae, pupae, and adults, as well as wings abnormality.

ACKNOWLEDGMENTS

We would like to express our thanks to the Collaborative Research Grant Lecturer - Students in 2019 contract number UGM/BI/1685/M/02/05.

REFERENCES

Amin, I., Norazaidah, Y. & Hainida, K.E., 2006, Antioxidant activity and phenolic content of raw and blanched *Amaranthus* species. *Food Chemistry* 94(1), 47-52.

Anbhudasan, P., Surendraraj, A., Karkhuzali, S. & Sathiskumara, S., 2014, Natural antioxidant and its benefit. *International journal of food and nutrition sciences* 3(6), 225-232.

Arlinda, M., 2019, Efektivitas bayam merah (*Amaranthus tricolor* L.) dan bayam hijau (*Amaranthus viridis* L.) Sebagai protektan radiasi UV B bagi *Bacillus thuringiensis* var. *kurstaki* pengendali *Spodoptera litura* Fab. [The effectiveness of red spinach (*Amaranthus tricolor* L.) and green spinach (*Amaranthus viridis* L.) as UV B radiation protection for *Bacillus thuringiensis* var. *kurstaki* controller *Spodoptera litura* Fab.] Undergraduate thesis, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta.

Bouda. H., Tapondjou, L.A., Fontem, D.A. & Gumedzoe Y.D., 2001, Effect of essential oils from leaves of *Ageratum conyzoides*, *Lantara camara* and *Chromolaena odorata* on the mortality of *Sitophylus zeamays* (Coleoptera, Curculionidae), *Journal of Stored Products Research* 37, 103-109.

Cagan, L. & Barta, M., 2008, Sublethal effect of Bt-maize in semi-artificial diet on european corn borer larvae, *Ostrinia nubilalis* (Hubner, 1796) (Lepidoptera, Crambidae). *Insect Pathogens and Insect Parasitic Nematodes IOBC Bulletin* 31, 127-130.

El-Sharkawey, A.Z., Ragaei, M., Sabbour, M.M., Afaf, A.A., Mohamed, H.A.L.A. & Samy, R., 2009, Laboratory evaluation of antioxidants as UV-protectants for *Bacillus thuringiensis* against potato tuber moth larvae. *Australian Journal of Basic and Applied Sciences* 3(2), 358-370.

Feitelson, J.S., Payne, J. & Kim, L., 1992, *Bacillus thuringiensis*: Insect and Beyond. *Biotechnology* 10, 271-275.

Finney, D.J., 1949, The adjustment for a natural response rate in probit analysis. *Annals of Applied Biology* 36(2), 187-195.

Griego, V.M. & Spence, K.D., 1978, Inactivation of *Bacillus thuringiensis* spore by ultraviolet and visible light. *Applied Environmental Microbiology* 35, 906-910.

Khasdan, V., Ben-Dov, E., Manasherob, R., Boussiba, S., & Zaritsky, A., 2001, Toxicity and synergism in transgenic *Escherichia coli* expressing four genes from *Bacillus thuringiensis* subsp. *israelensis*. *Environmental microbiology* 3(12), 798-806.

Khetan, S.K., 2001, Microbial pest control. Maecell Dekker, Inc. USA. p: 141.

Marwoto dan K.E. Neering, 1992, Pengendalian hama kedelai dengan insektisida berdasarkan pemantauan. [Soybean pest control with insecticides-based monitoring], Risalah Lokakarya Pengendalian Hama Terpadu Tanaman Kedelai, Balai Penelitian Tanaman Pangan Malang, 59−65.

Ningrum, A.D., 2019, Ekstrak daun teh (*Camellia sinensis* (L.) Kuntze) sebagai protektan *Bacillus thuringiensis* var. *kurstaki* terhadap sinar ultraviolet untuk pengendalian ulat grayak (*Spodoptera litura* Fab.) [Tea leaf extract (*Camellia sinensis* (L.) Kuntze) as a protectant *Bacillus thuringiensis* var. *kurstaki* against ultraviolet light for control of armyworm (*Spodoptera litura* Fab.)], Undergraduate thesis. Faculty of Biology, Universitas Gadjah Mada, Yogyakarta.

Schnepf, E., Crickmore, N., Van Rie, J., Lereclus, D., Baum, J., & Feitelson, J., Zeigler, D.R. and Dean, D., 1998, *Bacillus thuringiensis* and its pesticidal crystal protein. *Microbiology and Molecular Biology Reviews* 62 (3): 775-806.

Schunemann R., Knaak, N., & Fluza, L.M., 2014, Mode of action and specifity of *Bacillus thuringiensis* toxin in the control of caterpillars and stink bugs in soybean culture. *ISRN Microbiology* 2014, 1-12.

Shorey, H.H. & Hale, R.L., 1965, Mass-rearing of larvae of nine noctuid species on simple artificial medium. *Journal of Economic Entomology* 58: 522-524.

Sukirno, S., Priana, A.M., & Nayula, R.E.S., 2017, Effectiveness of dragon fruit extracts as UV protectant *Bacillus thuringiensis* cabbage caterpillar pest controller (*Crocidolomia binotalis*), [Efektifitas ekstrak buah naga sebagai UV protektan *Bacillus thuringiensis* pengendali hama ulat kobis (*Crocidolomia binotalis*)]. Scientific reports of students and lecturer collaborative research. Faculty of Biology Universitas Gadjah Mada. Yogyakarta.

Sukirno, S., Tufail, M., Rasool, K.G., El Salamouny, S., Sutanto, K.D., & Aldawood, A.S., 2018, The efficacy and persistence of *Spodoptera littoralis* nucleopolyhedrovirus (SpliMNPV) applied in UV protectants against the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) under Saudi field conditions. *Pakistan Journal of Zoology* 50(5), 1895-1902.

Sukirno, S., Tufail, M., Rasool, K.G., El Salamouny, S., Sutanto, K.D., & Aldawood, A.S., 2017, The effectiveness of spinosad and neem extract against *Spodoptera littoralis* (Boisd.) and *Spodoptera exigua* (Hubner): Exploring possibilities to enhance the bio- pesticide persistence with natural UV protectants under field-sunlight conditions of Saudi Arabia. *Pakistan Journal of Agricultural Sciences* 54 (4), 743-751.

Sutanto, K. D., El Salamouny, S., Tufail, M., Ghulam Rasool, K., Sukirno, S., Shepard, M., Shapiro, M., & Aldawood, A.S.A., 2017, Evaluation of natural additives to enhance the persistence of *Spodoptera littoralis* (Lepidoptera: Noctuidae) nucleopolyhedrovirus (SpliMNPV) under field conditions in Saudi Arabia. *Journal of Economic Entomology* 110(3), 924-930.

Tarigan, A.B., 2019, Efektivitas lidah buaya (*Aloe vera* L.) sebagai protektan *Bacillus thuringiensis* var *kurstaki* terhadap sinar ultraviolet dan agen pengendali hayati serangga ulat grayak (*Spodoptera litura* Fab.) [The effectiveness of aloe (*Aloe vera* L.) as *Bacillus thuringiensis* var *kurstaki* protectant against ultraviolet rays and insect biological control agent armyworm (*Spodoptera litura* Fab.)], Undergraduate thesis, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta.

USDA, 2020, Cabbage, raw nutrition facts and analysis per serving. https://www.nutritionvalue.org/Cabbage%2C_raw_nutritional_value.html. Accessed on June 29th, 2020.

Journal of Tropical Biodiversity and Biotechnology
Volume 05, Issue 02 (2020): 149 — 156
DOI: 10.22146/jtbb.43694

Research Article

The Diversity of Ray-finned Fishes (Actinopterygii) in Plio-Pleistocene Java

Donan Satria Yudha[1]*, Muhammad Ageng Prabowo[1], Rusyad Adi Suriyanto[2], Didit Hadi Barianto[3]

1) Laboratory of Animal Systematics, Faculty of Biology, Universitas Gadjah Mada, Jl. Teknika Selatan, Sekip Utara, Yogyakarta, 55281, Indonesia.
2) Laboratory of Bioanthropology and Paleoanthropology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Jl. Farmako, Sekip Utara, Yogyakarta, 55281, Indonesia.
3) Laboratory of Paleontology, Geological Engineering Department, Faculty of Engineering, Universitas Gadjah Mada, Jl. Grafika No. 2, Yogyakarta, 55281, Indonesia.

Submitted: 20 February 2019; Accepted: 10 July 2020; Published: 15 August 2020

ABSTRACT

Java has been known in the world of Paleontology as a contributor to the findings of *Homo erectus* fossils, but there are still other fossil findings that have not been identified until now, especially fossil fishes of the subclass Actinopterygii. This research was conducted to recognize the diversity of the actinopterygians fishes in Plio-Pleistocene of Java and to determine the diagnostic characters of each taxon group of fossils in the Plio-Pleistocene of Java. The study was carried out using comparative anatomical methods with present-day specimens and fossil findings collection of the Laboratory of Bioanthropology and Paleoanthropology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada; Bandung Geological Museum and Sangiran Early Man Site. The research found at least 8 species of fish fossils in Java which belong to three order, i.e., the order Siluriformes with 5 identified species: *Bagarius gigas, Hemibragus nemurus, Clarias macrocephalus*, family Ariidae with indeterminate genus or species, *Plotosus canius, Clarias batrachus*, and family Pangasiidae with indeterminate genus or species; the order Perciformes with two identified species: *Anabas testudineus* and *Sphyraena crassidens*; and the order Cypriniformes with one identified species: *Osteochilus vittatus*. Based on the fossil findings showed that the Java Island during the Plio-Pleistocene used to be a marine environment that gradually ascending into a lowland river which closes to mangrove swamps and estuaries while the ancient Bandung lake site was a lacustrine environment with calm currents and is overgrown with riparian vegetation.

Keywords: Actinopterygii fish, fossil, Java island, Plio-Pleistocene

INTRODUCTION

The fish fossils in Java belonged to the Trinil HK faunal group, which were 0.9 million years ago. Those fossils were members of the order Perciformes, such as; *Anabas testudineus* (Anabantidae) and *Channa* cf. *striata* (Channidae), members from order Siluriformes such as; *Clarias batrachus*, *Clarias leiacanthus* (Clariidae) and *Hemibagrus nemurus* (Bagridae) (Joordens *et al.*, 2009). Those identified fossils belonged to the subclass Actinopterygii. The actinopterygian fishes are characterized by pectoral radials (actinosts) and interopercle bones which can be fossilized (Nelson *et al.*, 2016).

The fossil fishes found in Java were inhabited the Bengawan Solo River during the Pleistocene, and far from the sea waters. At that time the terrestrial waters were murky lowland rivers, with several lakes, areas with sunken trees and aquatic vegetation, swamps with low oxygen levels, and estuaries with brackish water. This research was considered to be insufficient as a database in the inventory of fossil fish in Java because there were still many fossil fish specimens that have not been included in the study, therefore this study was conducted to complete the data from previous research.

The purpose of this study was to recognize the diversity of actinopterygians fossil of fish and to determine the diagnostic character of each group of

***Corresponding author**
Email: donan_satria@ugm.ac.id

fossil taxon in Pleistocene of Java and to understand the condition of the ancient environment where the fish lived.

MATERIALS AND METHODS

Materials

The study was carried out using comparative anatomical methods between present-day specimens with fossil findings collection of the Laboratory of Bioanthropology and Paleoanthropology, Faculty of Medicine, Public Health and Nursing UGM (abbreviated as LBP), Bandung Geological Museum (abbreviated as BGM) and Sangiran Early Man Site (abbreviated as SEMS).

The present-day material studied was preserved hard parts of the Actinopterygii fishes. The preserved hard parts of several recent fish species were specimens' collection of the Laboratory of Animal Systematics, Faculty of Biology UGM (abbreviated as LAS). Those specimens were *Arius nella*, *Clarias batrachus*, *Pangasius* sp, and *Hemibragus nemurus*. We also used a skeletal identification book titled Osteological Guide of Fishes from the Mekong System (Voeun, 2006).

Methods

The data taken were qualitative which obtained by objectively describing and observing the morphology by focusing on the characters that could be used as markers in each part of the skeleton. The character was based on the skeletal part. The anatomical parts were recorded by their general shape, ornamentation, surface, and special features of the specimen (Voeun, 2006).

Each fossil found was compared with the preserved specimens and the fish skeleton identification book by Voeun (2006). Identified species were then compiled into tables and lists of fauna on the island of Java at the time the fossils were discovered. Paleoecological data were secondary data obtained from sedimentary geological data and identified species information.

RESULTS AND DISCUSSION

Fossil Findings

There were a total of one hundred fragments of fossil fishes from three locations (LBP, BGM, and SEMS) have not been identified. The majority of fossil findings were members of the order Siluriformes because Siluriformes fishes possess a sturdy bone structure of pectoral spine and Neurocranium which was easier for the fossilization process. This structure also can be used as distinguishing characters of each species. At least 8 species of actinopterygians fish fossils were found in Java (Table 1), which belong to the order Siluriformes, Perciformes, and Cypriniformes.

Systematic Descriptions

Order Siluriformes G. Cuvier, 1817

Family Clariidae Bonaparte, 1846

Clarias batrachus (Linnaeus, 1758)

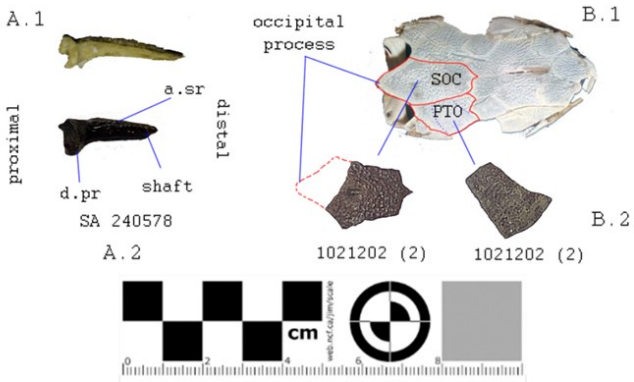


Figure 1. Fossils (A.2) pectoral spine and (B.2) neurocranium fragments of *Clarias batrachus* catfish with recent specimens as a comparison (A.1 & B.1). A.Sr = Anterior serration, D.Pr=Dorsal Process, SOC=Supraoccipital,PTO=Pterotic.

Material examined: A pectoral spine and two fragments of neurocranium (Figure 1) collections of Bandung Geology Museum.

Diagnostic characters: Pectoral spine, the base of the shaft is not too protruding compared to *Plotosus canius*, the anterior part serration is more dominant even though the fossil has been eroded (Figure 3, SA 240578). Characters resemble modern species but are not entirely identical types. The neurocranium is very identical due to the presence of occipital processes which are evident in the posterior part of the supraoccipital plate (Figure 3, 1021202 (2)).

Clarias macrocephalus Gunther, 1864

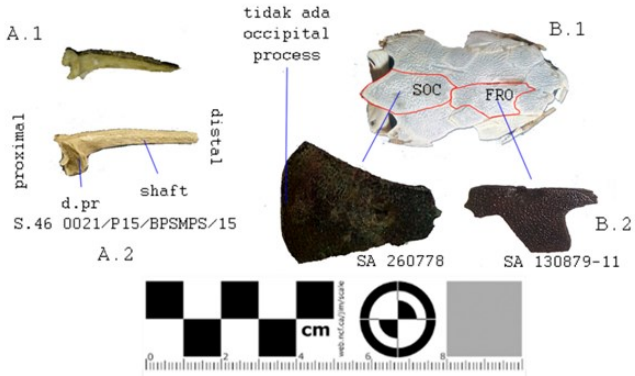


Figure 2. Fossil (A.2) pectoral spine and (B.2) fragments of neurocranium of *Clarias macrocephalus* with a recent specimen of *Clarias batrachus* as a comparison (A.1 & B.1). D.Pr=Dorsal Process, SOC=Supraoccipital, FROO=Frontal.

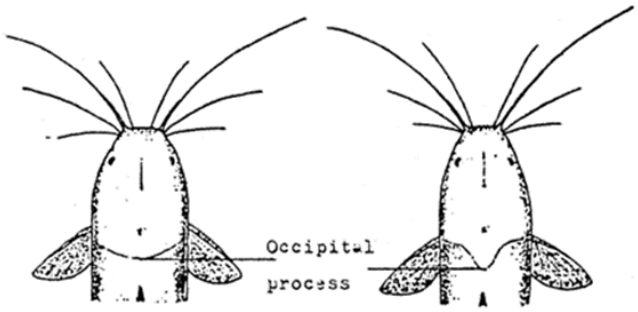


Figure 3. The distinguishing character of *Clarias macrocephalus* (left) and *Clarias batrachus* (right) is the presence of an occipital process on *Clarias batrachus*. (Srisuvantach, *et al*., 1985).

Material examined: 15 specimens of pectoral spine at Sangiran, 3 specimens of spina pectoralis at the Bandung Geological Musem along with 3 pieces of fragments of neurocranium.
Diagnostic characters: Pectoral spines are relatively thinner and finer in the surface, shafts bend in posterior direction, and dim serration (Figure 2, S.46 0021/P15/BPSMPS/15). Larger in size than modern *Clarias batrachus*. Neurocranium fragments are thin plates with a surface full of granules but they do not have an occipital process that extends posteriorly like *Clarias batrachus* (Figure 2, SA 260778).

ARIIDAE L.S. Berg, 1958
Indeterminate genus and species.

Figure 4. Several pieces of pectoral spine (A.2) fossil specimens from Ariidae's family with the recent specimen (A.1) as a comparison. a.Sr = Anterior serration, p.Sr = Posterior Serration, D.Pr = Dorsal Process.

Material examined: 3 pectoral spine specimens collection of Sangiran Early Man Site, 1 pectoral spine collection of Bandung Geological Museum along with a fragment of neurocranium.
Diagnostic characters: The shaft of pectoral spine is flat and straight, there are serration on both sides of the shaft but is more dominant on the posterior side. Groove is parallel horizontally along the shaft (Figure 4, S31 & S38), serration exists on both sides but the posterior side tends to be more evident (Figure 4, SA 100979).

PLOTOSIDAE Bleeker, 1858
Plotosus cf. *canius* (Hamilton, 1822)

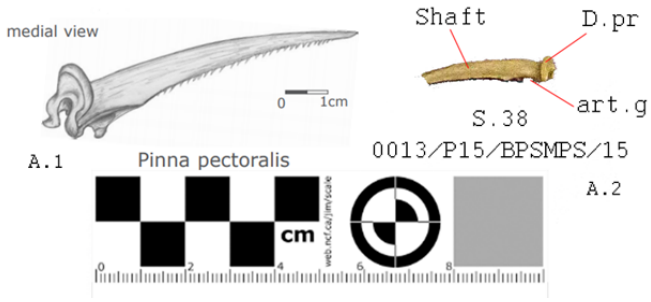


Figure 5. Pectoral spine fossil (A.2) of *Plotosus canius* fish with a comparison from Voeun (2006) (A.1). D.Pr=Dorsal Process. Art.g=Articular groove.

Material examined: A pectoral spine collection of Sangiran Early Man Site.
Diagnostic characters: Small pectoral spine, the proximal shaft protrudes anteriorly, shaft surface is smooth with small serration at the edges (Figure 5. S.38), but in the fossil specimen, the serration is absent or not fossilized.

PANGASIIDAE Bleeker, 1858
Indeterminate genus and species.

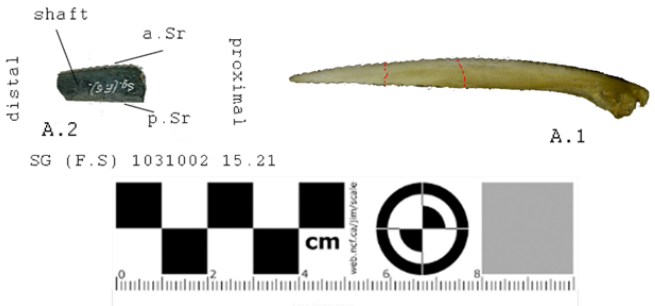


Figure 6. Pectoral spine fossil (A.2) of *Pangasius* sp. with a recent specimen as a comparison (A.1). a.Sr = Anterior serration, p.Sr=Posterior Serration.

Material examined: A pectoral spine collection of Bandung Geological Museum, Sangiran's finding.
Diagnostic characters: The pectoral spine is very flat and has a very smooth shaft. With a very small and relatively blunt serration compared to other species (Figure 6, SG (F.S) 1031002 15.21).

Material examined: 4 specimens of pectoral spine collection of Laboratory of Bioanthropology and Paleoanthropology UGM, 41 specimens of pectoral spine collection of Sangiran Early Man Site along with 1 piece of operculum, 19 specimens of pectoral spine collection of Bandung Geological along with 7 fragments of neurocranium. In 1876, this species was found in Padang (Figure 8).

Diagnostic characters: The part of the shaft located near the dorsal process of this species has a long indentation toward distal-anterior direction. On the dorsal side of the shaft is covered a small serration, then leads posteriorly toward a narrow groove that borders posterior serration. Whereas the ventral side is identical to the dorsal side but the groove is even narrower (Figure 7, SA 270778). Neurocranium tends to be flat dorso-ventrally like *Hemibragus nemurus* but is less ornamentative (Figure 7, K 1530 + K 1528). Rounded premaxilla like sickles (Figure 7, K 1526) and large operculare are trapezoidal with cavity near dorsal (Figure 7, PIS. 0026).

SISORIDAE Bleeker, 1858
Bagarius gigas Günther, 1876

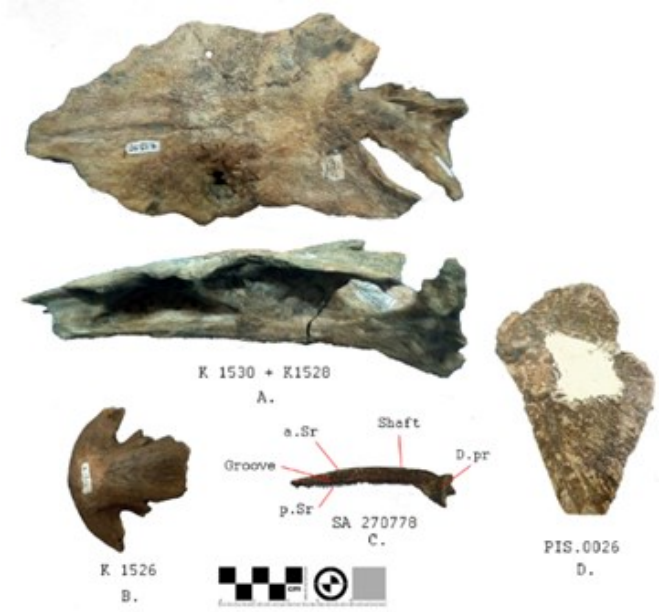


Figure 7. Fossils of (A) Nearly intact Neurocranium, (B) Premaxilla, (C) Pectoral spine, and (D) Opercularum of *Bagarius gigas*. a.Sr = Anterior serration, p.Sr=Posterior Serration, D.Pr=Dorsal Process.

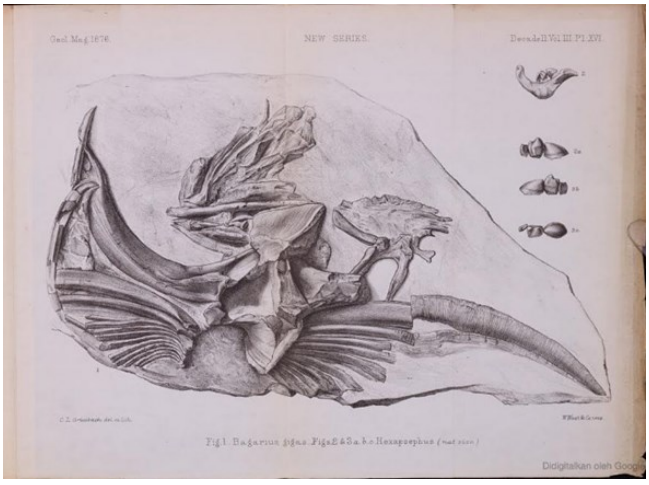


Figure 8. *Bagarius gigas* fossil, Günther, 1876 findings in Padang.

BAGRIDAE Bleeker, 1858
Hemibragus nemurus (Valenciennes, 1840)

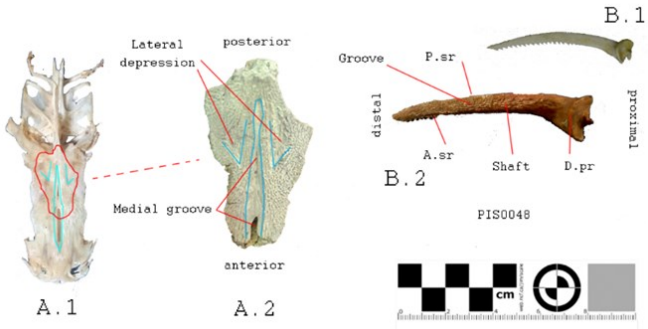


Figure 9. Fossils (A.2) fragments of Neurocranium and (B.2) pectoral spine of *Hemibragus nemurus* species compared with recent specimens of neurocranium (A.1) and pectoral spine (B.1). a.Sr = Anterior serration, p.Sr=Posterior Serration, D.Pr=Dorsal Process.

Material examined: 5 pectoral spines and 1 neurocranium fragment collection of the LBP UGM. Twenty four specimens of pectoral spine collection of the SEMS and 21 specimens of pectoral spine along with 1 fragment of neurocranium collection of the BGM.

Diagnostic characters: The shaft located near the dorsal process tends to be straighter, while the posterior part folds inward. The dorsal and ventral sides are relatively identical, the anterior and posterior sections are covered with serration, while its medial is covered with a fairly broad groove (Figure 9, PIS0048). The neurocranium is found to have lateral V-shaped depression upside down and medial groove along the central side of the neurocranium (Figure 9, A).

CYPRINIFORMES Bleeker, 1859
CYPRINIDAE Bleeker, 1859
Osteochilus vittatus (Valenciennes, 1842)

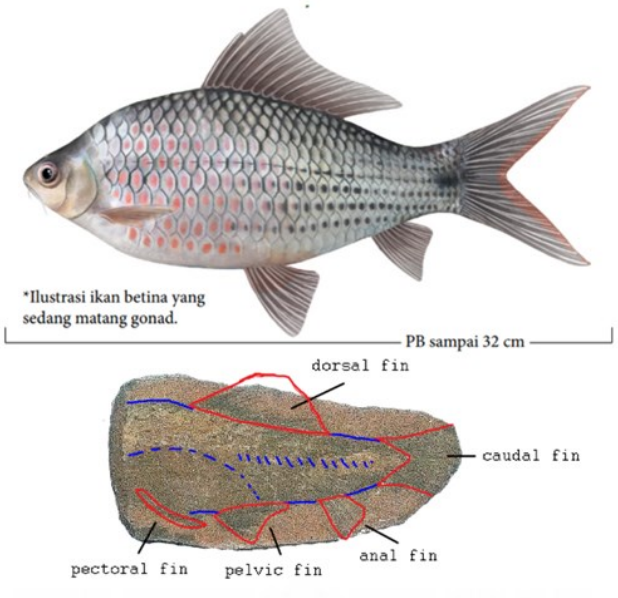


Figure 10. *Osteochilus vittatus* barb fossils in a stone plate show pectoral, pelvic, anal, caudal, and dorsal fins. The comparative figure is taken from Sukmono *et al.* (2017).

Material examined: 3 specimens of body parts cut off posteriorly and anteriorly in rocks.
Diagnostic characters: Latero-laterally flat body, hydrodynamically taper. In the anteriorly cut specimen, the dorsal, anal, and pectoral fins are sharp triangle in shape. The base of posterior dorsal fin must be opposite to anterior base of anal fin (Figure 10). Headpieces of moderate size compared to body proportions, unpreserved fins and body size generally differed drastically from both pieces it is possible that these three specimens were members of different types even though they were found in the same location.

PERCIFORMES Bleeker, 1863
SPHYRAENIDAE Rafinesque, 1815
Sphyraena crassidens (De Beaufort, 1926)

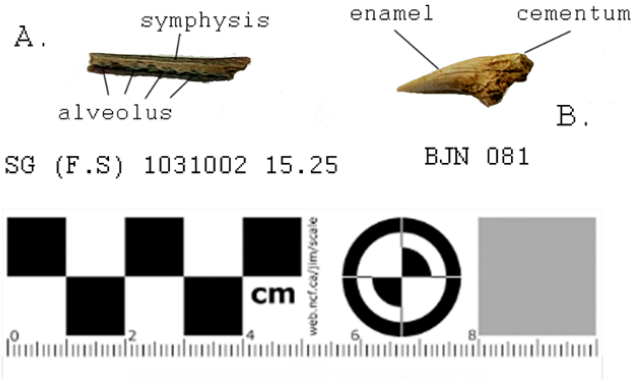


Figure 11. The jaw fossils of the *Sphyraena crassidens* species show the presence of a tooth gap alveolus and symphysis (A.) and sharply curved teeth with thin serration on the enamel surface (B.).

Material examined: 2 pieces of jaw specimens and a tooth.
Diagnostic characters: The jaws are thin and straight, with clear tooth sockets (Figure 11, SG (F.S) 1031002 15.25). The teeth are long, curved proximally with a very small serration on the sides of the teeth (Figure 11, BJN 081).

Material examined: 20 pieces of operculare and 1 piece of suboperculare collection of the Bandung Geological Museum.
Diagnostic characters: The operculare plate is the bony part of the operculum. The operculare and suboperculare pieces have an identical structure, thin in shape but very compact in structure with wrinkles on lateral surface and spurs at the posterior end of the operculare plate (Figure 12).

ANABANTIFORMES Britz, 1995
ANABANTIDAE Bonaparte, 1831
Anabas testudineus (Bloch, 1792)

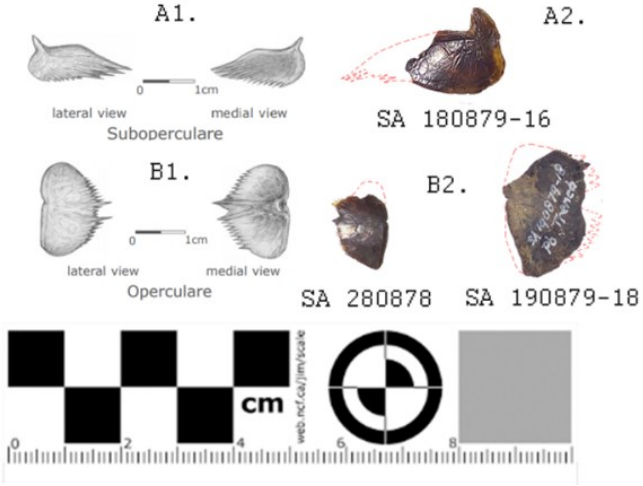


Figure 12. Operculare (B.2) and Suboperculare (A.2) fossils of *Anabas testudineus* perch with comparison from Voeun (2006) (A.1 & B.1).

Paleo-Environmental Implications

From the oldest site on the island of Java (Table 2); Cijurey in the southern Cirebon region found *Hemibragus nemurus* and many whale bones (not included in this study) indicate a lowland stream and estuary or mangrove swamps environment. This site has identical age to Satir faunal group in Bumiayu, Central Java when the island of Java was still a small island covered by mangroves during the Lower Pleistocene 1.5 to 1.2 million years ago (Louys *et al.*, 2007).

On the Sangiran site, divided into three layers which have different age respectively; Sangiran Black Clay, Sangiran Grenzbank, and Sangiran Kabuh (Laporte, 1990). The Sangiran Black Clay layer has an age that is identical to the Satir fauna group and the Cijurey site according to Laporte (1990). On this site, *Bagarius gigas* species were found to live in swamps that have short vegetation.

Next in the Sangiran Grenzbank layer which is identical to the H.K Trinil faunal group aged 1 million years ago in the Lower-Middle Pleistocene. In this layer, *Clarias macrocephalus* started to roam in lowland and wetland rivers. The Sangiran layer which is located above Grenzbank is Kabuh but no fossils of fish were found in this layer.

This study also found fossils on the Sangiran site with unclear layer of origin, namely; *Bagarius gigas*, *Hemibragus nemurus*, *Clarias macrocephalus*, Ariidae indeterminate, *Plotosus canius*, *Clarias batrachus, Sphyraena crassidens, Anabas testudineus*, and Pangasiidae indeterminate.

The existence of fish associated marine waters such as *Plotosus canius* and *Sphyraena crassidens*, fish that live in swamps/mangroves such as *Bagarius gigas*

Table 2. Actinopterygii fossil findings on several sites on Java and their age (Hardjasasmita, 1987; Theunissen *et al.*, 1990; Storm, 1995; Aziz *et al.*, 1999; Louys *et al.*, 2007; Cohen *et al.*, 2013; Arif *et al.*, 2014; Jayanti *et al.*, 2017).

← Older									Younger →
Age	Plio-Pleistocene	Lower Pleistocene	Lower Pleistocene	Middle-Lower Pleistocene	Pleistocene	Pleistocene	Upper-Middle Pleistocene	Upper-Middle Pleistocene	Holocene
	-	1.5 – 1-2 m.y.a.	1.5 - 1.2 m.y.a.	1.0 m.y.a.	1.5 – 0.8 m.y.a.	-	550-140 m.y.a.	550-140 m.y.a.	10-5 m.y.a.
Site / Species	Kalitidu	Cijurey	Sangiran Black Clay	Sangiran Grenzbank	Sangiran *assorted*	Cipatik/ Ciliin	Watualang	Sidoredjo (Ngandong)	Gua Lowo (Sampungian Site)
Bagarius gigas			✓		✓		✓		
Hemibragus nemurus		✓			✓			✓	✓
Clarias macrocephalus				✓	✓				
Ariidae					✓				
Plotosus canius					✓				
Sphyraena crassidens	✓				✓				
Anabas testudineus					✓				
Clarias batrachus					✓				
Pangasiidae					✓				
Osteochilus vittatus						✓			

and fish that live in freshwaters such as *Clarias batrachus* and *Anabas testudineus* show the environment of downstream river and estuary which was separated from the sea by swamps or mangroves.

In the ancient Bengawan Solo River, there were several excavation areas which were found Actinopterygii fish fossils, namely; Kalitidu, Watualang, Sembungan, and Pandean, Sidoredjo and Gua Lowo. At the Kalitidu Site, Bojonegoro, East Java, aged Plio-Pleistocene, a species of marine barracuda fish, *Sphyraena crassidens* were found. This fish was also discovered by de Beaufort in its publication "A collection of marine fishes from the Miocene of South Celebes in Maros, Sulawesi". This finding indicated this site originally in the sea or adjacent to the sea.

On other sites along the ancient Bengawan Solo River, in Watualang, Sembungan and Pandean were found *Bagarius gigas* cranium. The environment at this time showed a river that was close to a wetland swamp but not completely in the sea. This layer is younger than the Kaltidu site aged Middle-Upper Pleistocene.

In Bellwood, 2017 the Ngandong and Watualang sites were known to have the same age. Using Uranium radioactive age measurement, the age of both sites were dated 550 to 140 thousand years ago.

On the Sidoredjo site which on the formation with Ngandong at the edge of the ancient Bengawan Solo stream were found *Hemibragus nemurus* fossils and members of Clariidae that are different from the other species found in this study. Probably at this time the Bengawan Solo was flowing slowly and had a wide riverbank due to the presence of *Hemibragus* fish fossils. Not much has changed from the previous period at the Watualang, Sembungan,

Pandean site which tends to show the estuary river ecosystem near the mangrove swamp.

On the Gua Lowo site, which is part of the Sampung Site which has the youngest age in the ancient Bengawan Solo River aged 10-5 thousand years ago at the time of the Holocene. According to Storm (1995), the ancient Bengawan Solo River ecosystem was a Tropical Rain Forests. However, from the fish fossils found, *Hemibragus nemurus* shows the wide river waters with slow currents, not much different from the Sidoredjo site which has an adjacent age, even from Watualang.

On the Cipatik/Cililin site which used to be part of the ancient Bandung lake, found fossils of *Osteochilus vittatus* barb fish. The existence of this type of fish shows the calm Lake Bandung environment, and full of vegetation as the substrate for dwelling. Far different from other sites that tend to show the ecosystem of river estuaries.

CONCLUSION

Found at least 8 species of actinopterygians fish fossils in Java which belong to three orders. Those species are: *Bagarius gigas, Hemibragus nemurus, Clarias macrocephalus,* Ariidae indeterminate, *Plotosus canius, Clarias batrachus*, and Pangasiidae indeterminate from the order Siluriformes; *Anabas testudineus* and *Sphyraena crassidens* from the order Perciformes; and *Osteochilus vittatus* from the order Cypriniformes. Based on the fossil findings showed that Java Island during the Plio-Pleistocene used to be a marine environment that gradually became a lowland river close to mangrove swamps and estuaries. On the ancient Bandung lake site was a lacustrine environment that has a calm flow and is overgrown with riparian vegetation.

ACKNOWLEDGMENTS

Special thanks to Mr. Noorman Hendri, S.Si. who helped with sorting fossil fish in the Laboratory of Bioanthropology and Paleoanthropology, Ms. Marlia Yulianti Rosida, Ms. Rindy Gita Wahyuni, Mr. Ardian Yoseph and Mr. Albertus Nikko for helping with data collection and guiding in Sangiran Early Man Site, specifically to Mr. Aburizal Siregar who has provided accommodation in Bandung, Dr. Unggul Prasetyo Wibowo, M.T. and Mr. Indra Sutisna who has helped with data collection and guiding during writer stay in Bandung Geological Museum.

REFERENCES

Arif, Johan., Darwin Siregar., 2014, Analisis Kandungan Fluorine (F) di Dalam Fosil Tulang dan Gigi: Kasus dari Gua Pawon, Sangiran, dan Kalitidu [Analysis of Fluorine (F) Content in Fossil Bones and Teeth: Cases from Pawon Caves, Sangiran, and Kalitidu], *Forum Arkeologi* 27(3), 187-196.

Aziz, Fachroel., John de Vos., 1999, The Fosil Faunas from the Citarum Area, West Java, Indonesia, *Deinsea* 7, 21-32.

Cohen, K M. S C Finney., P L Gibbard., Fan P L., Fan J X., 2013, 'The ICS International Chronotratigraphic Chart, Episodes 36: 199-204' in Internasional Commision on Stratigraphy viewed 9 Januari 2019, from www.stratigraphy.org/ ICSchart/ ChronostratChart2018-08.jpg.

Günther, Albert., 1876, Contrubutions to our Knowledge of the Fish-Fauna of the Tertiray Deposits of the Highlands of Padang, Sumatra, *The Geological Magazine*, Decade II, Vol III, 434-440.

Hardjasasmita, H S., 1987, Taxonomy and phylogeny of the Suidae (Mammalia) in Indonesia, *Scripta Geologica* (85), 1-68.

Jayanti, Anita Galih Rangga., Rahajeng Ayu Permana Sari., Unggul Prasetyo Wibowo., 2017, *The Tertiary Mollusk Fosil Dispersion on the Southern Part of Cirebon Area*, Joint Convention Malang 2017.

Joordens J C A., F P Wesselingh., J de Vos., H B Vonhof., D Kroon., 2009, Relevance of aquatic environments for hominins: a case study from Trinil (Java, Indonesia), *Journal of Human Evolution* (57), 656–671.

Louys, Julien., Darren Curnoe., Haowen Tong., 2007, Characteristics of Pleistocene megafauna extinctions in Southeast Asia, *Palaeogeography, Palaeoclimatology, Palaeoecology* (243), 152-173.

Nelson, J.S., T.C. Grande and M.V.H. Wilson, 2016. *Fishes of the World*. 5th edition. Pp. 111.

Storm, Paul., 1995, The Evolutionary Significance of the Wajak skulls, *Scripta Geologica* (110), 1-247.

Sukmono, Tedjo. Mira Margaretha., 2017, Ikan Air Tawar di Ekosistem Bukit Tigapuluh [Freshwater Fish in the Bukit Tigapuluh Ecosystem], Frankfurt Zoological Society, Frankfurt. p: 68.

Theunissen, B. V., J de Vos., P Y Sondaar., F Aziz., 1990, 'The establishment of a chronological framework for the hominid-bearing deposits of Java; A historical survei', in Laporte, Léo F (eds.), *Establishment of a Geologic Framework for Paleoanthropology*,pp. 39-54. Geological Society of America, Colorado.

Voeun, Vuthy., 2006, *Osteological Guide of Fishes from the Mekong System*, Sursum, Tišnov.

Journal of Tropical Biodiversity and Biotechnology
Volume 05, Issue 02 (2020): 157 — 166
DOI: 10.22146/jtbb.55494

Research Article

Habitats Characteristic and the Resistance Status of *Aedes* sp. Larvae in the Endemic Areas of Dengue Haemorrhagic Fever in Sewon Subdistrict, Bantul Regency, Special Region of Yogyakarta

Soenarwan Hery Poerwanto[1]*, Defriana Lutfi Chusnaifah[1], Giyantolin[1], Dila Hening Windyarini[1]
1) Faculty of Biology, Universitas Gadjah Mada, Jl. Teknika Sel., Sekip Utara, Yogyakarta 55281, Daerah Istimewa Yogyakarta, Indonesia

Submitted: 17 April 2020; Accepted: 28 July 2020; Published: 15 August 2020

ABSTRACT
Dengue Hemorrhagic Fever (DHF) is caused by Dengue Virus and transmitted by female *Aedes* mosquito which spread almost all over the world. *Aedes* sp. mosquito lives cosmopolitan and breeds in wet environments. Panggungharjo and Bangunharjo villages were categorized as endemic and non-endemic DHF areas, respectively. The aims of this research were to study the characteristics of *Aedes* sp. mosquito breeding sites, the identity of presence species found in the sites, and the resistance status of *Aedes* sp. mosquitoes against organophosphate insecticide. The method was using a larval survey which consists of 200 houses as respondents located in Panggungharjo and Bangunharjo villages. The larval resistance was tested by a biochemical method since resistance could be associated with esterase enzyme activity. The characteristics of mosquito breeding sites that found were open containers, filled with clear and calm water, dark and rough wall surfaces, the bottom surface was not directly in contact with the ground, the water temperature was 27-29 °C, pH 6.5-7, and not directly exposed to sunlight. There was only one species of mosquito was found, *Aedes aegypti*. The resistance test of *Aedes* sp. larvae showed that *Aedes* sp. larvae population from Panggungharjo village were susceptible, and *Aedes* sp. larvae from Bangunharjo village were in moderate resistant against organophosphate insecticide.

Keywords: *Aedes* sp., breeding site, characteristics, resistance status

INTRODUCTION
Dengue fever or Dengue haemorrhagic Fever (DHF) is caused by the virus Flavivirus; type DENV I, II, III, and IV (Knipe & Howley, 2007) and type I, II, and IV occur more often in Indonesia (WHO, 2009). Dengue haemorrhagic fever is spread by female mosquitoes of the genus Aedes that are scattered in the 112 tropical and subtropical countries in the world (WHO, 2013). *Aedes* has cosmopolitan lifestyle and easily adapt to the urban environment and the countryside. Some member species of *Aedes* that are often found in Indonesia is *Aedes aegypti* and *Aedes albopictus* (Suksesi & Surahma, 2007). *Aedes* females generally suck blood in the morning and towards evening. Adult mosquitoes can survive for two weeks and are capable of laying at least 100 eggs each spawn. The eggs of *Aedes* solitary, with a dark colour and can survive in dry conditions for up to 9 months and can hatch if they are in optimal conditions (Service, 1996).

The Ministry of Health of the Republic of Indonesia noted that the Hemorrhagic fever ever is an extraordinary occurrence in Indonesia. Bantul regency is an area that has regions of endemic and non-endemic infected with the Dengue Hemorrhagic Fever (DHF). According to the Department of Health (Health office) Bantul Regency, the number of DHF cases in 2015 as many as 1.441 cases, then increased to 2.442 cases in 2016. Every year DHF cases always occur, especially in Sewon sub-districts such as Panggungharjo and Bangunharjo villages which are included as urban

***Corresponding author**
Tel.: +628164889999
Email: soenarwan@ugm.ac.id

environments that continues to grow and has the potential to become an endemic area of DHF (Department of Health Bantul Regency, 2016; 2017).

Responding to outbreaks or extraordinary events of dengue in several regions in Indonesia, the government, through the Health Service, has taken various steps to deal with and control the disease. Programs that are encouraged include the 3M Plus movement (*Menguras*: Drain, *Menutup*: Cover, and *Memanfaatkan kembali*: Reuse items; plus mosquito control with larvacide, plants, and fish), efforts to use insecticides through space spraying (thermal fogging / fumigation or Ultra Low Volume/ULV) with insecticides in DHF-prone areas (Widiarti *et al.,* 2011). The use of insecticides with active ingredients organophosphate (Malathion) in the long term with high frequency for insect control causes an increase in mosquito resistance to these active compounds (Stojanovich & Scott, 1966). Therefore, it is important to do habitat mapping, observation of breeding site characteristics, and resistance status of mosquito larvae to support the eradication and control program of infectious disease vectors, especially dengue fever/DHF.

MATERIALS AND METHODS

This research was conducted in the field and laboratory. Research was done with survey method in Panggungharjo and Bangunharjo Villages, Sewon Sub-District, Bantul Regency, Special Region of Yogyakarta, which were endemic and non-endemic areas of Dengue Fever. The survey was conducted to observe the characteristics of mosquito breeding sites and sampling larvae in one hundred (100) houses in each village. The manufacture of preparations and identification of mosquitoes and the larvae held in the Laboratory of Systematics of the Animal Section of Parasitology, Faculty of Biology, Universitas Gadjah Mada. Treatment and test status of the resistance carried out in the Laboratory of Parasitology, Faculty of Medicine, Universitas Gadjah Mada. Research conducted in March – June 2013.

Observation of Habitat Characteristics and Larva Collection

Observations were carried out by roaming methods or surveys to places that could potentially be breeding grounds for mosquitoes, such as water puddles, tanks, buckets, open water jars, spring water sources, rivers, sewers, plantations or vacant land. Observations were made on 100 houses in each Panggungharjo and Bangunharjo Villages randomly. The houses surveyed were recorded and seen whether or not there were larvae in containers in their environment. These positive containers or breeding grounds were recorded and described that covers: basic material and habitat edges, water turbidity, and vegetation or shade. Data was used to determine the number of houses and characteristics of larva positive containers and monitor *Aedes* population with two indicators, namely HI (House Index) and CI (Container Index).

The calculation of House Index (HI) and Container Index (CI) (WHO, 2009).

a) House Index (HI)

$$HI = \frac{\textit{The number of houses positive for larvae or eggs}}{\textit{The whole number of houses examined}} \times 100\%$$

b) Container Index (CI)

$$CI = \frac{\textit{The number of Container positive for larvae or eggs}}{\textit{The whole number of Container examined}} \times 100\%$$

Mosquito larvae or pupae found in the location were counted and taken with a dipper or pipette to be identified. Houses with positive containers with larvae, one or two ovitrap were installed. Positive water storage environmental parameters contained larvae/pupae were observed, includes: water temperature, air temperature, and water acidity (pH). Data from observations of types and characteristics of mosquito breeding sites and types of mosquito larvae were analyzed descriptively.

Questions were proposed to 200 residents in the villages of Panggungharjo and Bangunharjo. Data were analyzed quantitatively to compare community knowledge in the two villages.

Identification of Larvae

Larvae obtained from the field were identified in the Laboratory of Systematics of the Animal Section of Parasitology, Faculty of Biology, Universitas Gadjah Mada, with the identification book "Illustrated Key to Mosquitoes of Vietnam" by C. J. Stijanovich and H. G. Scott (1966). Mosquito larvae other than the *Aedes* genus were killed and discarded, while *Aedes* larvae were taken to test their resistance.

Status of Resistance

Larvae identified as *Aedes* genus were taken of three (3) individuals for each region (hamlets) in every village. One final or 3rd instar *Aedes* larvae were taken and homogenized with 500 µl phosphate buffer solution (PBS) pH 7.0. Each larva made two replicates for test material. The substrate was prepared with 3 mg α-Naphtyl acetate dissolved in 500 µl acetone and added with 50 ml PBS solution, homogenized. Reagent coupling was made with 30 mg of Fast blue which was homogenized with 7 ml of 5% SDS and 3 ml of distilled water. The prepared homogenates were taken 50 µl with a micropipette and put into a microplate well, then added with 50 µl

of the substrate, let stand for 60 seconds. A total of 50 µl reagent coupling solution was added to the microplate well and let stand for 10 minutes. Color changes were observed (colorimetry). The color change reaction was stopped by adding 50 µl of 10% Acetic Acid. Determination of color intensity that occurs, analyzed quantitatively by ELISA, by reading the Absorbance Value (AV) with a Microplate Reader at a wavelength of 450 nm.

Data Analysis

Testing results data on the status of resistance were measured qualitatively and quantitatively. Qualitative measurements were carried out based on color changes (colorimetry), while quantitative resistance status measurements were based on absorbance values (AV).

The resistance status figures according to Lee (1991) are as follows:

- Vulnerable (not resistant or very vulnerable (VV)): AV < 0.70
- Medium resistance (moderate susceptibility (MS)): 0.70-0.90
- High resistance (not vulnerable (NV)): AV > 0.90

Control of substrate or medium was used as a comparison of the treatment or method used.

RESULTS AND DISCUSSION

Data collection of mosquito larvae were conducted from each village in 3 (three) hamlets. The village of Panggungharjo represented hamlets: Garon, Geneng, and Krapyak Wetan. The village of Bangunharjo represented hamlets: Tarudan, Wojo, and Jotawang. At all points of data retrieval, larvae of *Aedes* were found. The village location selection was based on 2012 annual larvae monitoring data and a history of fogging/fumigation treatments at the research location. The sixth data retrieval location had 60-70% free larvae and was recommended by the officers of the local health center as a location prone to mosquito breeding.

Observation of Habitat Characteristics and Larva Collection

The results of the data collection on mosquito larvae in Panggungharjo village showed that in Garon, Geneng, and Krapyak Wetan Aedes mosquito larvae were positive. In Garon hamlet, there were 8 houses positive for mosquito larvae in 8 baths. In Geneng

Table 1. Types of mosquito larvae habitat found in Panggungharjo Village.

No.	Hamlets	Habitat/Container	Total	Larva Found	The Number of Larvae
1.	Garon	Bath	8	*Aedes aegypti*	+ + +
		Bath in Water Closet	-	-	-
		Water tank	-	-	-
		Bucket	-	-	-
		Used tires	-	-	-
		Flower pot	-	-	-
		Bird cage	-	-	-
		Pool	-	-	-
		Clay Water Vessel	-	-	-
		Cans/garbage	-	-	-
2.	Geneng	Bath	3	*Aedes aegypti*	+ + + +
		Bath in Water Closet	1	*Aedes aegypti*	+ + + +
		Water tank	-	-	-
		Bucket	-	-	-
		Flower pot	-	-	-
		Bird cage	-	-	-
		Pool	-	-	-
		Clay Water Vessel	-	-	-
		Cans/garbage	-	-	-
3.	Krapyak Wetan	Bath	3	*Aedes aegypti*	+ + +
		Bath in Water Closet	2	*Aedes aegypti*	+ + +
		Water tank	-	-	-
		Bucket	-	-	-
		Cans/garbage	-	-	-

Description of the number of larvae:
+ : 1-10 + + + : 21-30
+ + : 11-20 + + + + : > 30

Figure 1. Types of containers that could potentially become a breeding ground for *Aedes* in Garon, Geneng, and Krapyak Wetan hamlets, Panggungharjo Village. Description: a. Clay Water Vessel; b. Bath in Water Closet ceramic; c. Bath ceramic; d. Bath cement; e. Bath in water closet cement; f. Potted plants; g. Open wells; h. Bird cages; i. Bucket/ reservoir open water; j. Garbage and a crock; k. pool; l. The place of ablution.

hamlet, there were 4 houses positive for larvae in 3 baths and 1 tub of water closet. In Krapyak Wetan hamlet, there were 5 positive houses contained the larvae of *Aedes* in 3 baths of water closet and 2 baths (Table 1).

Types of containers with a positive sign were ceramic bathtub, bathtub cement, and bath in a water closet (Table 1). Ceramic bathtubs with positive mosquito larvae are in public facilities such as mosques and residential houses (Figure 1). Generally, there are deposits of dirt or sand at the base of the water or the crust. Bath cement with positive larvae was found in citizens home (Figure 1). Bath in a water closet with positive larvae contained in the residents ' houses and used cement tub (Figure 1e).

From interviews with residents, it was known that the dengue fever case in Panggungharjo Village at the beginning of this year had experienced an increase. Garon Hamlet has 3 cases, Geneng Hamlet has 4 cases, and in Krapyak Wetan Hamlet has 5 cases. In 2012 there were only 9 cases recorded at the Sewon Health Center 2.

The results of larvae data collection in Bangunharjo Village showed that Wojo, Jotawang, and Tarudan hamlets were positive for *Aedes* larvae. In Tarudan hamlet, there were 4 houses with positive mosquito larvae in 3 baths and 1 well. In Wojo hamlet, there were 8 houses positive for larvae in 8 baths. In Jotawang hamlet, there were positive larvae *Aedes* in in 1 bath (Table 2).

Containers observed for positive of mosquito larvae were bathtubs generally located in a house with a tub of cement or ceramic (Figure 2) and open wells inside the house (Figure 2) (Table 2). The habitats observed and positive for larvae of *Aedes* were mostly in baths.

Bangunharjo Village is a village with a non-endemic status of dengue fever cases in 2012, as dengue fever tends not to be continuous every year. However, it could potentially become an endemic area considering its located in the border of the city and is directly adjacent to Panggungharjo Village which is an endemic area of dengue Fever. In addition, low numbers of free larvae of *Aedes* in Bangunharjo village increase the potential spread of dengue fever. Bangunharjo village has a distribution region, which tend to cluster and are divided into

Table 2. Types of mosquito larvae habitats found in Bangunharjo Village.

No.	Hamlet	Habitat/Container	Total	Larva Found	The Number of Larvae
	Tarudan	Bath	3	*Aedes aegypti*	+ + +
		Bath in Water Closet	-	-	-
		Well	1	*Aedes aegypti*	+ + + +
		Bucket	-	-	-
		Water tank	-	-	-
		used tires	-	-	-
		Clay Water Vessel	-	-	-
		Flower pot	-	-	-
		Others	-	-	-
	Wojo	Bath	8	*Aedes aegypti*	+ + +
		Bath in Water Closet	-	-	-
		Well	-	-	-
		Bucket	-	-	-
		Water tank	-	-	-
		used tires	-	-	-
		Flower pot	-	-	-
		Others	-	-	-
	Jotawang	Bath	1	*Aedes aegypti*	+ +
		Bath in Water Closet	-	-	-
		Well	-	-	-
		Bucket	-	-	-
		Water tank	-	-	-
		used tires	-	-	-
		Clay Water Vessel	-	-	-
		Flower pot	-	-	-
		Others	-	-	-

Description of the number of larvae:
+ : 1-10 + + +: 21-30
+ + : 11-20 + + + + : > 30

several complexes that are separated by rice fields. From the results of interviews with residents, there was an outbreak of dengue fever at the beginning of 2013. In Jotawang, Wojo, and Tarudan hamlets, each has two cases of dengue fever.

In Wojo Hamlet in 2013, a chikungunya outbreak was also spread by *Aedes* mosquitoes. Therefore, in March, fogging/fumigation was carried out. During the research period, which was in May 2013 in Jotawang, fogging/fumigation was also held, but *Aedes* larvae and eggs were still found in one of the resident's house. This situation shows that fogging is less effective.

Based on the results above, habitat characteristics of the *Aedes* is a container containing clean water, water with clear to slightly turbid, quiet, with rough walls, well-lit/with shade, and the base of the water is not in direct contact with the ground. In addition, used tires and used goods in the vicinity of the source of water were found in the Bangunharjo Village. These items could potentially become a breeding ground for *Aedes*.

The majority Habitat of *Aedes* found in both villages were baths in the house or closed baths. In this case, it is defined as the location/venue for the whole activity or the majority of mosquito life cycle. Including mating and laying eggs.

On Garon Hamlets, one container was found positive for larvae but has murky water and almost translucent. Such circumstances were also found in the Wojo Hamlets. Turbidity of water is related to the difference in the structure of the soil and water management in such environments that cause cloudy water or leave the crust is colored yellow or black. The content of dissolved substances and water turbidity limits the ability of light to penetrate water and can be used effects of aquatic biota. However, the discovery of the larvae of *Aedes* showed that it can adapt in an environment that is not beneficial.

Environmental parameters review of *Aedes* larvae habitats in the Panggungharjo Village, i.e. an average water temperature 28 °C, air temperature of 33.67 °C, and water pH of 6.8. While in Bangunharjo Village, the average water temperature was 28.13 °C, air temperature of 33.67 °C, and water pH of 6.8. The environmental conditions of the container with positive larvae in both villages tended to be the same or close to and within the normal

Figure 2. Types of containers that could potentially be the habitat of *Aedes* in Tarudan, Wojo, and Jotawang hamlets, Bangunharjo Village. Description: a. Bath Ceramic; b. Bath cement; c. Bath in Water Closet ceramic; d. Bath in Water Closet cement; e. Bucket; f. Open wells; g. The enclosure of livestock; h. Pot plants; i. Clay Water Vessel.

range. The difference in air temperature was due to fluctuations in the weather at the time of data collection. The temperature of the air tends to heat into warm water temperature make the development of mosquitoes eggs, larvae, and pupae faster.

The water temperature still in the optimum temperature range becomes the breeding places and larval development of *Aedes*. *Aedes* larvae are capable of developing optimum at a water temperature of 25 -30 °C. Tolerance and range of the optimum temperature depend on the type/species of mosquito and its adaptation. They also capable of growing optimally at a pH of 4.4 to 9.3, and the conditions in the second study site were at the optimal pH. Environmental conditions are supported by the temperature of the air for it tends to be warm in the dry season, so *Aedes* mosquitoes can complete the whole stage of its life cycle more quickly.

In terms of the HI and the CI (Table 3), it is known that Panggungharjo and Bangunharjo villages have the potential of prone to cases of Yellow fever. In Garon, Krapyak Wetan, as well as Wojo hamlets, they potentially have the emergence of DHF cases given the high HI. Although the value of CI tends to be low or below 10%, the potential spread of Yellow fever disease remains high given the presence of the vector in the form of *Aedes* mosquito. Therefore, it is worth the effort to seriously addressing and combating infectious disease vectors, in this case mosquitoes.

At the beginning of 2013, an outbreak of Chikungunya occurred in Wojo hamlet. This was followed by fogging. Panggungharjo village, which was a high endemic area in 2012, the value of CI in the three villages observed had relatively low or less than 10% (Table 3). The value of HI in two hamlets, namely Garon and Krapyak Wetan were relatively high of 26.67% and 16.67%. Although the value of HI was relatively high, in 2012-2013 Panggungharjo village did not experience outbreaks of diseases transmitted through vector, but from year to year the number of dengue fever/DHF sufferer or who are experiencing symptoms of dengue fever/DHF continued to increase.

The success of government programs in control and eradication of infectious disease vectors can be known through citizen's level of knowledge of about the dengue fever (DHF). Recap results of 200 respondents consisted of 100 residents of Panggungharjo Village and 100 citizens of Bangunharjo Village knew that the knowledge of

Table 3. The value of House Index (HI) and Container Index (CI) in Panggungharjo and Bangunharjo villages.

Village	Hamlets	House Checked	House Positive Larvae	HI (%)	Containers Are Inspected	Containers Positive For Larvae	CI (%)
Panggungharjo	Garon	30	8	26.67	113	8	7.08
	Geneng	40	4	10	104	4	3.85
	Krapyak Wetan	30	5	16.67	97	5	5.15
	SUBTOTAL	**100**	**17**	**17**	**314**	**17**	**5.41**
Bangunharjo	Tarudan	32	4	12.50	102	4	3.92
	Wojo	31	8	25.81	101	8	7.92
	Jotawang	37	1	2.70	102	1	0.98
	SUBTOTAL	**100**	**13**	**13**	**305**	**13**	**4.26**
TOTAL		**200**	**30**	**15**	**619**	**30**	**4.85**

Panggungharjo society about DHF, its causes, as well as the eradication of mosquito nests was higher compared to the citizens of Bangunharjo.

Bangunharjo Village residents knew that dengue fever/DHF and its rising symptoms are equal to (80%), but they who didn't knew the cause reach (40%) and its disease vector to (44%) about the dengue fever/DHF. However, community awareness of cleanliness is quite high up to (74%). Another case with Panggungharjo Village community, out of 100 respondents, 95 respondents had knowledge of dengue fever disease. Knowledge about the causes and carriers of dengue fever disease were (90%) and understanding of family hygiene and health were (100%). This is reinforced by the public's awareness to use the biological method (fish) as an effort to control mosquito vectors compared to using abates or other chemicals.

However, the intensification of extension program by the government to the community, especially in endemic regions should be improved. Because the value of HI in Panggungharjo and Bangunharjo villages still relatively high. These circumstances need to be the highest attention of the government in dealing with outbreaks of infectious diseases through vectors in Panggungharjo and Bangunharjo villages, such as through the improvement of human resources, environmental cleanliness, and with the extermination of vector directly.

The Identification of Larvae

The larvae obtained from the 30 containers positive for the larvae were *Aedes aegypti* larvae (Figure 3). They were found in all containers of positive larvae with the amount varies from little (10-20 tails) to very many (>>100). *Aedes* larvae identified from its activities which is able to dive at the base of the old containers. *Aedes* larvae found in large quantities in containers made of cement or with a rough surface, even found in open wells in Tarudan Hamlet.

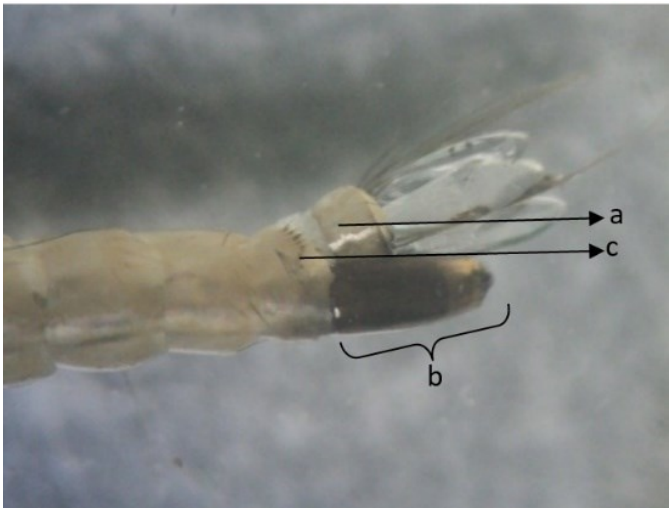


Figure 3. The larvae of *Aedes aegypti* magnification 4x100. Description: a. Saddle; b. Siphon; c. Sex comb.

Aedes aegypti larvae can be identified from the presence of the siphon, it is a self-contained breathing apparatus in the larvae of the Culicidae members with length tend to be the same with the saddle. The saddle is a thickening or sclerotized caudal part contained mobility aids on mosquito larvae, namely tracheal gill and the caudal seta/anal brush (Figure 3a). On *Aedes* larvae, the saddle is only partially experienced thickening. On *Aedes aegypti* larvae posterior parts there are two rows of sex comb whereas in *Aedes albopictus*, there is only one line. Sex comb of the posterior/the second line on the larvae of *Aedes aegypti* have a thorn on the side (Figure 3c).

The identification can be simply observed by

looking at the posterior or last segment on mosquito larvae. Obtained *Aedes* larvae have a siphon (Figure 3b) with medium size and length which is relatively the same with the saddle. Saddle experienced a thickening or sclerotized most and there are two lines of sex comb with the second line branching and barbed side (Figure 3c).

Status of Resistance

Resistance Status of *Aedes* larvae in Panggungharjo and Bangunharjo Villages against organophosphate compounds tested by the method of Elisa Assay based on the value of the Absorbance Value (AV) using ELISA reader. Testing with biochemical methods was conducted to determine the activity of the enzyme esterase non-specific with regard to the mechanism of the resistance onset, namely the onset of the allele for resistance in populations (Macoris *et al.*, 2003). The results of research analysis were done by 2 ways, namely qualitative and quantitative analysis. Qualitative analysis was done by comparing the intensity of sample color with the positive control and negative. Quantitative analysis was done by reading the absorbance value (AV) using ELISA reader at λ = 450 nm. To determine the Resistance Status of mosquito larvae the standard used was Lee (1991) (Lee, 1991).

Based on Table 4 can be seen that *Aedes* larvae in the Village of Panggungharjo has average status of Susceptible (SS) with the mean AV 0.539 ± 0.083. The general condition indicates that control of the spread of dengue fever in Panggungharjo Village with insecticides organophosphates can still be done with a dose that is generally applied. However, the potential change of the Susceptible (VV) Resistant Medium (MS) to watch over the treatment and use of insecticides, especially of insecticides with the active ingredients of organophosphate, on a continuous basis. In general, the difference AV in the three villages shows that the activity of the control and eradication of infectious disease vectors has been done yet cause effects that lead to active resistance mechanisms of mosquitoes especially *Aedes* against insecticide type of organophosphate.

Larvae of Bangunharjo Village have an average AV 0.777 ± 0.150 status Resistant Medium (MS) (Table 5). The Tarudan hamlet has AV which is the highest 0.848 ± 0.130. Mosquito larvae in the three hamlets in Bangunharjo village have a higher potential to be Resistant to High (NV) given the absence of a treatment-spraying/fogging which took place on two consecutive years. In 2012, Bangunharjo Village has the fumigation in the three hamlets observed related cases of *Chikungunya* and at the beginning of 2013, in Tarudan, Wojo, and Jotawang hamlets has also carried out spraying. However, there are still adult mosquitoes can survive and lay eggs in the ovitrap installed. It shows that fogging has been done less effectively, for there began to be resistance that resulted in the mosquito life cycle is not interrupted and the possibility of the spread of infectious disease through mosquito vector is high.

Fogging had been carried out for approximately two months prior to the study. It was done related to the outbreak of *Chikungunya* in the region. Insecticides were often used for fogging in Panggungharjo and Bangunharjo villages were a type of Malathion made active organophosphate with a target of adult mosquitoes. For the eradication of mosquito larvae, citizens/ordinary people use tilapia as a biological control and chemical use of Temefos or abate.

The resistance nature of mosquitoes to an insecticide was caused by the presence of genetic aspects with the R-genes (Mulyaningsih, 2004).

Table 4. Resistance Status of Larvae *Aedes* of Panggungharjo Village.

No.	Sample (Hamlets)	Absorbance Value (AV)	Resistance Status	Average (AV)
1	Garon	0.601 ± 0.113	susceptible (VV)	**0.539 ± 0.083 Susceptible (VV)**
2	Geneng	0.499 ± 0.038	susceptible (VV)	
3	Krapyak Wetan	0.517 ± 0.044	susceptible (VV)	
4	Substate / medium Control	0.383 ±0.025	susceptible (VV)	

Table 5. Resistance Status of Larvae *Aedes* of Bangunharjo Village.

No.	Sample (Hamlets)	Absorbance Value (AV)	Resistance Status	Average (AV)
1	Tarudan	0.848 ± 0.130	Resistant Medium (MS)	**0.777 ± 0.150 Resistant Medium (MS)**
2	Wojo	0.718 ± 0.167	Resistant Medium (MS)	
3	Jotawang	0.767 ± 0.146	Resistant Medium (MS)	
4	Substate / medium Control	0.383 ±0.025	susceptible (VV)	

Genetics aspects influence on the physiological system of the mosquito, includes affecting the work of enzymes esterase that is able to neutralize the insecticides, especially the type of organophosphate. Other aspects such as mobilization, adaptation and regeneration of the mosquito broad quickly also affect the ability of the mosquito in spreading the disease without being affected by the insecticide.

Resistant mosquitoes have the potential to pass down their resistant genes to their offspring, as well as mosquitoes with medium resistance status. Vulnerable mosquitoes have the potential to become medium resistant and then become resistant due to the use of the same type of insecticide for the control of DHF vectors in a long time. Seeing the increasing trend of resistance that occurs in Panggungharjo and Bangunharjo Villages, there needs to be more serious handling from the government in terms of eradicating vectors, especially mosquitoes. This is to anticipate the increasing number of resistant mosquitoes. The higher the level of resistance of the mosquito, the more difficult to control, where in addition to the dosage should be increased and should be sought new insecticide to eradicate the mosquitoes that have been resistant to such.

The ability of resistance in mosquitoes to the active compound organophosphates leads to the mechanism of nerve impulses inhibition to not occur. This is due to the physiological basis of mosquito which has enzymes esterase capable of degrading such active compounds. Enzymes esterase such as malathion carboxylesterase, oxidases or glutathione S-transferase (GST) are enzyme that can detoxify to the insecticidal ester type, in this case the organophosphorus (Macoris *et al*., 2003; Pimsamam *et al*., 2009).

CONCLUSION

The habitats characteristics of mosquitoes, in general, are open containers with long time-stagnant water, crystal clear water, and calm, with surface of rough and dark walls, while surface of the base are not directly related to the soil, the water temperature ranged between 25°C – 29°C and pH of the water ranged between 6-8, and also not exposed to direct sunlight. The type of mosquito that is found in Panggungharjo and Bangunharjo villages, Sewon District, Bantul Regency, Special Region of Yogyakarta is *Aedes aegypti*. Resistance Status of larvae *Aedes* in Panggungharjo village is Susceptible (SS) against organophosphorus compounds, while the resistance status of larvae *Aedes* at Bangunharjo is a Resistant Medium (RS) against organophosphorus compounds.

ACKNOWLEDGMENTS

Thank you to Mrs. Dr. Dra. Rr. Upiek Ngesti W. A., DAP&E, M. Biomed for their guidance and input during the research and creation of research reports.

REFERENCES

Department of Health Bantul Regency, 2016, *Profil of Health Bantul Regency*, [Dinas Kesehatan Kabupaten Bantul], Special Region of Yogyakarta.

Department of Health Bantul Regency, 2017, *Profil of Health Bantul Regency,* [Dinas Kesehatan Kabupaten Bantul], Special Region of Yogyakarta.

Knipe, D. M., and Howley, P. M., 2007, *Fields Virology: Flaviviridae "The Viruses and Their Replication", 5th Edition*, Lippincott-Raven Publishers, Philadelphia.

Lee, H. L., 1991, *Esterace Activity and Temephos Susceptibility in Aedes aegypti (L.) Larvae*, Mosq. Borne Dis. Bull, Pp. 8, 91-94.

Lidia, Kartini dan Widyaningrum, E. 2008, Deteksi Dini Resistensi Nyamuk *Aedes albopictus* Terhadap Insektisida Organofosfat di Daerah Endemis Demam Berdarah Dengue Di Palu (Sulawesi Tengah) [Early Detection of *Aedes albopictus* Mosquito Resistance to Organophosphate Insecticides in Endemic Dengue Fever Areas in Palu (Central Sulawesi)], *Media Kesehatan Masyarakat*, 3, 2 pp. 1-6.

Macoris., Maria de, L. G., Andrighetti, Maria, T. M., L. Takaku, C. M. Glasser, Garbeloto, V. C., Bracco, J. E., 2003, Resistance of *Aedes aegypti* from the State of São Paulo, Brazil, to Organophosphates Insecticides. *Mem Inst Oswaldo Cruz*. 98(5), pp. 703-708

Mulyaningsih, B., 2004, Variation in Susceptibility Status of Organophospate Insecticide Among Several Geographic Population of *Aedes albopictus* Skuse in Indonesia. *Berkala Ilmu Kedokteran,* 36, 2.

Pimsamarn, S., Sornpeng, W., Akksilp, P., Paeporn, and Limpawitthayakul, M., 2009, Detection of insecticide resistance in *Aedes aegypti* to organophosphate and synthetic pyrethroid compounds in the north-east of Thailand. *Dengue Bulletin*. Vol. 3 Pp. 194-200.

Service, M. W., 1996, *Medical Entomology for Students*, London Chapman and Hall, London.

Stojanovich, C. J. and Scott, H. G. 1966. *Illustrated key to mosquitoes of Vietnam*, U.S.Communicable Disease Center, Atlanta.

Sukesi, T. W. dan Surahma, A. M., 2007, Kerentanan Nyamuk *Aedes aegypti* L. Terhadap Senyawa Organofosfat temephos dan Malathion di Kelurahan Wirobrajan Kecamatan Wirobrajan, Yogyakarta [Vulnerability Against *Aedes aegypti* L. temephos and Malathion Organophosphate compounds in the Village Wirobrajan Wirobrajan subdistrict, Yogyakarta], *Kesehatan Masyarakat* 1,1. pp. 21, 22 – 25.

Widiarti, Heriyanto, B., Boewono, D. T., Mujiono, U. W., Lasmiati and Yuliadi, 2011, Peta Resistensi Vektor Demam Berdarah *Dengue Aedes aegypti* Terhadap Insektisida Kelompok Organofosfat, Karbamat, dan Pyrethroid di Provinsi Jawa Tengah dan Daerah Istimewa Yogyakarta [Resistance map Dengue vector *Aedes aegypti* Group Against Organophosphate insecticides, carbamates, and pyrethroids in Central Java and Yogyakarta]. *Buletin Penelitian Kesehatan,* 39, 4. pp. 176-189.

World Health Organization (WHO), 2009, *Dengue: guidelines for diagnosis, treatment, prevention and control - New edition,* WHO Press, Switzerland.

World Health Organization (WHO), 2012, *Monitoring and evaluation indicators for integrated vector management,* WHO Press, Switzerland.