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Editors :
RE Prabowo, AR Maharning, ER Ardli, H Pramono, GE Wijayanti, MH Sastranegara, Y Sistina

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The Roles of Biology in Utilization of Indonesian Bioresources to Improve Nation competitiveness

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PREFACE

This book is produced as a compilation of the presented papers during the International Symposium on Indonesian Biodiversity held by Indonesian Biological Society from August 30 to September 1, 2013 at Jenderal Soedirman University, Purwokerto, Indonesia.

Several manuscripts were submitted to the editorial board. Three papers were presented by the keynote speakers covering themes of mollusc diversity, mangrove diversity, and biodiversity ethics. Thomas von Rintelen of Naturkunde Museum, Berlin, Germany focused on the origin and distribution of biodiversity, Tadashi Kajita of Chiba University, Chiba, Japan emphasized the conservation genetics of mangroves, whereas Daryl Macer of American University of Sovereign Nation, Scottdale, Arizona, USA, highlighted the ethical world views of nature. Other presented papers were written by the participants. These included wide variety of biota discussed, e.g., microbial diversity and their roles in the environment; marine, mangrove, or fresh water diversity and its community structure; insect, bird, mammal, or plant diversity and its distribution.

This symposium would not have been happened without the supports of many parties. Acknowledgements were addressed to the Rector of Jenderal Soedirman University for his encouragement and endorsement, the Dean of Faculty of Biology, Jenderal Soedirman University for providing the necessity in the preparation of symposium, and all participants without whom this symposium would not have occurred.

It is hoped that this proceeding may provide the readers with the up to date information of the field.

Purwokerto, Januari 2014

Editors

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Distribution and Habitat of Endemic Fish Biodiversity in Ancient Lake Towuti, South Sulawesi

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Lake Towuti is a tectonic-oligotrophic located in Malili Complex, South Sulawesi. This lake sustains the life of several endemic fish resources, as well as utilized for various human purposes i.e. hydroelectric power plant, capture fishery, navigation, ecotourism and source of water for domestic uses. On the other side of this lake also supports the life of the kinds of resources, especially fish species that are endemic genetic resources that can be utilized surrounding communities. Therefore, the diversity of fish resources in these waters should be protected from a variety of activities that can reduce the population of the fish resources. The aim of research is to know the distribution and habitat of endemic fish biodiversity in ancient Lake Towuti as a basic for formulating the zoning criteria of endemic species in Lake Towuti. This research was conducted in Lake Towuti from June to October 2009 at Tominanga, Cape of Manu, Loeha Island, Hola-hola downstream, Kawatang downstream, Beau and Cape of Bakara. Fish sampling using experimental gill net with four mesh size. The connection of the fish resources with environmental parameters were analyzed using principal components analysis. Habitat characteristics viewed from the environmental factors that are important for fishes were high in pH, total phosphorus, total organic matter and suspended solid; substrate type is silt and low clay and sand; high riparian vegetation cover of the group *Ottelia mesenterium* and *Cyperus*. Stations that have high species diversity for fishes were Tominanga and Hola-hola downstream.

Keywords: distribution, habitat, fishes, ancient Lake Towuti

INTRODUCTION

Lake Towuti located in Malili Complex, Regency of East Luwu, South Sulawesi. This lake has a vast 56 000 ha, maximum depth of 203 m, transparency as deep as 22 m and classified oligotrofik (Haffner et al. 2001). This lake is a tectonic lake and has been designated as a recreation park according to Agriculture Minister Decree No. 274/Kpts/Um/1979. This lake sustains the life of several endemic fish resources. There are 29 species of fish from 13 families (Wirjoatmodjo et al. 2003). Of the 29 species of fish there are 19 species of endemic fish that are listed in the IUCN (IUCN, 2003 and Froese and Pauly, 2004).

More concrete data how much level of exploitation of the fish has not been obtained, but based on the results of interviews with some fishermen on Lake Towuti, the catch from year 2000-2006 tending to decline. According to Samuel et al. (2005), the number of a fishing gear especially dipnet operating in Lake Towuti in 2003 is only four others subsequently increased to 15 in 2004. An increasing number of the fishing gear is not selective, potentially in lowering populations of fish in the lake.

Besides having an endemic fish resource as economics potentially, Lake Towuti also used for hydroelectric power plant, capture fishery (ornamental fish, consumption and animal feed source), navigation, ecotourism and source of water for domestic uses (Nasution, 2006). In addition to the above problems, there are indications that the polluted waters of Lake Towuti has been biologically characterized the entry of exotic fish species.

In line with the population and other activities around Lake Towuti, suspected to affect the resources of endemic fish that live in the lake. Based on the functions of fishery resources for food and livelihoods, should conserve the fish resources. Additional reasons why the fish resources in Lake Towuti must to conserved and managed is the fish caught are endemic species. The aim of research is to know the habitat characteristics of ichthyofauna in Lake Towuti as a basic for formulating the zoning criteria of endemic species in Lake Towuti.

MATERIAL AND METHOD

The study to be done in Lake Towuti, South Sulawesi (Figure 1) from June to October 2009 with descriptif methods. Research stations, namely: A) Tominanga, B) Cape of Manu, C) Loeha Island, D) Hola-hola, E) Kawatang, F) Beau and G) Cape of Bakara.

Measurement of environmental parameters conditions of fish resources consist of parameters that directly measured in the field namely: temperature, turbidity, conductivity, dissolved oxygen and pH using Water quality checker Horiba U-10. Environmental parameters

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measured in the laboratory include nuisance parameters ($N\text{-}NO_2$, $N\text{-}NH_4$) and nutrients (TN, $N\text{-}NO_3$, TP, $P\text{-}PO_4$) and chlorophyll-a. Water sampling was conducted as much as two liters using vandorm bottle sampler and then preserved following the method of Anonymous (1998). The method of analysis can be seen in Table 1.

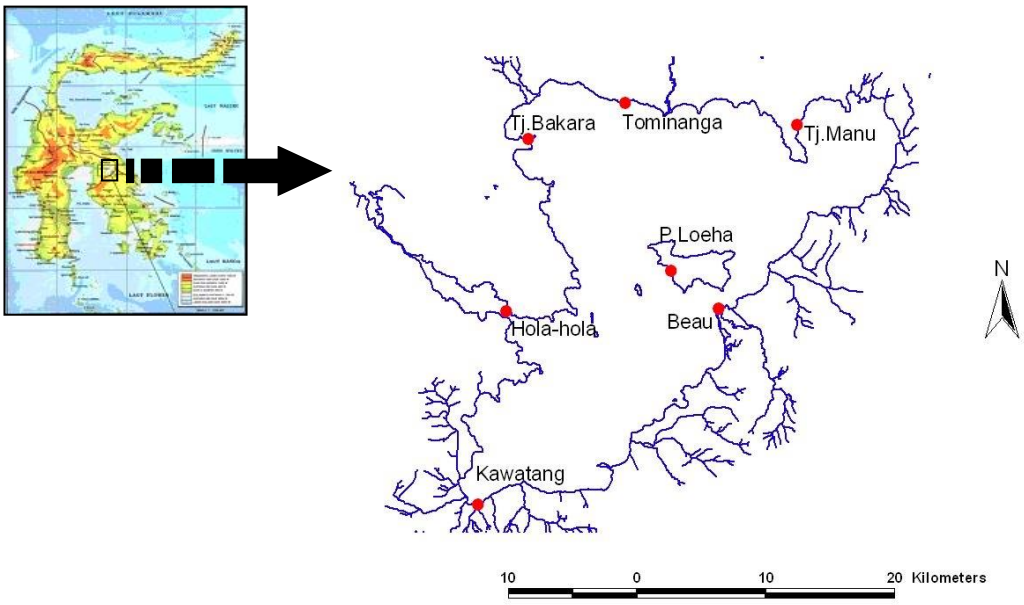

Figure 1. Research station in Lake Towuti

The presence of vegetation also plays a role in setting the parameters of physical habitat diversity. Vegetation sampled using 10 x 10 m transects placed parallel to the coast of each two replications. Furthermore, the documentation prepared and preserved using 70% alcohol, then dried in an oven at a temperature of 70 ^oC. Samples of vegetation identified in the Herbarium Bogoriense, Research Center for Biology-Indonesian Institute of Sciences, Cibinong.

For the determination of sediment structure, substrate samples were taken by Ekman grab, put into plastic containers which have been labeled. Furthermore, the sample included in the metal tray, dried in oven at 105 ^oC until dry, then cool, and weigh it. If the sample to clot, crushed with mortar until the actual size depending on the type of sediment. Graded using a sieve shaker more less 10 minutes. Weigh each fraction and calculated the cumulative percentage of each grain size (Head, 1981). Method of texture analysis using hydrometer refers to Sulaiman et al. (2005).

Table 1. Methods of water quality parameters analysis

No.	Parameters	Methods
1.	Temperature	In situ, Water Quality Checker-Horiba U-10
2.	pH	In situ, Water Quality Checker-Horiba U-10
3.	Dissolved oxygen (DO)	In situ, Water Quality Checker-Horiba U-10
4.	Turbidity	In situ, Water Quality Checker-Horiba U-10
5.	Conductivity	In situ, Water Quality Checker-Horiba U-10
6.	$N\text{-}NO_2$	Sulfanilamid, spectrofotometri
7.	$N\text{-}NO_3$	Brucine, spectrofotometri
8.	$N\text{-}NH_4$	Fenate, spectrofotometri
9.	Total N	Brucine with $K_2S_2O_8$ as ocsidator, spectrofotometri
10.	$P\text{-}PO_4$	Ascorcic acid, specrofotometri
11.	Total P	Ascorcic acid, spectrofotometri
12.	Chlorofil-a	Extraction with aceton, spectrofotometri
13.	Suspended solid	Spectrofotometri

Fish samples were collected from using experimental gillnet with mesh sized ¾, 1, 1¼ and 1½ inches. The total length of the net is 200 m (4 x 50 each mesh size). Net equipped with the float at top and sinker at the bottom. Net is positioned vertical to the coastline in each station

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(Nasution et al. 2007). Fish samples preserved using 4% formalin solution then soaked in 70% alcohol solution. To determine the type of fish species identified using Weber and Beaufort (1913), Weber and Beaufort (1916), Weber and Beaufort (1922) and Kottelat et al. (1993).

RESULT AND DISCUSSION

Habitat characteristics of the research station can be seen in Table 2. From these tables, each research station has different characteristics. At some stations, such as Tominanga, Kawatang, Hola-hola and Beau existing ecological connectivity as a source of incoming water (inlet) and the out water (outlet). Station of inlet of lake Towuti from Tominanga River, inlet of lake Towuti from Kawatang River and Beau existing water sources, each of which originated from Kawatang River and Babasalo River. Hola-hola station an outlet of Lake Towuti that flows into Larona River and empties into the Gulf of Bone.

The existence of the source of inlet and outlet, macrophyte and riparian vegetation, and different types of substrates. As Beau station existing of river water into the lake is Babasalo River as source water for the three villages were Beau, Bantilang and Loeha. Habitat of Beau station is also very unique because it was found six types of macrophyte and riparian vegetation inhabited by bats and birds. Water quality parameters are presented in Table 3. $N\text{-}NO_2$ (nitrite) and $N\text{-}NH_4$ (ammonia) parameters were classified as nuisance parameters (Hartoto et al, 1998).

The availability of sufficient dissolved oxygen in the range between 4.7 - 7.4 mg/L appear to be able to oxidize ammonia and nitrite to nitrate. The biggest concentration of nitrite is 0.007 mg/L occurred at Beau station in October 2009. Turbidity describe optical properties of water which is determined based on the amount of light absorbed and emitted by the materials in water. Conductivity (electrical conductivity) is a numerical illustration of the ability of water to continue the flow of electricity. Therefore, the more soluble salts that can be ionized, the value of conductivity is higher. Reactivity, valence numbers, and the concentration of dissolved ions influence on conductivity (Greenberg et al, 1998). Most likely caused by a number of organic and inorganic materials that are suspended in that location which may be colloid and fine particles. The parameters of $N\text{-}NO_3$, total nitrogen, $P\text{-}PO_4$ and total phosphorus is a parameter that indicates the concentration of nutrients in Lake Towuti.

The kinds of fish found used a catch experimental gillnet can be seen at Table 4. The number of fish caught were 11 species. The highest abundance was found on Rough Pangkilang or Yellow Pangkilang (*Telmatherina celebensis*) as many as 881 fishes, followed by Anggori (*Glossogobius celebius*) as many as 103 fishes.

Table 2. Habitat characteristics of research station in Lake Towuti

No	Station	Coordinate	Habitat characteristics Environmental condition
1.	Tominanga (A)	E 02° 39.365’ S 121° 29.935’	There is inlet of Tominanga River, there is no macrophyte
2.	Cave of Manu (B)	E 02° 40.406’ S 121° 37.081’	There is no macrophyte
3.	Loeha Island (C)	E 02° 46.505’ S 121° 31.830’	Island in the middle of the lake, there is no macrophyte
4.	Hola-hola (D)	E 02° 48.187’ S 121° 24.941’	There is a outlet that flows into Larona River; there is no lake plant grass
5.	Kawatang (E)	E 02° 56.377’ S 121° 23.720’	Swamps, there is flood plain, there was a relationship lakes with the river flows into the Kawatang River, there is lake plant grass (smooth)
6.	Beau (F)	E 02° 48.091’ S 121° 33.848’	There is inlet of Babasalo River; swamps; there is no macrophyte
7.	Cave of Bakara (G)	E 02° 40.893’ S 121° 25.873’	There is a high-trunked vegetation (Pandanus) in the lake

In Lake Towuti, substrate type is dominated by hard substrate that is sand. Sand substrate is important for benthic organisms, especially benthic fish, shrimp, crabs and molluscs. Organic matter also plays an important role as substrate and food resources of benthic organisms. High organic matter found in Hola-hola station and Kawatang station.

In Table 2, the high percentage of organic matter in this station, can be associated with nutrient inputs from rivers that enter and exit of Lake Towuti. An upper limit of $N\text{-}NO_2$ for fisheries

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set by the Government Regulation No.82 in 2001 are 0.06 mg/L and N-NH_4 which is 0.02 mg/L. N-NO_2 concentration in Lake Towuti it ranged between 0.001 – 0.007 mg/L and N-NH_4 concentration between 0 - 0.009 mg/L, did not threaten aquatic life in the lake because its value is still below the threshold the maximum limit according to the above regulation.

Greenberg et al. (1998) said that, turbidity is caused by the presence of organic material and suspended and dissolved anaorganik (ie. silt and sand), as well as inorganic and organic materials in the form of plankton and microorganisms of water. The range of turbidity in Lake Towuti is 0 - 8 NTU, the largest turbidity in Kawatang station, with a value of 8 NTU. Concentration of suspended solid (SS) in these locations ranged from 0 - 8.8 mg/L and the concentration of total organic matter (TOM) ranged from 8.233 - 25.198 mg/L (Table 3). The range of conductivity values in Lake Towuti are 0.139 - 0.156 mS/cm.

Table 3. The range value of water quality in Lake Towuti

Parameters	Unit	Station						
		A	B	C	D	E	F	G
pH		7.69 - 8.40	7.70 - 8.10	7.51 - 8.30	8.00 - 8.30	7.75 - 8.30	7.51 - 7.60	7.46 - 8.10
Conductivity	mS/cm	0.140 - 0.146	0.141 - 0.156	0.142 - 0.148	0.139 - 0.146	0.142 - 0.149	0.143 - 0.153	0.143 - 0.150
Turbidity	NTU	0 - 2	0 - 2	1	0 - 2	0 - 8	4	0 - 2
DO	mg/L	6.11 - 7.30	5.45 - 7.00	6.07 - 6.60	5.65 - 7.00	6.05 - 7.40	4.70 - 5.96	5.70 - 6.53
Temperature	^oC	29.3 - 31.0	28.7 - 29.3	28.7 - 30.2	28.7 - 30.8	29.6 - 30.6	28.7 - 29.9	28.6 - 31.3
SS	mg/L	0	0	0	0	0 - 8.8	1.6 - 11.2	0 - 0.4
Chlorofil-a	mg/m^3	0 - 0.002	0 - 0.145	0 - 0.241	0 - 0.344	0 - 2.019	0 - 1.847	0
N-NO_2	mg/L	0.001 - 0.003	0.001 - 0.004	0.001 - 0.004	0.001 - 0.002	0.001 - 0.004	0.004 - 0.007	0.001 - 0.004
N-NO_3	mg/L	0.006 - 0.068	0.007 - 0.697	0 - 0.004	0 - 0.132	0 - 0.088	0 - 0.179	0 - 0.038
N-NH_4	mg/L	0 - 0.001	0	0	0 - 0.006	0 - 0.007	0	0 - 0.009
TN	mg/L	0.110 - 0.325	0.118 - 0.793	0.087 - 0.165	0.099 - 0.603	0.042 - 0.299	0.005 - 0.218	0.012 - 0.480
P-PO_4	mg/L	0 - 0.012	0 - 0.014	0 - 0.012	0 - 0.016	0.007 - 0.018	0 - 0.012	0 - 0.022
TP	mg/L	0 - 0.143	0 - 0.169	0 - 0.111	0 - 0.114	0.021 - 0.127	0 - 0.211	0.020 - 0.097
TOM	mg/L	5.240 - 13.472	8.483 - 13.722	23.451 - 27.942	8.483 - 29.938	8.233 - 25.198	18.212 - 28.940	12.225 - 17.464

Description: A = Tominanga, B = Cave of Manu, C = Loeha Island, D = Hola-hola, E = Kawatang, F = Beau dan G = Cave of Bakara

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In June, August and October 2009, the water temperature in Lake Towuti that sampling was 28.6 - 31.3 ^{o}C with an average of 29.6 ^{o}C. The range of water between 7.46 - 8.4 mg/L, with an average of 7.93, it tends to be alkaline, while dissolved oxygen concentration between 4.7 - 7.4 mg/L with an average of 6.26 mg/L. N-NO_3 concentration ranged from 0 - 0.697 mg/L, with an average of 0.076 mg/L. Total nitrogen concentrations ranged from 0.005-0.793 mg/L, with an average of 0.254 mg/L. P-PO_4 concentrations ranging from 0 - 0.022 mg/L, with an average of 0.008 mg/L. Total phosphorus concentrations ranged from 0 - 0.211 mg/L, with an average of 0.063 mg/L. Concentrations of N-NO_3 and P-PO_4 is still below the quality standard regulation of PP No.82 of 2001 for the fishery that is N-NO_3 maximum of 10 mg/L and P-PO_4 is 0.2 mg/L. The average value of those parameters still meet the quality standard regulation of PP No.82 of 2001 for the fishery.

Table 4. Fish species which are caught using Experimental gill net

Fish species	Station							Number (fish)
	A	B	C	D	E	F	G	
Telmatherina celebensis	289	42	35	65	175	246	29	881
Paratherina striata	9	3	3	1	2	5	1	24
P.cyanea	22	0	0	11	6	0	0	39
Glossogobius celebius	22	2	3	32	15	25	4	103
Glossogobius flavipinnis	3	1	4	45	0	0	0	53
Glossogobius intermedius	2	0	3	4	3	12	7	31
Glossogobius matanensis	0	0	0	0	0	0	1	1
Oreochromis niloticus	3	0	0	0	0	1	0	4
Channa striata	1	0	0	0	0	0	0	1
Anabas testudineus	5	0	0	0	0	2	1	8
Aplocheilus panchax	0	0	0	0	0	5	0	5
Number (fish)	356	48	48	158	201	296	43	1150

Description: A = Tominanga, B = Cave of Manu, C = Loeha Island, D = Hola-hola, E = Kawatang, F = Beau dan G = Cave of Bakara

In Lake Towuti found as many as 116 species of riparian vegetation. High diversity of riparian vegetation species found at Tominanga, Kawatang and Loeha Island stations. The high percentage of riparian vegetation cover is *Cyperus*, *Glochidion arborescens* Blume, *Xanthophyllum tennipetalum*, *Kjelbergerdendron celebicum*, *Ottelia mesenterium*, *Ficus microcarpa*. Riparian vegetation has an important role as a source of organic material input from outside the water system (allochthonous) as a source of insects that feed several endemic fish species in these waters and litter that can act as a substrate or food source for benthic organisms (fish, shrimp, crab and molluscs). As an example of the *Kjelbergerdendron celebicum* species that are fruits and seeds of *Ficus microcarpa* can be used as fish feed in the lake. Therefore ecologically, Lake Towuti poor nutrients (oligotrofic) feed source organism supplied from riparian vegetation, so that riparian vegetation has important ecological connectivity of Lake Towuti aquatic systems.

The results of research Wirjoatmodjo et al. (2003), a species of endemic fish in Lake Towuti there are nine species. In this research the number of fish are caught equal to the catch based on Nasution (2008a) research, namely 11 species but its different species. Fish species not caught on this research were Sepat rawa (*Trichogaster trichopterus*), Dui-dui (*Dermogenys megarhamphus*), Red Pangkilang (*Tominanga* sp.), and Rice fish (*Oryzias marmoratus*). On this research result found Cork (*Canna striata*) which is a competitor of Butini (*Glossogobius matanensis*) which is an endemic fish in Lake Towuti. Tilapia (Oreochromis *niloticus*) are also found in these waters (Table 4). Likewise fish caught by fishermen that gill net, has many species of fish are caught, Tilapia (*O. niloticus*), Mujair (*Oreochromis mosambicus*), Osang or Betok (*Anabas testudineus*) and Goldfish (*Cyprinus carpio*). This indicates that has happened is the biological pollution in Lake Towuti due to the influx of more exotic species. It 'll eventually is expected to threaten the existence of endemic fish inhabiting the waters. Therefore zoning conservation area of endemic fish in this lake be important to be fixed .

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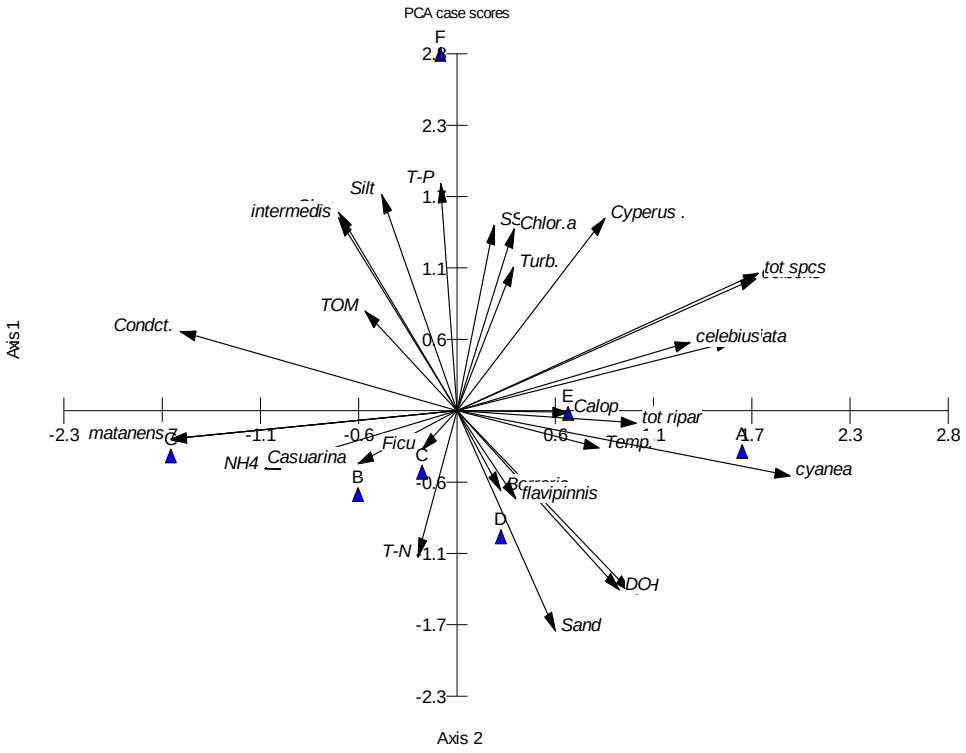
Linkage between endemic fish species with water quality parameters, riparian vegetation and substrate type

From the principle components analysis (PCA), the total endemic fish species in Tominanga encountered station and environmental parameters that play a role is a total phosphorus (TP) and *Cyperus* sp. (Figure 2). For some of endemic fish species as *Telmatherina celebensis* play important roles in parameter that is suspended solid, total phosphorus and percent of *Cyperus* sp. covering and *Ottelia mesenterium* is also important that determines the parameters of the distribution of endemic fish species.



Vector scaling: 5.73

Figure 2. Result of PCA analisys between fish species with environmental parameters

For *Paratherina striata* of parameters is really important is ammoniac (where the smaller concentration, the more the fish), total of riparian vegetation and percent of *Cyperus* sp. covering. For *P. cyanea* parameters that determine the importance or its distribution is TOM. For Glossogobius flavipinnnis which determine its distribution were pH, TN, total of riparian and percent of *Borreria* sp. covering.

Table 5. Some important environmental parameters associated with endemic fish in Lake Towuti

Fauna	Zonation criteria	Important environmental parameters that determine the distribution of endemic species	Stations that have high fish diversity
Ichthyofauna	Ecologi caliIntegrity: The suitability of environmental Conditions (water quality and substrate type)	1. High diversity of fish species: pH and concentration of TP is high 2. Several endemic fish species in bentic habitat: TP and TOM are high, SS is high Type of substrate: low percentage of clay, silt and sand	Tominanga and Hola-hola (A dan D)
	Ecological connectivity: Riparian types	High diversity of fish species: Percent of riparian vegetation cover height of the *Ottelia mesenterium* dan *Cyperus* group	

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For *G.celebius* which play important roles in parameter that is play important roles in parameter that is *Borreria* sp. covering. For *G.intermedius* played an important role, which is a pH, Suspended Solid (SS), Total Organic Matter (TOM) and sand. That determines its distribution is smooth substrates i.e. mud and clay. For *G.matanensis* played an important role is ammonium (NH_4).

On Rough Pangkilang or Yellow Pangkilang (*Telmatherina celebensis*) that abundance highest, recruitment been successful, and spawning site is in habitats that have vegetation or aquatic macrophyte are in Hola-hola station (Nasution, 2005; Nasution et al. 2007 and Nasution 2007). At the station there are a many aquatic macrophyte a place to put the Yellow Pangkilang fish eggs. It is another on bonti-bonti blue (*Paratherina striata*), this fish has abundance highest, recruitment been successful and spawning site is in habitats that have vegetation or aquatic macrophyte are in stone habitat like Tominanga and Loeha Island (Nasution, *et al.* 2007; Nasution, *et al.* 2008 and Nasution, 2008b).

Linkage between endemic fish species with water quality parameters, riparian vegetation and substrate type have some environmental parameters that are important in formulating criteria for zoning endemic biota in Lake Towuti can be seen in Table 5.

CONCLUSION

1. Habitat characteristics viewed from the environmental factors that are important for endemic fish were high in pH, total phosphorus, total organic matter and suspended solid; substrate type is silt and low clay and sand; high riparian vegetation cover of the group *Ottelia mesenterium* and *Cyperus*.
2. The average value of those parameters still meet the quality standard regulation of PP No.82 of 2001 for the fishery.
3. Stations that have high species diversity for endemic fish biodiversity were Tominanga and Hola-hola downstream.

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Population Structure of Bagrid Catfish *Hemibagrus nemurus* Collected at Five Different Rivers

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Hemibagrus nemurus is one of the most exploited freshwater fish in Java. They are commonly found in the rivers, lakes, and reservoirs. However, a study on population structure and connectivity among rivers population of *H. nemurus* in Java Island was not available. Therefore, not much is known about connectivity of river populations in Java Island, despite the fact that such information is essential in determining the choice of genetic conservation unit for conservation purposes. This study aimed to investigate the population structure of the green catfish *H. nemurus* collected at five rivers in Java Island. The analysis was based on 470 bp fragment of the cytochrome c oxidase 1 gene from 79 individuals. *H. nemurus* showed high level of genetic diversity. Pairwise comparion showed high levels of genetic different among populations, indicating strong population structuring and limited gene flow. Each river population can be regarded as one gentic conservation unit. This pattern of population structure has important implication for sustainable management.

Keywords: Green catfish, genetic diversity, connectivity, over-exploitation

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Cytotoxic Assay of Soil Actinomycetes Isolated from Purbalingga Indonesia

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This research is a preliminary study to obtain active compounds with potential as cytotoxic agents from Actinomycetes. Two Actinomycetes strains were isolated from soil and identified based on 16S rRNA sequence. The Isolates are LP and RP have similarity with *Micromonospora chersina* and *Micronomospora aurantica*, respectively.The identified isolates were fermented in liquid media for one weeks. The liquid and mycelium were extracted using ethyl acetate. Whole extract of each fermented isolate was partitioned and evaporated to obtain ethyl acetate extract. The cytotoxicity was evaluated on HeLa cells by MTT assay. The result showed the LC_{50} value were 260 µg/ml and 320 µg/ml for LP and RP.

Keywords : Actinomycetes; MTT Assay

INTRODUCTION

Actinomycetes widely distributed in soil and play an important role in digesting and recycling organic compounds (Presscot, 2002). Abundance and types vary according to ecological factors such as vegetation, soil pH, humus content and water content (Hayakawa *et al*., 2010). They have provided many important bioactive compounds of high commercial value and continue to be rountinely screened for new bioactive substance. Actinomycetes are the main producers of antibiotics and other secondary metabolites (Hayakawa *et al*., 2004). These products have diverse biological activities, including antifungal, antibacterial, antiviral, antitumor, immunosuppressive, and cytotoxic (Lazzarini *et al*., 2000).

In screening for Actinomycetes able to produce bioactive compounds, the exploration of new soils and habitats has been recommended (Takahashi and Omura, 2003). In this context, Indonesian soil might be a rich source of Actinomycetes species, and here we have focused on searching for strain possesing citotoxic potential. In tropical environments, it is possible to find the high diversity and high population of Actinomycetes and open opportunities to acquire new metabolite (Nurkanto *et al*., 2010).

Prevalence of cancer tends to increase from time to time. According to IARC (2005), cervix cancer is an important public health problem. It is the third cancer in frequency in women worldwide and the most or second most common cancer among women in developing countries. Thus new drugs from natural resorces such as soil Actinomycetes are still required. This research is preliminary study obtain active compounds with potential as cytotoxic agents from Actinomycetes. We screened Actinomycetes as potential cytotoxic based on the observation of viability of cervix cancer cells (HeLa cells) using in vitro assay with MTT method.

MATERIAL AND METHOD

Soil sample collection

Soil sample were collected from Purbalingga, Middle Java, Indonesia and were transported aseptically in sterile plastic container to the laboratory. Each total of 100 grams of soil samples was taken from a depth of 0-10 cm. Filtered using a 2 ~ mm mesh sieve and dried at room temperature for 7 days.

Isolation of Actinomycetes

The samples were first treated with heat at 120℃ for 1 hour. Sample of each soil were first mixed, suspended in sterile distilled water (1 g in 10 ml) homogenized by vortexing. After allowing the tube to stand for 1 minute, 1 mL of supernatant was transferred to 9 mL of 0,5% phenol solution. Then the mixture was maintained at 30˚ C with occasional stirring. After incubation for 30 minute,the treated samples were spread (0.1 ml) over the surface of plates of Soil Extract Agar. Soil Extract Agar made as follows, 1000g soil (Andosol soil from Lembang was selected) was dissolved in 2 liters of 50mm NaOH and incubated overnight at room temperature. Solution was filtered and centrifuged for 60 minutes at 18,000 rpm. Supernatan was filtered through 0.2 µM sieve. Soil Extract Agar medium contains 500 ml/L soil extract and 15 g/L agarose. Then was added 50 mg/L Cycloheximide (Hamaki *et al*., 2005). The plates were

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incubated for 3 weeks. After the incubation periode, the plates were examined for the presence of Actinomycetes colony. The suspected colonies were picked up and purified on ISP-2 media, and incubate at 30℃ for about 7 days. All observed colonies were isolated, purified and conserved in 20% glycerol in water at -20℃.

Culture characteristics

Purified isolates of Actinomycetes were used to study the morphology of spore bearing hyphae with the entire spore chain with the Actinomycetes morphologies as describe by Holt, 1994. This was done by using cover slip method in which individual culture were transferred to the base of cover slips buried in ISP2 agar medium.

Gram staining

A smear of culture was taken in clean glass slide and heated gently over a flame. The smear was covered with a thin film of crystal violet for 1 min and washed gently in slow running tap water. Gram's iodine solution was flooded over the smear for 1 min and washed with tap water. Alcohol was used to decolorize the smear until the violet color ceased to flow away. The slide was washed with tap water and counter stain safranine was flooded over the smear for 2 min, then the slide was washed, drained, air dried, and viewed under microscope. The culture retaining the violet color indicated that it was Gram-positive organism.

Identification of isolate based on 16S rRNA gene sequencing.

Selected isolate was subjected for sequence analysis of 16S rRNA gene. Identification using the 16S rRNA was conducted by PCR using 9F forward primer,GAGTTTGAT(C/T)(C/A)TGGCTCAG; and 1541R reverse primer, AAGGAGGTG(A/T)TCCA(A/G)CC. The reaction mixture (50µL total volume) contained 30 µL ddH_2O, 5 µL of $MgCl_2$, 5 µL of 10X buffer 4 µL of deoxynucleoside triphosphates, 1 µL of each primer, 0,25 µL of Takara Taq Polymerase (Takara Bio Inc, Japan), and 5 µL of cell lysis as template. PCR conditions were as follow: denaturation at 98˚C for 20 s, annealing at 52˚C for 45 s, and elongation at 72˚C for 2 min. A total of 30 cycles were performed, followed by a final elongation for 4 min at 72˚C. PCR products were purified with a Geneaid PCR Fragments Extractio Kit according to the manufacturer's instruction (Geneaid, Taiwan). Amplicon was sequenced with an automatic sequence analyzer (Applied Biosystems 3130 3130 DNA Analyzer; Applied Biosystems, CA, USA) using the BigDye_Terminator v3.1 Cycle Squencing Kit (Applied Biosystems). Related sequence was identified by performing sequence database searches using BLAST. Sequence data for related species was retrieved from GenBank (Istianto *et al*., 2012).

Fermentation condition

A loopful of a selected strain was inoculated into a 500-mL Erlenmeyer flask containing 250 ml of YMB and incubated on a rotary shaker at 150 rpm, 30℃ for 7 days. After the incubation periode, the broth was filtered using Whatman No.1 filter paper.

Extraction of metabolites

The culture broth was extracted with ethyl acetate. 25 ml of the crude extracelullar extract was taken in tubes and 25 ml of respective solvent was added. Gentle mixing was done for 1 hour and the tube were spun at 5000 rpm for 15 minutes, ethyl acetate phase containing dissolved metabolites was collected and the solvent was evaporated using vacuum rotary evaporator.

Cytotoxicity Test in vitro with Methyl Thyazole Tetrazolium (MTT) Assay

The ethyl acetate extract fraction was tested in vitro for cytotoxicity againts cancer cells using HeLa cell line (cervix cancer cell line) using MTT assay based on the protocol from Cancer Chemoprevention Research Center, Gajah Mada University.

The MTT assay is commonly used to evaluate cell survival, based on the ability of viable cells to convert MTT, a soluble formazan precipitate, which quantified by spectrophotometry. HeLa cells was treated by each ethyl acetate crude extract with several concentration (1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, and 62,5 µg/ml) in 96-well tissue culture dishes and incubated with MTT (5 mg mL^{-1}) for 4 h. HeLa cells not treated by the extract was used as a control. The amount of MTT dye reduction was calculated based on its absorbance at 570 nm. The LC50 value was calculated by probit analysis.

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RESULT AND DISCUSSION

Isolation of Actinomycetes

Two Actinomycetes were isolated from soil. Isolation of Actinomycetes were performed based on observation on macroscopic and microscopic level. The Actinomycete isolates show different morphological characteristic (Tabel 1 and pig 1). The isolated strains were Gram +, non motile and aerobic bacteria. The isolates have septate mycelium. The aerial mycelium appears irregularly, black in color. Nonmotile spores are borne singly.

Tabel 1. The colony characteristic of Actinomycetes on ISP2 medium

Isolate code	Size (mm)	AM	SM	DP	Gram
LP	2-4	Black	Yellow	-	Positif
RP	2-4	Black	Orange	-	Positif

AM aerial mycelium; SM substrate mycelium; DP diffusible pigment

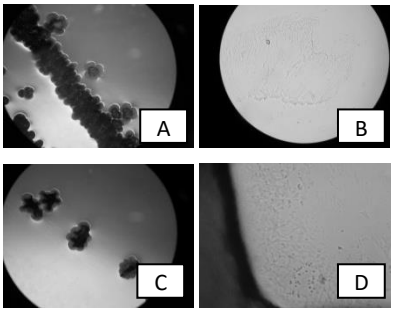

Figure 1. Microscopic characteristic of LP and RP (A. Colony morphology of LP; B. Spore characteristic of LP; C. Colony morphology of RP; D. Spore characteristic of RP)

PCR Amplification and Identification of Actinomycetes

The 16S rRNA regions were amplified using the by 9F and 1541R primer. PCR products showed single visible band about 1500 bp on agarose gel electrophoresis (Fig 2). Homology value of the LP and RP isolates based on BLAST search using 16S rRNA gene sequence data as query showed that was 99%, respectively, as *Micromonospora chersina* and *Micronomospora aurantica*

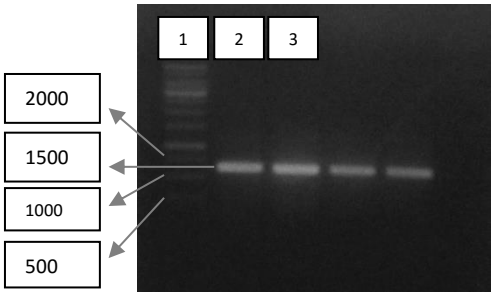

Figure 2. PCR amplification of sequence DNA by 9F and 1541R primer (Lane 1: ladder, Lane 2 and 3: LP and RP)

Cytotoxicity Assay in vitro with MTT Method on HeLa Cells

Cytotoxicity assay of ethyl acetate extract was carried out at several concentration (1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, and 62,5 µg/ml) by MTT viability test toward HeLa cell (cervix cancer cell). The extract showed growth inhibition againts HeLa cells. The LC_{50} values of the crude extract were determined using probit analisis (MINITAB 16) and were found 260 µg/ml for LP and 320 for RP, in comparison to untreated controls cells. According to Gerand *et al.* (1972) in Ampasavate *et al*. (2010), based on National Cancer Institute standard, crude extracts possessing an IC_{50} less than 20 µg/ml are considered active against the tested cancer cells. In this

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study, the LC_{50} is higher. This result show that the crude extracts of LP and RP were not selective against HeLa cell, but it still possibility posses selectivity againts another cell line.

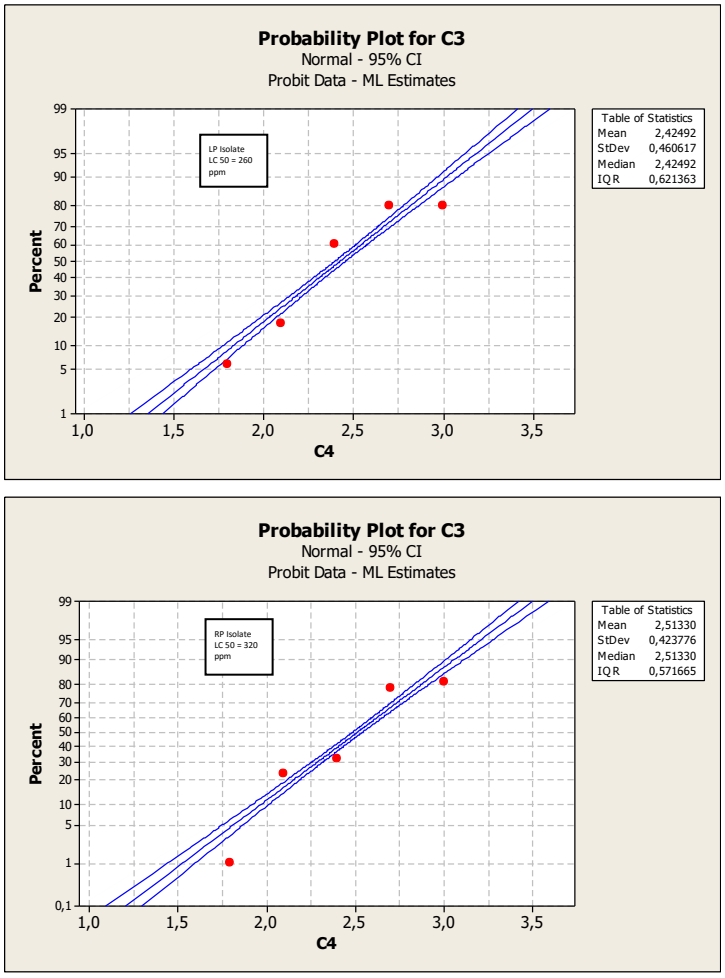

Figure 3. LC50 graphic plot using probit analisis

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Distribution of Higher Termites (Isoptera: Termitidae) in Bandung City

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Termite surveys of 30 districts in Bandung City yielded three species of higher termites (family Termitidae) i.e. *Ancistrotermes pakistanicus* Ahmad, *Macrotermes gilvus* Hagen, and *Odontotermes javanicus* Holmgren. Termite *A. pakistanicus* was found in Mandalajati only; whereas *O. javanicus* was noticed in two districts i.e. Cibiru and Bandung Wetan. *M. gilvus* was reported in 19 of 30 districts in Bandung City and this species is one of the most important destructive insect pest in urban environment. Soldiers of *A. pakistanicus* and *O. javanicus* were found monomorphic, while *M. gilvus* dimorphic. Observation on enteric valve armatures of worker castes of those species showed that they have six sides spiny and shaped in symmetry.

Keywords: distribution, Termitidae, Bandung City, enteric valve armature

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Phytochemical Screening and Cytotoxic Activities of Brown Algae *Sargassum duplicatum* and *Turbinaria ornata*

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Sargassum duplicatum and *Turbinaria ornata* as the marine bioactive compounds resources that used for antibacterial, antioxidant, anticancer etc, without scientific validation of its bioactivity and toxicity. The aims of this research were to determine the phytochemical screening and their cytotoxic activities from marine brown algae *S. duplicatum* and *T. ornata* with various extracts using different solvent (ethyl acetat and 96% ethanol). Phytochemical screening was carried out by Harborne methods, while the cytotoxic activities using Brine Shrimp Lethality Test (BSLT). The extract of *S. duplicatum* showed the presence of alkaloids, triterpenoids, steroids, saponins, phenolics, flavonoids and quinons. The extract of *T. ornata* showed the presence of alkaloids, triterpenoids, steroids, phenolics, flavonoids and quinons. The LC_{50} values of the extracts were determined by probit analysis method. The result of this research showed that all of the extract from those species are active in BSLT, indicated by LC_{50} values was less than 1000 mg/l. The extract of *T. ornata* that dissolved in 96% ethanol is the most active extract and more potent than the others extracts with LC_{50} values was 58,31 mg/l.

Keyword : phytochemical, BSLT, LC_{50}, *Sargassum duplicatum* and *Turbinaria ornata*

INTRODUCTION

Algae are rich source of biologically active metabolites, especially secondary metabolites with various chemical structures and properties (phytochemical). This phytochemical has been used as a traditional medicine and provide new pharmaceutical compounds.

On other hand, only a small species of brown algae has been reported of its bioactivity and toxicity. La Barre et al. (2010) adds that several species of brown algae play an important role in health, microbiology, enzymology and ecotoxicology.

The brine shrimp lethality test (BSLT) was considered for preliminary assessment of toxicity from plant extracts (Krishnaraju et al., 2005; Tamat et al., 2007). BSLT developed by Meyer et al. (1982) is general bioassay which has been developed for screening, fractionation and monitoring of physiologically active natural products, simple, easy to do, inexpensive, fast and requires few extracts. BSLT method can be followed up with other bioassay methods, that are more complex and expensive once the active compound was isolated (Pisutthanan et al., 2004).

A number of previous studies have demonstrated the phytochemical and cytotoxic activity of *Sargassum* and *Turbinaria* from different geography. In Indonesia, Teluk Awur, Jepara has abundant resource of algae, especially brown algae *Sargassum* and *Turbinaria* with various biological activities. In light of this, the present study was designed to determine the phytochemical screening and their cytotoxic activities of *S. duplicatum* and *T. ornata* with various extracts using different solvent (ethyl acetat and 96% ethanol).

MATERIALS & METHODS

Chemicals and reagents

Chemicals and reagents used in this experiment : n-hexane, ethyl acetate, 96% ethanol, distilled water, H_2SO_4, dragendorff reagents, meyer reagents, chloroform, anhydrate acetate, $FeCl_3$, HCl, sodium hydroxide, magnesium, *Artemia salina* and seawater.

Plant materials

The brown algae, *S. duplicatum* and *T. ornata* were collected from the intertidal region of Teluk Awur, Jepara, Central Java, Indonesia. Algae samples were cleaned of epiphytes and extraneous materials were removed with seawater and fresh water. The samples were transported to the laboratory in sterile polythene bags at 20° C temperature. The samples were dried for ten days and ground in an electric mixer and obtained the dried algae powder.

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Preparation of algae extracts

Three type of solvents with different polarity were used in this experiment : n-hexane, ethyl acetate and 96% ethanol. The procedures of extraction were conducted according to the Santoso *et al*. (2012) with modification. The dried powder of *S. duplicatum* and *T. ornata* (125 g) were extracted for 24 hours in 500 mL of each solvent using automatic shaking for maseration process at room temperature. Then the extraction was filtered by using Whatman no. 42 filter paper. Each filtrate was concentrated to dryness at 40° C temperature using rotary evaporator to perform paste. Each crude extract was kept at -20° C until ready to analysis. Crude extract of ethyl acetate and 96% ethanol from *S. duplicatum* and *T. ornata* were used for further analysis.

Phytochemical screening

Phytochemical screening of the crude extract of *S. duplicatum* and *T. ornata* were carried out using standard phytochemical methods described by Harborne (1987).

Brine Shrimp Lethality Test (BSLT)

The assay was carried out according to Meyer et al. (1982), McLaughlin and Rogers (1998) and Krishnaraju et al. (2005), with slight modifications. Brine shrimp eggs (*Artemia salina*) were hatched in sterile seawater under aeration and illumination at room temperature for 48h. After 48h, active nauplii *A. salina* was ready used for BSLT.

Ten nauplii were transferred with Pasteur pipette and placed in each vial which containing 4 ml of brine solution. Samples for testing were prepared by initially dissolving 50 mg of crude extract in organic solvent (96% ethanol) up to 50 µl per 5 ml of sea water and further diluted with sea water to produce the required concentrations.

In each experiment, 1 ml of the sample extract solution of *S. dupicatum* and *T. ornata* were added to 4 ml of brine solution and incubated at room temperature for 24 h under the light and the died nauplii were counted. Different concentrations were used in this test (0,63; 3,98; 25;11, 158,43 and 999,63 mg/l) and control and for each concentration were done in triplicate. The percentage mortality of each concentration and control was determined using the equation :

$$\%\ \text{mortality} = \frac{\text{no. of dead nauplii}}{\text{no. of nauplii in test}} \text{ x } 100\ \%$$

Probit analysis was used to determine the concentration at which lethality to brine shrimp represents 50% (LC_{50}).

RESULTS AND DISCUSSION

Phytochemical screening

The results of phytochemical screening of various extracts from *S. duplicatum* and *T. ornata* showed varied degree of bioactive compounds (Table 1). Phenolic compunds, flavonoids, quinones and steroids were present in all of the crude extract. The bioactive compounds of those species were influenced by different solvent.

Table 1. Phytocemical Screening of *S. duplicatum* and *T. ornat*

Bioactive Compounds	*S. duplicatum*		*T. ornata*	
	Ethyl acetate	96% Ethanol	Ethyl acetate	96% Ethanol
Phenolic compounds	+	+	+	+
Flavonoids	+	+	+	+
Quinons	+	+	+	+
Steroids	+	+	+	+
Alkaloids	+	+	-	+
Triterpenoids	-	-	-	+
Saponins	+	+	-	-

Semi-polar solvent (ethyl acetate) were able to extract phenolic compounds, terpenoids, alkaloids, aglycones and glycosides. Polar solvent (ethanol) were able to extract alkaloids, phenolic compounds, carotenoids, tannins, sugars, amino acids and glycosides (Harborne, 1987). Phenolic compunds, flavonoids and quinones are often found in the form of glycosides, present in

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the cell vacuole and easily soluble in polar compounds (ethanol), as presented by Harborne (1987), Lenny (2006), Sirait (2007) and also Hayati and Halimah (2010).

Phenolic compounds showed as antioxidant activity, antitumor, antiviral and antibiotic (Harborne, 1987; Apak et al., 2007). Flavonoids have potential as antioxidant (Bhat et al., 2009), antitumor and anticancer (Ren et al., 2003), anti-inflammatory, antiviral, lipase inhibitors and hormones (Lee et al., 2007). Quinone has been used as an anticancer and antioxidant (Bolton et al., 2000).

Alkaloids, triterpenoids and saponins were only detected in some extracts of *S. duplicatum* and *T. ornata*. Alkaloids can only be formed from a few amino acids, such as ornithine, lysine, phenylalanine, tyrosine and tryptophan (Lenny, 2006). Alkaloids were not detected in ethyl acetate extract of *T. ornata*, indicate that ethyl acetate extract of *T. ornata* not has these amino acids.

The formation of triterpenoids and steroids needed of cholesterol as precursors that are non-polar (Harborne, 1987). However, triterpenoids are also found in ethanol extract of *T. ornata.* This is confirmed by Hayati and Halimah (2010), that triterpenoids compounds which have -OH groups is polar, so it can be extracted in ethanol solvent (polar).

Alkaloids compounds have potential as an antioxidant (Rao et al., 2011). Alkaloids, flavonoids and saponins have a function as source of lipase inhibitor (Ruiz et al., 2005). Triterpenoids are known have a bitter taste which protects from insect and microbial attack (Harborne, 1987), plays a role in the treatment of breast cancer (Bishayee et al., 2011) and melanoma (Sarek et al., 2012). Saponins are used an antimicrobial, anticancer, antifungal and antiviral (Francis et al., 2002; Singh et al., 2003). However, at high concentrations, it can lead cells damage and induce apoptosis (Singh et al., 2003).

The results obtained were in line with Rachmat (1999), Senthilkumar and Sudha (2012) and also Jeyabalan and Marimuthu (2012). Rachmat (1999) reported that alkaloids, steroids and phenolics compound were present in *Sargassum* sp. *Turbinaria sp.* (*T. conoides*) contains alkaloids, flavonoids, steroids, terpenoids and tannins (Senthilkumar and Sudha, 2012). Jeyabalan and Marimuthu (2012) adds that the extract of *T. ornata* and *Sargassum* sp. (*S. myriocystum*) from Tamil Nadu, India contains steroids, alkaloids, phenols, flavonoids, saponins and tannins.

Brine Shrimp Lethality Test (BSLT)

The results of BSLT on crude extract of *S. duplicatum* & *T. ornata* are shown in Table 2. The percentage mortality increased with an increase in concentration. The extract from those species are active in BSLT, indicated by LC_{50} values was less than 1000 mg/l.

Table 2. BSLT of *S. duplicatum* & *T. ornata*

Species	Solvent	% Mortality in Different Concentration (mg/l)					LC50
		0.631	3.98	25.11	158.43	999.63	mg/l
S. duplicatum	Ethyl acetate	13.33	16.67	26.67	40	90	98.81
S. duplicatum	96% Ethanol	16.67	23.33	33.33	50	86.67	62.87
T. ornata	Ethyl acetate	13.33	16.67	20	26.67	86.67	193.86
T. ornata	96% Ethanol	13.33	23.33	30	53.33	90	58.31

Brine Shrimp Lethality Test (BSLT) used nauplius *A. salina* which was 48 hours. At these stadia, nauplius *A. salina* has had a respiratory and complete digestion (Albutana et al., 2011). The mortality of nauplius *A. salina* induced by extract of *S. duplicatum* and *T. ornata*, which diffuses into the body of *A. salina* through the gills. This condition will disturb homeostasis and enzymatic metabolism of *A. salina*, such as respiration and osmoregulation.

The extract of *T. ornata* that dissolved in 96% ethanol is the most active extract and more potent than the others extracts with LC_{50} values was 58,31 mg/l. The variation in BSLT results may be due to the different bioactive compounds which have cytotoxic substances (i.e flavonoid, triterpenoid and saponins). Extract of *T. ornata* that dissolved in 96% ethanol is the most active extract and presence of phenolic compounds, flavonoids, quinons, steroids, alkaloids and triterpenoids.

These bioactive compounds as antifedant. When these compounds entry into the body of *A. salina*, it will inhibit the sensory receptors in his mouth. So, the *A. salina* was unable to identify his food (Rita et al., 2008).

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Cytotoxic property of plant material is due to the presence of antitumor compounds. Many of the secondary metabolites produced by the marine brown algae are well known for their cytotoxic property. As noted by Rafaela et al. (2006) and Fajarningsih et al. (2008).

BSLT results may be used to guide the researchers to prioritize for further fractionation and isolation of these bioactive compounds. Other cytotoxicity tests and specific bioassays may be done on the isolated bioactive compounds (Peteros and Uy, 2010). This bioassay has good correlation with cytotoxic activity in some human solid tumors, and has led to the discovery of novel natural active antitumor and anticancer agents (McLaughlin *et al.*, 1998).

CONCLUSION

In conclusion, the result of the present study showed that *S. duplicatum* and *T. ornata* potential as producer of cytotoxic secondary metabolites. Therefore, these spesies might be utilized for development a novel antitumor and anticancer agents. Thus, the mechanism and isolation of these bioactive compounds is needed for further studies.

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Study of Mangrove Forest Community Structure, Carbon Stocks Estimated, and The Human Behavior Around The Mangrove Forest at Segara Anakan Cilacap

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Mangrove forest can reduce CO_2 concentration at atmosphere through photosynthesis process and saved on their biomass. Unfortunately, this process was disturbed by human activities such as illegal logging; land use change; and installing agriculture and ponds land. This study aims to compare the community structures of mangrove forest, carbon stocks estimated, and the human behavior between Center East (CE) and Center West (CW) at the Segara Anakan mangrove forest, and its relation among them. This study was used survey method. Purposive sampling was used to decide sampling stations. The plot sampling was used to get the data of mangrove vegetations. To get social data, purposive sampling and snow ball method were used. The results show that 12 mangrove species were found (10 species in CE area and 8 species in CW area). The conditions of community structure in CE area (10 species of mangrove, 478 individuals, distribution pattern is random) was better than the conditions of community structure in CW area (8 species of mangrove, 120 individuals, distribution pattern is random). Similarly, the amount of carbon stocks in CE area (tree = 5.8742 tons/ha, sapling = 14.2887 tons/ha) larger than in CW area (tree = 0.3844 tons/ha, sapling = 4.9050 tons/ha). Human behavior around the CE area (60% good, 40% bad) is better than in the CW (40% good, 60% bad). In general, the good human behavior around the CE area tends to produce a good mangrove forest community structure, so that the carbon stocks tend to be higher than in the CW area.

Keywords: *Mangrove, Carbon Stocks, Human Behavior, Segara Anakan*

INTRODUCTION

Global warming is a condition of increasing of Green House Gases such as CO2, CH4, NOx, SOx, and CFC at the atmosphere (Sutamihardja and Mulyani, 2011). CO2 gas is one of green house gas that increase quite high, approximately 30 ppm during 17 years only which was never occurred before (Sutamihardja, 2009). The concentration of CO2 in the atmosphere can be reduced due to the role of mangrove forests through the process of photosynthesis. CO2 gas is absorbed by mangrove plants through photosynthesis then stored in the form of biomass. Mangroves are known to be higher than phytoplankton in reducing the concentration of CO2 per unit of area in the ocean with the same area (Kathiresan and Bingham, 2001).

One of the mangrove forests in the southern island of Java is the Segara Anakan mangrove forest, Cilacap. Segara Anakan mangrove forest conditions based on several studies showed such as a reduction in area (Ardli and Wolff, 2009; Sastranegara, 2004), cause by human activities such as illegal logging (Yowono et al., 2007; Sastranegara *et al*., 2007), land used change into ponds (Sastranegara, 2004), and installing agricultural areas (Ardli, 2007). Sapuregel area (Center East/CE) and Montean (Center West/CW) are two areas of the mangrove forest in Segara Anakan that begin to be degraded due to illegal logging (Pribadi, 2007; Hinrichs *et al*., 2009). This condition is relating to the community structure of mangrove forest in both areas. The community structure of mangrove forest influence the biomass of mangrove forest, where the amount of mangrove biomass will influence the amount of carbon stocks in mangroves.

Many studies on the community structure of mangrove forest have been published, but similar study that associated with carbon stocks and the human behavior around mangrove forest never been published. Therefore, this study aims to compare the community structure of mangrove forests, carbon stocks estimated, and the human behavior between Center East (CE) and Center West (CW) at the Segara Anakan mangrove forest, and its relation among them.

MATERIALS AND METHODS

The object studied was mangrove forest and human communities around in Segara Anakan Cilacap. Equipments are GPS (Global Positioning System); boat; the meter 1.5 m and roller meter 100 m; ice box; soil tester; thermometer; salt-refractometer; mangrove identification book (Kitamura *et al*., 1997 and Giesen *et al*., 2006), as well as questionnaires and writing materials.

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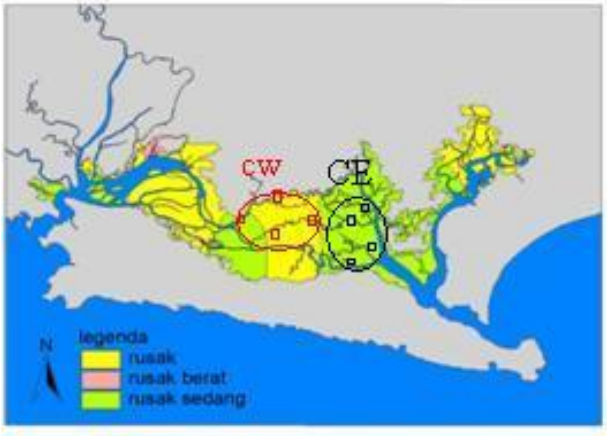

Figure1. Map of Research Study at Segara Anakan Cilacap (Ardli et al., 2010).

The method used in this study is survey method. Sampling location determination using purposive sampling method, data collection mangroves used Plot-Sampling method (Mueller-Dumbois and Ellenberg, 1974), the human behavior data collection using purposive sampling method and Snow Ball method. This study was conducted for 6 months.

The community structure of mangrove forest analysis using calculations total species, number of individuals and distribution patterns. Carbon stocks mangrove forests analyzed with allometric equation to determine biomass of mangroves and used the calculation formula of carbon (carbon content (C) = Biomass (B) x0, 46) (Kauffman and Donato, 2012). Descriptive analysis was used to analyze the human behavior data.

RESULT AND DISCUSSION

The Mangrove Forest Community Structure

The results showed that there were 12 species of mangrove found in the location of the study (10 species in CE area and 8 species in CW area). Pribadi (2007) found 26 species of mangrove in Segara Anakan, while Hinrichs *et al*. (2009) found 21 species of mangrove trees only and 5 species of understorey genera in Segara Anakan.

Mangrove trees in CE area based on the data of species richness, abundance, and density (3, 14, 116 ind/ha, respectively) is higher compared to the CW area (1, 1, 8 ind/ha). The same thing is also showed by the levels of sapling, in the CE area based on the data of species richness, abundance, and density (10, 442, 3023 ind/ha, respectively) is higher than in CW area (8, 109, 909 ind/ha). The level of seedling in CE area based on the data of species richness and abundance (3, 22 respectively) is higher than in CW area (4, 10). As for the understorey show different things, understorey in CE area based on the data of species richness and abundance (2, 28 respectively) is lower than in CW area (3, 111).

The Distribution pattern at sapling level in both areas showed a random pattern except for two species in the CE area, *Rhizophora apiculata* and *Aegiceras corniculatum* showing aggregate distribution pattern. This is thought due to two species are available and have not the main target to be used as firewood by the local peoples around at the CE area. Pribadi (2007) reported that in Sapuregel area (CE) the sapling level dominated by *Rhizophora apiculata, Aegiceras corniculatum, Scyphiphora hydropyllacea* and *Aegiceras floridum.*

Carbon Stocks Estimated

The amount of carbon stocks of mangroves tree in the CE area (5.8742 tons/ha) were significantly higher than in the CW area (0.8357 tons/ha). The amount of carbon stocks in each area is strongly influenced by the biomass of mangrove plant. The higher biomass in the CE area (12.7701 tons/ha) compared to the CW area (0.08357 tons/ha) was produced higher carbon stocks too. Similarly, in level of sapling, the mangrove biomass in the CE area (30.2897 tons/ha) were significantly higher than in the CW area (10.676 tons/ha). The same thing showed by carbon stocks at sapling level. Carbon stocks in the CE area (14.2887 tons/ha) is three times higher than in the CW area (4.9050 tons/ha). Haririah and Rahayu (2007) reported that the higher biomass of a plant can produce higher carbon stocks.

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Table1. Mangrove Species were found in Segara Anakan Cilacap

No.	Species	CE Area			CW Area		
		Abundance	Density (ind/ha)	Distribution Pattern	Abundance	Density (ind/ha)	Distribution Pattern
							Tree
1	*Avicennia alba*	1		Random	0	0	-
2	*Aegiceras corniculatum*	12	100	Random	0	0	-
3	*Ceriops decandra*	1	8	Random	1	8	Random
	Total	14	116	-	1	8	-
	Total Species in CE area (3) and CW area (1)						
				Sapling			
1	*Avicennia alba*	3	25	Random	4	33	Random
2	*Avicennia marina*	1	8	Random	20	167	Random
3	*Sonneratia caseolaris*	2	17	Random	0	0	-
4	*Sonneratia alba*	0	0	-	2	17	Random
5	*Rhizophora apiculata*	131	597	Aggregate	36	300	Random
6	*Rhizophora mucronata*	0	0	-	14	117	Random
7	*Bruguiera gymnorizha*	8	67	Random	0	0	-
8	*Aegiceras corniculatum*	204	1700	Aggregate	15	125	Random
9	*Ceriops tagal*	43	358	Random	0	0	-
10	*Ceriops decandra*	7	59	Random	1	8	Random
11	*Xylocarpus granatum*	2	17	Random	0	0	-
12	*Nypha fruticans*	21	175	Random	17	142	Random
	Total	442	3023		109	909	
	Total Species in CE area (10) and CW area (8)						
				Seedling			
1	*Aegiceras corniculatum*	5		Random	2		Random
2	*Ceriops sp.*	8		Random	2		Random
3	*Nypha fructicans*	0		-	2		Random
4	*Rhizophora apiculata*	9		Random	4		Random
	Total	22			10		
	Total Species in CE area (3) and CW area (4)						
				Understorey			
1	*Acanthus ilicifolius*	15		Random	26		Random
2	*Acanthus ebracteatus*	0		-	48		Random
3	*Derris trifoliata*	13		Random	38		Random
	Total	28			111		
	Total Species in CE area (2) and CW area (3)						

Aegiceras corniculatum have the highest biomass and carbon stocks in the CE area at tree level (7.8472 tons/ha; 3.6097 tons/ha) and sapling level (20.8939 tons/ha; 9.6112 tons/ha), while in the CW area, *Rhizophora apiculata* had the highest biomass and carbon stocks (6.0569 tons/ha; 2.7801 tons/ha) at sapling level compared with other species. The high carbon stocks in mangrove species *Aegiceras corniculatum* is in CE area because has the highest of the number and density (204 individuals, 1700 ind/ha) compared to other mangrove species. Similarly, *Rhizophora apiculata* (36 individuals, 300 ind/ha) in the CW area has the highest carbon stocks compared to other mangrove species. Pribadi (2007) reported that around the CE area is dominated by *Aegiceras corniculatum* and *Rhizophora apiculata*.

The Human Behavior around the Mangrove Forest

Human behavior around the mangrove forests Segara Anakan, Cilacap (CE and CW areas) indicate a difference. Human behavior in the CE area (60% good, 40% bad) is still considered better than the human behavior in the CW area (40% good, 60% bad). The human knowledge in the CE area (80% good, 20% quite good) is better than the human knowledge in the CW area (40% good, 60% quite good), as well as by the attitude shown by the people of the two areas (CE: 60% positive, 40% negative; CW: 20% positive, 80% negative). Human behavior is closely related to knowledge. Then this is will affect the attitudes and generate specific action. Good knowledge will result in a positive attitude and then manifest in a good action, and vice versa.

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Based on interviews with local people, mangrove species most commonly harvested is *Rhizophora* sp. (bakau), but local people also begin cutting down mangrove species *Aegiceras corniculatum* (Gedangan). This is because species *Aegiceras corniculatum* is still available, while species *Rhizophora* sp. is less. Sastranegara *et al*. (2007) reported that *Rhizophora apiculata*, *Rhizophora mucronata* and *Bruguiera gymnorhiza* was cut by local people. *Rhizophora apiculata* and *Rhizophora mucronata* tend to use for firewood and charcoal (Sastranegara *et al*., 2007), whereas *Aegiceras corniculatum* used for firewood only.

In CE area, mangrove wood used as firewood in the process of making palm sugar, whereas in the CW used as firewood in the process of cooking shellfish in a large number. Mangrove wood cut with a machetes and then transported by small boats. Mangrove wood then sold at a price range of Rp.50.000-Rp.80.000 per boat (2-3 cubic). Number of small boats used to bring mangrove wood per day increased from 5-8 in 2005 (Sastranegara and Marhaeni, 2005) to 6-14 per day in 2006 (Sastranegara *et al*., 2007).

Figure 2. Mangrove wood cut by local people and used for firewood

The livelihoods of local people around the CE area are fishermen, farmers, Indonesian workers abroad (TKI) and partly as a maker of palm sugar, while people's livelihoods around the CW area is fishermen. However, when income from the catch of fish or shrimp are no longer able, that's making them look for additional income and one of them is by cut the mangrove trees for sale or personal use for firewood. Reichel *et al*. (2009) reported that the loss of the main income from fishing making the fishermen seek other income by illegal logging of mangrove forests to be sold to nearby industries.

The Relationship among the Mangrove Forest Community Structures, Carbon Stocks Estimated, and the Human Behavior around the Mangrove Forest at Segara Anakan, Cilacap

The human behavior influences the condition of mangrove forest community structure, then affects to the amount of carbon stocks in mangrove forest. The good human behavior around the CE area (60% good, 40% bad) shows the condition of the community structure of mangrove forests in the CE area are still good and high enough carbon stocks (tree = 5.8742 tons/ha, sapling = 14.2887 tons/ha). While the human behavior around the CW area that is not good, (40% good, 60% bad) produce the less well condition of mangrove forest community structure and produce low carbon stocks also (tree = 0.3844 tons/ha, sapling = 4.9050 tons/ha).

CONCLUSION

The conditions of mangrove forest community structure based on the species richness, abundance, and distribution patterns in the CE area are still better than the conditions in the CW area. Amount of carbon stocks in the CE area (tree = 5.8742 tons/ha, sapling = 14.2887 tons/ha) higher than in the CW area (tree = 0.3844 tons/ha, sapling = 4.9050 tons/ha). Human behavior in the area around CE area (60% good, 40% bad) is better than in the CW area (40% good, 60% bad). In general, the good human behavior around the CE area, produce a good mangrove forest communities, so that storage of carbon stocks tend to be higher than the conditions in the CW area. Therefore, the CE area needs to be conserved, while the CW area needs to be restored.

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Phenomenon of Diversity and Preferency of Sialang Tree (Nest of *Apis dorsata* Fabr.) at Rokan Hulu and Rokan Hilir Regency (Riau Province)

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The Asiatic giant honeybees (*Apis dorsata* Fabr.) are abounded with the lowland rainforests in Riau province. The colonies of *A. dorsata* are found nesting in most tall bee trees (sialang trees; local name). In Riau province there are about 50 species of sialang tree. There are two type of land in Riau (peat land and mineral land) that might be directly/indirectly affected to distribution of sialang tree. The conversion of conservation forest over to palm oil and plantation forest industry that planted by *Acacia mangium* and *Acacia crassicarpa* was also one factor that probably giving the unique phenomenon of distribution of sialang tree. Determining the vegetation distribution and the number of Sialang trees, colony distribution, and *A. dorsata* density colonies at Rokan Hulu regency (0° 25’ 20’’ - 01° 25' 41’’N and 100° 02’ 56’’ - 100° 56’ 59’’ E) and Rokan Hilir regency (01° 14' – 02° 45' N and 100° 17' – 101° 21' E). The farmer behavior was also documented to compare the sustainable *A. dorsata* harvesting method (Purnomo, 2008). The correlation showed that all diversity parameter (except richness index) of sialang tree gave negative correlation to *A. dorsata* colony significantly, but showed the positive correlation to honey production significantly. The Rokan Hulu (Hs: 1.17) had highest diversity of sialang tree than in Rokan Hilir (Hs: 1.05). The honey productivity also showed that in Rokan Hulu (28.8 ton/year) had highest than in Rokan Hilir (8.27 ton/year). Mean while the species of sialang tree that is preferenced by *A. dorsata* were Kempas (*Koompassia malaccensis*) and Julang (*Afzelia javanica*) (average of 100 colony of *A. dorsata* in each sialang tree).

Key word: *Apis dorsata, sialang tree diversity, preferency,*

INTRODUCTION

The exploitation of forests, soils, rivers, lakes, and seas which are needed excessively and temporarily is not a wise action to make. Because, it is possible that the flora and fauna and the microorganism hosting those ecosystems can be used as human welfare (Kompas, may 22nd, 2013). One of the Indonesia diversity forms is found in Asiatic giant honeybee (*A. dorsata*) which represents fauna, and sialang tree which represents flora where the existences are more marginalized because of the rapid deforestasion.

Asiatic giant honeybee (*A. dorsata*) is the most productive honeybee producing honey which has the percentage of honey production nearly 60% of all honey produced in Indonesia (Ditjen RLPS, 2006). The characteristic of Asiatic giant honeybee hive is a hive with one stroke that hangs in a branch and a twig of a tree. The hive stroke can be measured until 2x1 meter with 20 kg honey production per hive. This species only develops in sub tropical and tropic Asia (around Pakistan to Indonesia) and can not be found outside of Asia. In Indonesia, it can be found in Sumatra, Kalimantan, Sulawesi, West Nusa Tenggara and East Nusa Tenggara (except Irian) (Starr *et al.*, 1987).

Sialang is a term for a big, tall tree which has diametre reached 100 cm or more, and the height can reach 25 to 30 meter and is hosted by *A. dorsata*. In Riau, it has at least 50 species of the biggest sialang trees which spread in peat and mineral soil. Sialang tree is a kind of plant which is protected by law, both government law and customary/ community law. It is intended to preserve those trees as the place which the group of bees produces honey as one of incomes of the people who lives near the forests (Mujethid, 2007).

Two unique regencies in Riau province experiencing the rapid change of natural forest is Rokan Hulu and Rokan Hilir. Based on the data of Riau provincial forestry office (2006), the rapid change of the nature forest was from the conversion of Plantation Forest Industry/PFI and palm tree plantations. The problem occured the reduction of numbers and diversity of sialang trees in that regency. Another problem was the type of soil that presence on the Rokan Hilir that tends to ombrogen/ peat land. This soil type is becoming the limiting factor for many sialang tree to exist.

The impact of natural forests deforestation being PFI is appeared to be a unique phenomenon. The tendency of *A. dorsata* colonies is more getting away to the forest boundary of HTI *Acacia crassicarpa, A. mangium,* and *Eucalyptus* sp. (Purnomo *et al.*, 2007). The similar tendency appeared in palm tree plantation that showed the existence of the colonies withdrawing

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from the forest boundary. This issue was related to the availability of food resources of honeybee *A. dorsata*, which extrafloral nectar is produced by the *Acacia* plant (Sihombing, 1997).

Therefore, the objectives of the study are (1) to determine the diversity level of sialang tree in Rokan Hulu and Rokan Hilir regency and (2) to identified the dominant sialang tree species in Rokan Hulu and Rokan Hilir regency.

DIVERSITY OF SIALANG TREE IN ROKAN HULU AND ROKAN HILIR REGENCY

A tree is called sialang tree if the tree is hosted by *A. dorsata*. Although the tree is with height about more than 30 meters, but if it is not hosted by *A. dorsata*, it will not be called sialang tree. The analysis result of sialang tree diversity showed that a district which had the highest diversity parameter (Shanon weiner and abudancy index) was at Rokan Hulu regency (Hs: 1.17 and N: 3.16) than in Rokan Hilir regency (Hs: 1.05 and N: 2.83). Analysis of sialang tree diversity parameter in Rokan Hulu showed that the sialang tree diversity located in the area that was located around boundary of natural forest (undisturbed forest) and concession areas of Plantation Forest Industry (PFI) which were planted with *Acacia mangium*. Mean while the diversity parameter in Rokan Hilir showed that the sialang tree diversity located in the village that was located around boundary of natural forest and along side of Rokan river (Figure 1)

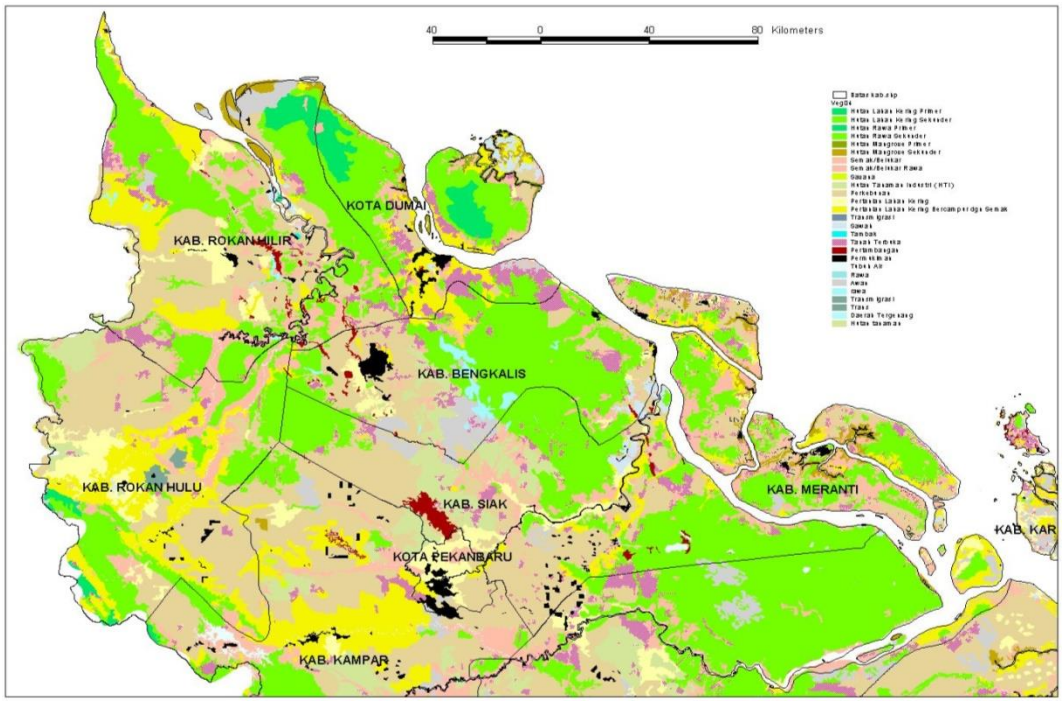

Figure 1. Land cover of Riau province in 2008

It is suspected that the limit factor which formed the fertility level of peat soil in Rokan Hilir affected the number and diversity of sialang tree which are adaptable in that area. Peat type that found in east coast of Sumatera is ombrogen. This peat soil possibly first appeared from the mangrove sediment soil which is then dried. This peat soil contains of high salt and sulfide, so only fewer decomposer organisms inhabit it. Research in Sarawak showed that peat started forming on mangrove mud about 4,500 years ago in the beginning with depth rate about 0.475 m/ 100 years (at 10-12 m depth of peat), later shrank to approximately 0.223 m/ 100 years at the depth of 0-5 m. Probably, the older the forest of peat soil, the fewer the availabilty of nutrients (Wikipedia, 2012). Therefore, it is thought that types of sialang trees growing much in rural forest area and river boundary are hard to grow in coastal area and only specific types that can tolerate to this boundary factor. The sialang tree that appeared in Rokan Hilir are existed as long as the Rokan river that is argued by the sedimentation of such mineral that deposited in the river side.

Based on the results of an inventaritation of the sialang trees, there are only 8 species of trees with a number of 30 trees in Rokan Hilir and dominated by Kempas (*Kompssia parvifalia*) and Arau. Whereas in Rokan Hulu had 12 types of sialang trees that the number of 515 and dominated by *Acacia mangium* and Benda. Interesting phenomenon is the existence of *Acacia*

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mangium as an invasive tree species and that used as the species for pulp and paper raw material in plantation forest industry especially in dry land. *A. mangium* had been introduced to Riau province for the last 20 years and became the most invasive species together with *A. crassicarpa*. This *Acacia* species had light seed which easy to spread by wind and adaptable to marginal land and the growth became massive. So, it is suspected that existence of *Acacia mangium* will give the positive effect to colony of *A. dorsata* but give negative impact to the local tree species.

The other assume is that the type of land that located in the Rokan Hilir (peat soil), especially areas adjacent to the sea become limiting factor for the spread of the sialang tree. Based on observations, vegetation in areas adjacent to the sea is dominated by coconut (*Cocos nucifera*). This type of tree has no branches to accommodate the *A. dorsata* to nest. In addition to the conversion of natural forests into palm oil plantations (Figure 1) also led to population *A. dorsata* is very low in this regency and this is indicated by low colony *A. dorsata* (Table 1). According to Liow et al. (2001) revealed that the proportion of stingless bees and honey bees (Hymenoptera: Apidae) was very low in oil palm plantation areas and very high in undisturbed areas, roomates implies that palm oil plantations are not suitable in terms of either fulfilling the preferences of honey bees or the ability to support them. Palm trees do not produce nectar and their dense leaves rendering them Unsuitable for nest building by *A. dorsata* (Oldroyd & Nanork, 2009).

Table 1. Sialang tree species in Rokan Hilir and Rokan Hulu

No.	Sialang tree species	Total tree that found	
		Rokan Hilir	Rokan Hulu
1	Arau	7	
2	Rengas (*Glupta aptera*)	2	
3	Kempas (*Kompssia parvifalia*)	8	2
4	Makeluang (*Heriteria tarrieta*)	2	6
5	Kayu Ara	3	4
6	Kayu Batu (*Homalium tomentosum*)	3	
7	Cempedak Air (*Artocarpus maingayi*)	3	
8	Beringin (*Ficus benjamina*)	2	9
9	Randu (*Ceiba petandra*)		89
10	Akasia (*Acacia mangium*)		253
11	Pinong (*Pencace* sp.)		4
12	Julang (*Afzelia javanica*)		4
13	Benda		121
14	Jati (*Tectona grandis*)		14
15	Durian (*Durio zibethinus*)		6
16	Sengon (*Parasienthes falcataria*)		3
	Total	30	515

PREFERENCY OF *A. dorsata* TO SIALANG TREE IN ROKAN HILIR AND ROKAN HULU

Based on the analysis, the preference level/ the fondness of honeybee *A. dorsata* to the sialang tree in Rokan Hilir representing the vegetation structure (coast, ombrogen peat soil and river flow area) showed that sialang tree type which is the best is arau (average of 25.72 colony per tree) and Rengas (*Glupta aptera*) (average of 32.5 colony per tree). Mean while in Rokan Hulu that representing red-yellow podzolic showed the highest of aggregation of *A. dorsata* were Kempas (*Kompssia parvifalia*) (average of 100 colony per tree) and Julang (*Afzelia javanica*) (average of 100 colony per tree) (Table 2).

Some factors influenced the high and low of colony preference of honeybee *A. dorsata* to sialang tree relatively are many horizontal branchings. The tall of tree reaching 27 m with branching fewer than 15 are not found vegetation/ another tree which is as big as the sialang tree, and branching that is far from plants of epifit and liana (Starr *et al.*, 1987) and located around the sustainable forest (Purnomo *et al.*, 2007). It can be seen that sialang tree located in the center of concession area of PFI was not inhabited by honeybee *A. dorsata* which was caused of the micro climate change (Purnomo *et al.*, 2007).

The honey productivity also showed that in Rokan Hulu (28.8 ton/year) had highest than in Rokan Hilir (8.27 ton/year). Honey productivity also showed positive correlation to diversity index

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of sialang tree (0.583). It means that the increasing of diversity of sialang tree will effect to increase of honey productivity that produced by *A. dorsata.* This is contrast to Pachepsky (2001) which stated that the increase of diversity will decrease the productivity level of a community, especially tropical area, the great diversity level has low productivity. While in subtropical area and temperate regions, even though it has low diversity level, the productivity level is high.

Table 2. Sialang tree species and average of *A. dorsata* colony aggregation in Rokan Hilir and Rokan Hulu

No.	Sialang Tree Species	Rokan Hilir		Rokan Hulu	
		Σ tree species	Σ colony	Σ tree species	Σ colony
1	Arau	7	180		
2	Rengas (*Glupta aptera*)	2	65		
3	Kempas (*Kompssia parvifalia*)	8	160	2	200
4	Makeluang (*Heriteria tarrieta*)	2	25	6	180
5	Kayu Ara	3	60	4	265
6	Kayu Batu (*Homalium tomentosum*)	3	50		
7	Cempedak Air (*Artocarpus maingayi*)	3	45		
8	Beringin (*Ficus benjamina*)	2	45	9	660
9	Randu (*Ceiba petandra*)			89	3402
10	Akasia (*Acacia mangium*)			253	1824
11	Pinong (*Pencace* sp.)			4	130
12	Julang (*Afzelia javanica*)			4	400
13	Benda			121	2895
14	Jati (*Tectona grandis*)			14	286
15	Durian (*Durio zibethinus*)			6	140
16	Sengon (*Parasienthes falcataria*)			3	30
	Total	30	630	515	10412

Observations indicate that the total number of *A. dorsata* colony in Rokan Hilir colony numbered only 630 in 2008, mean while in Rokan Hulu regency reached 10412 colonies. Development of honey bee colonies affected by various factors, one of which is the availability of food, especially pollen. According to Cale and Ruthenbuhler (1975), the bee population development is influenced by some factors, one of those is the ability of a queen bee to keep laying eggs. The ability of laying eggs is strongly influenced by the food (royal jelly) given from the worker bees to the queen bee, and to produce royal jelly, the bee colony needs pollen in sufficient amount. Royal jelly formed by the worker bees is also influenced by the existence of hypopharengeal gland which is located in the heads of worker bees. This gland needs nutrion such as protein, and in that way, the amount of pollen will impact to the development of bee colony.

CONCLUSION

1. The analysis result of sialang tree diversity showed that a district which had the highest diversity parameter (Shanon weiner and abudancy index) was at Rokan Hulu regency (Hs: 1.17 and N: 3.16) than in Rokan Hilir regency (Hs: 1.05 and N: 2.83). Analysis of sialang tree diversity parameter in Rokan Hulu showed that the sialang tree diversity located in the area that was located around boundary of natural forest (undisturbed forest) and concession areas of Plantation Forest Industry (PFI) which were planted with *Acacia mangium.*
2. Based on the results of an inventaritation of the sialang trees, there are only 8 species of trees with a number of 30 trees in Rokan Hilir and dominated by Kempas (*Kompssia parvifalia*) and Arau. Whereas in Rokan Hulu had 12 types of sialang trees that the number of 515 and dominated by *Acacia mangium* and Benda. Interesting phenomenon is the existence of *Acacia mangium* as an invasive tree species and that used as the species for pulp and paper raw material in plantation forest industry especially in dry land

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Phylogenetic Analysis of *Mangifera* Based on RBCL Sequences, Chloroplast DNA

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Genus of Mangifera has 69 species that are mostly distributed around Borneo, Sumatra, Java and Malay Peninsula. Phylogenetic study of the genus was conducted to investigate the ancestor trait and relationships among those species. Phylogenetic tree is constructed based on nucleotide variation in rbcL gene within 16 samples of Mangifera : 13 species from Indonesia and 3 species from Thailand. Two species were added from other genera as outgroups. Genomic DNA was extracted using CTAB protocol and amplified with rbcL primers. Sequencing result was analyzed using BLAST function on NCBI. Multiple sequence alignment from all samples of rbcL sequences are generated using Bioedit and ClustalX program. Subsequently, phylogenetic was constructed by using Maximum Parsimony method in PAUP* 4.0b10 software. The aligned rbcL comprise 905 characters and it has 72 characters of parsimony informative with consistency index (CI) 0,889 and retention index (RI) 0,962. Phylogeny generated four main groups. Group I consist of M. cochinchinensis and M. macrocarpa (Thailand); group II : M. indica M. cesia, M. aplanata and M. altisima ; group III : M. laurina, M. longipes, M. similis, and M. gedebe ; group IV : M. laurina (Thailand), M. foetida, M. caesia, Mangifera spp, and M. odorata. Phylogenetic analysis revealed that Mangifera is monophyletic. There is a diversification between M. laurina from Indonesia and Thailand, as well as M. macrocarpa. Phylogenetic analysis also provides information to support the assumption that M. odorata is a hybrid of M. indica and M. foetida, and strongly support the assumption that M. longipes is a synonim of M. laurina.

Keywords: *Mangifera*, *Phylogenetic*, rbcL.

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Anatomical Structures of Leaf Surface and Physiological Properties of *Synedrella Nodiflora* Related to Resistance Against Fomesafen

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Fomesafen is one of widely used herbicides to control *Synedrella nodiflora*, a broad-leaf weed species commonly found in several estates with potential of causing serious problems in crop productivity. After 30 years of use, however, resistance against this herbicide in waterhemp was reported. Here we present our description on anatomical structures of leaf surface and physiological properties of *S. nodiflora* associated with its resistance against Fomesafen. It is notified that anatomical structures of leaf surface in resistant *S. nodiflora*, particularly the number of trichomes and stomata at leaf lower surface, correlate significantly to Fomesafen absorption, while it is not the case in susceptible wildtype. In general, resistant *S. nodiflora* shows better plant vigor than it is in susceptible wildtype.

Key words : *Synedrella nodiflora*, Fomesafen, resistance

INTRODUCTION

Synedrella nodiflora is a broad-leaf weed species commonly found in several estates, e.g. coffee, cacao, tea and legume crop systems. It has potential of fast growing as one individual can produce up to 100 inflorescences, each of which may bear 30 fertile seeds without dormance. This large number of seeds enables sufficiently high variability resulting in high adaptability to environmental stress including herbicide exposure (Dwiati *et al*., 2003).

One herbicide recommended to control *S. nodiflora* is Fomesafen, which may be applied either pre- or post-emergently. This belongs to systemic selective group of herbicides inhibiting photosynthesis (Ross and Childs, 2000) and is commonly applied to control broad-leaf weeds in soybean (Dwiati *et al.*, 1997; Dwiati and Budisantoso, 2005) and peanut (Dwiati, 2013).

Fomesafen commercially formulated as Reflex was firstly released in 1970, but after 30 years of use, resistance in waterhemp *(Amaranthus tuberculatus)* in Kansas was reported (Anonymous, 2006). Santhakumar (2002) noticed that weed resistance against herbicides may be stimulated by those of long persistance in soil, while Dwiati *et al.* (2003) reported that Fomesafen has sufficiently long persistance in soil, i.e. four months, indicating high potential of giving rise to weed resistance. Nevertheless, *S. nodiflora* resistance against Fomesafen has only been slightly characterized as yet. Here we are reporting our study on anatomical structures of leaf surface and physiological description of *S. nodiflora* related to its resistance against Fomesafen.

MATERIALS AND METHODS

Seeds of both resistant and susceptible *S. nodiflora* were planted simultaneously until they were 50 days old with five to six leaves when Fomesafen, provided as Reflex by Syngenta, was applied foliarly at 50 mgL^{-1} and 100 mgL^{-1}. Two hours later when the susceptible weeds were still alive, data on anatomical structures of leaf surface and physiological properties were collected.

Data on anatomical structures of leaf surface consisted of number of trichomes per mm^2 leaf area, number of stomata per mm^2 leaf area and cuticle thickness. These were used to see whether a correlation between leaf surface structures and Fomesafen absorption occurs. On the hands, physiological features depicted as plant vigor including plant height, leaf weight, stem weight, root weight, leaf surface area and leaf area index (LAI) were also measured in relation to plant resistance against Fomasafen.

Second, fourth and sixth leaves were picked up two hours after herbicide application to assess the absorption process. These were then washed in ethanol 70% and analyzed for Fomesafen content. Meanwhile, the ethanol was evaporated from residual wash where n-hexan was subsequently added for Liquid Gas Chromatography analysis. Absorption was calculated as the subtraction of Fomesafen left in the residual wash from total amount applied at the respective concentration. Then, regression analysis of anatomical data on Fomesafen absorption was performed.

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RESULTS AND DISCUSSION

Foliar absorption is a considerably complicated process depending on leaf surface characteristics, physico-chemical properties of herbicide, herbicide concentration and environmental condition. Leaf surface with all structures existing both at lower and upper surfaces may affect herbicide absorption. Number of trichomes and stomata as well as cuticle thickness at both leaf surfaces have their respective role in the absorption process. In this case we proposed multiple regression model of equation

$$Y = \beta0 + \beta1X1 + \beta2X2 + \beta3X3 + \beta4X4 + \beta5X5 + \beta6X6 + E$$

to describe the individual factor contribution on Fomesafen absorption, where
X1: number of stomata at leaf upper surface
X2: number of trichomes at leaf upper surface
X3: cuticle thickness of leaf upper surface
X4: number of stomata at leaf lower surface
X5: number of trichomes at leaf lower surface
X6: cuticle thickness of leaf lower surface
Y: absorbed Fomesafen

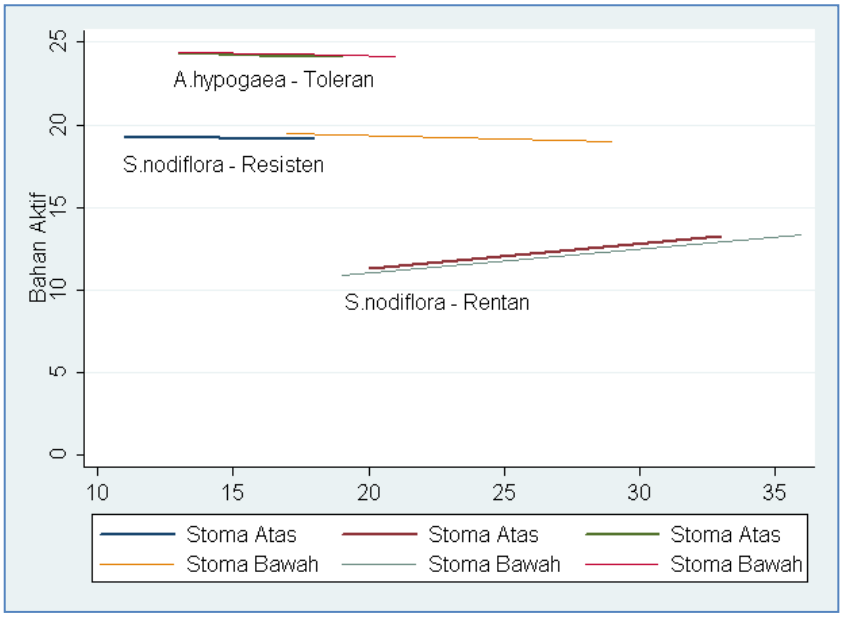

Figure1. Relationship between stomata number at both leaf surfaces and Fomesafen absorption

Table 1. The effect of plant type on plant height (cm), young leaf weight (g), mature leaf weight (g) and total leaf weight (g)

Plant type	Plant height (cm)	Young leaf weight (g)	Mature leaf weight (g)	Total leaf weight (g)
Susceptible *S. nodiflora*	12.800 b	0.286 b	2.113 b	2.946 b
Resistant *S. nodiflora*	21.300 a	0.953 a	4.106 a	6.226 a

Numbers followed by the same letters within column are not significantly different at level of confidence of 0.05.

Table 2. The effect of plant type on leaf area and LAI

Plant type	Leaf area (mm^2)	LAI
Susceptible *S. nodiflora*	294.667 b	0.650 b
Resistant *S. nodiflora*	622.667 a	1.375 a

Numbers followed by the same letters within column are not significantly different at level of confidence of 0.05

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Table 3. The effect of plant type on stem weight (g)

Plant type	Stem weight (g)
Susceptible *S. nodiflora*	1.206 b
Resistant *S. nodiflora*	4.240 a

Numbers followed by the same letters within column are not significantly different at level of confidence of 0.05

Table 4. Interaction between plant type and Fomesafen concentration on root weight (g)

Plant type	Control	Fomesafen of 50 mgL^{-1}	Fomesafen of 100 mgL^{-1}
Susceptible *S. nodiflora*	2.020 a	1.440 p	1.260 p
Resistant S. nodiflora	10.240 b	6.940 q	6.780 q

Numbers followed by the same letters within column and row are not significantly different at level of confidence of 0.05

Following analysis of variance and subsequent stepwise analysis the regression model proved to be acceptable in resistant *S. nodiflora*, particularly for number of trichomes at leaf lower surface ($Y = 11.556 - 0.373X5$ with $R^2 = 0.504$) and number of stomata at leaf lower surface ($Y = 11.556 - 0.373X5 + 0.094X4$ with $R^2 = 0.615$). This means that X5 inhibits Fomesafen absorption as much as 50.4%, while X4 increases it to 11.1% (61.5% - 50.4%). Conversely, all the six factors had no significant effect on Fomesafen absorption in susceptible *S. nodiflora*.

Trichomes are vigorous structures existing at both leaf surfaces, impeding herbicide active substance to attach at leaf surfaces after herbicide application. Even in case of *S. nodiflora* they can disperse herbicide because of their upright position to leaf surface with non glandular properties. In addition, Chachalis *et al.* (2001) noticed that trichome density plays important role in inhibiting herbicide absorption, since those with high density may result in the formation of air sacs under herbicide particles preventing their contact with leaf surfaces.

Number of stomata at both leaf upper and lower surfaces of resistant *S. nodiflora* appeared proportional to Fomesafen absorption (Figure 1 on separate sheet). This corresponds to Suh *et al.* (2005) that stoma is a leaf surface structure easily penetrated by herbicide, because there are no epicuticle crystals around it, but only a smooth epicuticle layer instead, facilitating herbicide to get through area around it. Wang and Liu (2007) notified that pesticide foliar absorption in broad bean proceeds in two pathways, i.e. apolar via cutin and wax and polar by crossing aqueous pores. The later locates around guard cell, glandular trichomes, and trichome basal area.

The more stomata at leaf lower surface of resistant *S. nodiflora*, the higher Fomesafen absorption (Figure 1 on separate sheet). The number of stomata at leaf lower surface of resistant *S. nodiflora* found higher than that at leaf upper surface.

Audus (1976) suggested that in order to penetrate leaf via stoma, herbicide must cross a barrier, which is a very complex process influenced by surface tension of herbicide, contact angle of herbicide and morphological and chemical structure of stoma hole wall. Penetration can occur only if surface tension of applied herbicide is equal or less than critical surface tension. Besides, herbicide must have contact angle of zero, meaning that complete wetting on leaf surface should occur.

In respect of physiological properties, ANOVA showed that plant type had significant effect on plant height. Further analysis revealed that significant difference between both plant types was found (Table 1 on separate sheet). Resistant *S. nodiflora* noticeably looks more defensive against herbicide stress than its susceptible wildtype does, where almost twice in height was clearly observed. Similar results seemed likely to be obtained in leaf weight where it was also significantly affected by plant type. Subsequent analysis indicated that resistant *S. nodiflora* has significantly more leaf weight, both in case of young and mature ones, than that of its susceptible wildtype (Table 1 on separate sheet). Either plant height or leaf weight implies that resistant biotype may develop better vigor as reflected by higher values of both. This is in accordance with Duff (2007) noticing that resistant *Amaranthus rudis* is more vigorous than its susceptible wildtype is. At seven to 28 days old resistant *A. rudis* has more leaf weight than that of susceptible wildtype. Nevertheless, susceptible *A. rudis* has more trunks, so that nearly equal total weight as that of resistant biotype was observed.

Both improved plant height and leaf weight of resistant *S. nodiflora* should lead to higher capacity of photosynthesis, so that the weeds could compete favorably with the main crops. Although the respective leaf weight at 42 days old was still low, the resistant weeds might increase

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their leaf number. This is truely the case as the leaf area of resistant *S. nodiflora* was as twice as that of its susceptible wildtype (Table 2 on separate sheet), which corresponds to Duff (2007) reporting that leaf area of resistant *A. rudis* at 20 days old was three times higher than that of its susceptible wildtype, while that of resistant *A. rudis* at 30 days old was twice higher than that of its susceptible wildtype.

It seems likely that higher leaf weight followed by higher total weight in resistant *S. nodiflora* resulted in not only better plant vigor but also preferable phylotaxis. This could be seen from its LAI, which was as about twice as that of susceptible wildtype (Table 2 on separate sheet). The higher LAI of resistant biotype individuals enables them to predominate and compete better with the main crops.

Application of Fomesafen on resistant *S. nodiflora* leaves can affect their form in that the leaves may fold up, particularly when they were still young. As the leaves are getting mature and older the folds reduce. This represents the symptoms attributable to application of PPOase inhibitor like Fomesafen (Shoup and Al-Khatib, 2005; Falk *et al.*, 2006). Lermontova and Grimm (2000) denoted that young and developing leaves serve as active sites of PPOase inhibitor, while old leaves are more tolerant to this types of herbicides.

Plant type had significant effect on stem weight, where further analysis proved that resistant *S. nodiflora* had stem weight of nearly four times in compare to that of susceptible wildtype (Table 3 on separate sheet). This obviously contributes to better plant vigor as well.

In various concentrations of Fomesafen plant type showed significant effect on root weight. In this case resistant *S. nodiflora* has root weight of about five times as that of its susceptible wildtype (Table 4 on separate sheet). Accordingly, this implies that resistant *S. nodiflora* has potential of better competition with main crops, since they exploit more soil than the susceptible wildtype could do. The latter showed root weight of only 2.020 g or five times less than the resistant biotype had both in the absence and presence of Fomesafen application.

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Characterization of *Synedrella nodiflora* (L.) Gaertn. Resistance against Fomesafen using PPX2L Partial Gene as Molecular Marker

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Fomesafen inhibits protox, an enzyme playing important role in chlorophyl biosynthesis. Deletion of three bases at gene encoding protox, i.e. *PPX2L*, was reported as one of resistance mechanisms against protox inhibiting herbicides in waterhemp *(Amaranthus tuberculatus)*. Nevertheless, only a few studies on molecular characterization of *Synedrella nodiflora* resistance against Fomesafen were reported. Therefore, a homology study on the sequences of *PPX2L* partial genes isolated from both resistant and susceptible *S. nodiflora* against Fomesafen was carried out. Unfortunately, the PCR products of resistant *S. nodiflora* remained unclear in spite of the optimation on annealing temperature. On the other hands, the PCR products of susceptible *S. nodiflora* showed three bands, in that of 500 bp was strongly assumed as *PPX2L* partial gene. Susceptible *S. nodiflora* was found genetically different from susceptible *A. tuberculatus*, indicated by the absence of three base pairs at position 834, 835 and 836 in susceptible *S. nodiflora*, where in susceptible *A. tuberculatus* this position was CAG. Then, in both susceptible *S. nodiflora* and *A. tuberculatus* there was C at position 919 but T in resistant *A. tuberculatus*. At amino acid level this position was CCC (proline) in susceptible *S. nodiflora*, CTA (leucine) in susceptible *A. tuberculatus* and TTA (leucine) in resistant *A. tuberculatus*. Therefore, in spite of base alteration from C in susceptible *A. tuberculatus* to T in resistant *A. tuberculatus*, the encoded amino acid remained constant, i.e. leucine. Significant difference was, however, observed in susceptible *S. nodiflora* because there was proline at the same position.

Keywords : *Synedrella nodiflora* (L.) Gaertn, Fomesafen, *PPX2L* gene, herbicide resistance

INTRODUCTION

Fomesafen can be used as both pre- and post-emergence herbicide to control broadleaf weeds in crops such as cotton, tobacco and tomatoes (Bridges and Stephenson, 1991), watermelon and cucumber (Johnson and Talbert, 1993), potato (Sloik, 2002), and soybean (Dwiati et al., 1997; Dwiati and Budisantoso, 2005). Fomesafen, belonging to protox inhibiting herbicide, was first released in 1970 and after being used for 30 years resistance in waterhemp *(Amaranthus tuberculatus)* was reported (Anonymous, 2006).

Shantakumar (2002) notices that weed resistance against herbicides can be due to the use of herbicides of long persistence in soil. Dwiati et al. (2003) find that Fomesafen proves still active in soil four months after application as the presence of phytotoxic symptomps in weeds grown on soil previously exposed to it are observed.

Synedrella nodiflora (L.) Gaertn.commonly found in legume cropping systems is one of broadleaf weed species which can be controlled with Fomesafen. Recurrent application of Fomesafen can, however, lead to resistance of the weeds. Dayan and Duke (1997) suggest seven different mechanisms of weed resistance against protox inhibiting herbicides. They are reduction in herbicide absorption, translocation of active substance from leaves to roots or stems, protective mechanism against toxic singlet oxygen, accumulation of enzymes reducing protoporphyrin IX, differences in membrane plasm sensitivity to singlet oxygen, enhancement of herbicide degradation and changes in herbicide binding site of protox.

Shoup and Al-Khatib (2005) ensure that enhancement of herbicide degradation do not occur in a population of *A. tuberculatus* resistant against protox inhibiting herbicide. On the other hands, Patzoldt et al. (2005) report that an alteration of herbicide binding site of protox is observed in resistant *A. tuberculatus*. Then, Patzoldt et al. (2006) find that the alteration is due to the change in enzyme conformation as a result of one amino acid deletion, i.e. glycine at position G210. This results from three-base deletion in *PPX2L* gene, which encodes both mitochondrial and plastidal protox.

Although molecular feature of *A. tuberculatus* resistance against protox inhibiting herbicide has already been well elucidated, there is still no report on that of *S. nodilflora*. Therefore, this study aims to know the sequence of *PPX2L* partial gene isolated from both resistant and susceptible *S. nodiflora* against Fomesafen and to analyze this partial gene sequence.

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MATERIALS AND METHODS

Wild type *S. nodiflora* were collected from three different locations, i.e. Karangwangkal Purwokerto, Tanah Baru Bogor and Ecopark Cibinong, while the resistant biotype individuals were produced by repeated application of Fomesafen on the wildtype plants at sublethal doses for ten generations. Fomesafen was formulated as Reflex by Syngenta, the company providing the herbicide in this study. Foliar application was carried out on both resistant and susceptible *S. nodiflora* when the weeds were 50 days old.

The study was basically designed into several steps, i.e. (1) isolation and amplification of *PPX2L* partial gene of *S. nodiflora*, (2) sequencing of *PPX2L* partial gene, (3) homology study of *PPX2L* partial gene of some plant species available in database, (4) analysis of the sequence of *PPX2L* partial gene. mRNA isolation as well as *PPX2L* partial gene amplification were performed using Invitrogen kit. The isolated mRNA was used as RT-PCR template since it was transcribed reversely into cDNA. The PCR mixture consisted of 4 µl mRNA, 12.5 µl 2x master mix, 1 µl forward primer (5'-AAAAGGGTTGCTGTTGTTGG-3') and reverse primer (5'-GGGGATAACTCCGAAGC-3') of 10 µmol respectively, 1 µl Superscript TM III RTase, 2 µl $MgSO_4$ and aquabidest up to a volume of 25 µl. Both forward and reverse primers were designed using *PPX2L* sequence of *A. tuberculatus*. This reaction was carried out in a condition as follows: 1 cycle of cDNA synthesis at 55°C for 30 mins, 1 cycle of predenaturation at 94°C for 4 mins, 40 cycles of PCR consisting of denaturation at 94°C for 30 secs, annealing at 50.6°C for 30 secs and extension at 72°C for 1 min respectively, proceeded by 1 cycle of final extension at 72°C for 7 mins. The PCR products were sent to First Base Singapore for sequencing using terminator dye method. The sequences were then subject to Blast and Clustal W for homology study. Finally, any differences observed in conserved areas were analyzed both in nucleotide and amino acid level.

RESULTS AND DISCUSSION

The *PP2XL* partial gene of resistant *S. nodiflora* could not be amplified with the oligonucleotide primers used in spite of the optimation on PCR annealing temperature. On the other hands, those of susceptible biotypes collected from three different locations, i.e. Tanah Baru Bogor, Ecopark Cibinong, and Karangwangkal Purwokerto, were successfully amplified resulting in three bands of 250 bp, 500 bp and 700 bp respectively (Figure 1).

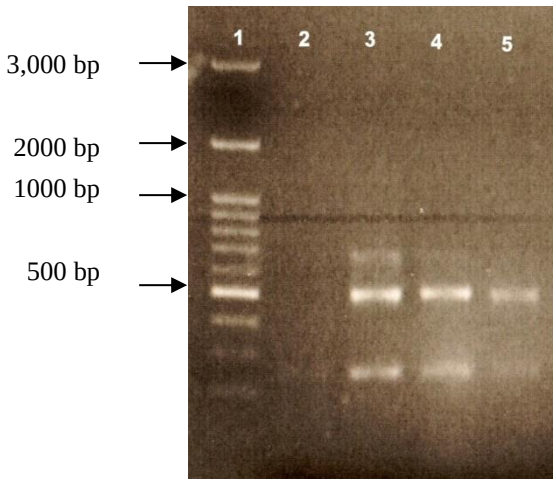

Figure 1. PCR products of *S. nodiflora*

Lane 1 DNA marker
Lane 2 PCR product of resistant *S. nodiflora* from Karangwangkal Purwokerto
Lane 3 PCR product of susceptible *S. nodiflora* from Karangwangkal Purwokerto
Lane 4 PCR product of susceptible *S. nodiflora* from Tanah Baru Bogor
Lane 5 PCR product of susceptible *S. nodiflora* from Ecopark Cibinong

All the PCR products were then subjected to sequencing. Blast analysis on 250 bp and 700 bp bands of susceptible *S. nodiflora* showed that they are partial genes encoding glutamine synthetase, while the 500 bp band showed 97% homology with total chloroplast genom of various plant species, especially *Helianthus annus*. Since there was no information on homology with

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PPX2L sequences of any plant species, Clustal W analysis was performed between *PPX2L* sequences of resistant and susceptible *A. tuberculatus* which were used to design the oligonucleotide primers in this study. This revealed a three-base deletion at positions 679, 680 and 681 as Patzoldt et al. (2006) reported, where the 500 bp sequence of susceptible *S. nodiflora* are not included, indicating that the primers designed from *PP2XL* sequence of *A. tuberculatus* are not compatible to those of susceptible *S. nodiflora* (Figure 2 on separate sheet).

Furthermore, Clustal W analysis among 500 bp bands of the three susceptible *S. nodiflora* and *PPX2L* sequence of susceptible *A. tuberculatus* proved that they are genetically different, though some conserved areas were observed, i.e. from position 749 to 756 and from position 847 to 858. The first was CCTTAGAG in both plant species, while the later was AAAACCTAAGGG in susceptible *S. nodiflora* and AAA-CCTAAGGG in susceptible *A. tuberculatus* showing a single A deletion in susceptible *A. tuberculatus*. There is C at position 919 of both susceptible *S. nodiflora* and *A. tuberculatus*, but it is T in resistant *A. tuberculatus*. When this, along with the next two bases (919, 920, 921), is translated into amino acid, there will be proline (CCC) in susceptible *S. nodiflora*, leucine (CTA) in susceptible *A. tuberculatus* and leucine (TTA) in resistant *A. tuberculatus* (Figure 3 on separate sheet).

The high homology of 500 bp bands of the three susceptible *S. nodiflora* with total chloroplast genom of various plant species indicates that the DNA fragments can be strongly assumed as part of a protox encoding gene, i.e. *PPX2L*. Patzoldt et al. (2006) report that *PPX2L* is a gene responsible for protox inhibiting herbicide resistance in *A. tuberculatus*, which is a longer version of *PPX2* gene with additional 90 bases or 30 amino acids in the N-end of its translation product. This extra fragment presumably encodes signal sequence required for plastidal import, so that *PPX2L* gene isolated from *A. tuberculatus* seems likely to encode both plastidal and mitochondrial protoxs. Lee et al. (2008) notices that *PPX2L* is not a different gene from *PPX2*, but instead they are allelic genes located at the same locus. On the other hands, Che et al. (2000) states that plastidal protox of 548 amino acids is encoded by *PPX1* gene, while mitochondrial protox of 504 amino acids is encoded by *PPX2* gene. Both amino acid sequences show only 27.3% homology with each other.

The incompatibility between primers used with *PPX2L* sequence of *S. nodiflora* revealed that this plant species is genetically different from *A. tuberculatus*. Therefore, unlike the mechanism of resistance against protox inhibiting herbicides in *A. tuberculatus*, that in *S. nodiflora* remains unclear. Lee et al. (2008) suggest that since there are two sites of actions of protoxs in plants, mechanism of resistance against protox inhibiting herbicides should involve simultaneous mutation of both protox encoding genes. However, as *A. tuberculatus* has *PPX2L* gene encoding both mitochondrial and plastidal protoxs, single mutation in this gene may result in resistance against protox inhibiting herbicides. This, as reported by Patzoldt et al. (2006), is due to a three-base or one amino acid deletion at position 210, i.e. glycine.

Weeds becoming resistant against herbicide may develop their capability to degrade active substances rapidly before they reach phytotoxicity threshold and to modify herbicide binding site or to shorten the binding process (Kendig, 2006). Menalled and Dyer (2005) adds that weeds subjecting to herbicide resistance is capable of preventing herbicide activity displacement from sensitive to tolerant site. In addition, Murata et al. (2004) suggest another mechanism of herbicide resistance in weeds, i.e. amplification of enzymes in a certain level that herbicide is converted into a non toxic form.

Although it is proved that *S. nodiflora* is genetically different from *A. tuberculatus*, some conserved areas are observed enabling alignment of both nucleotide and amino acid sequences between both plant species. The presence of proline in susceptible *S. nodiflora* instead of leucine in both susceptible and resistant *A. tuberculatus* is interesting to elucidate in a further study. Parlich et al. (1983) state that proline is often associated with plant adaption to environmental stress.

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Rhizobacteria for Plant Growth Promotion and Their Tolerance to Drought Stress

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Rhizobacteria have been known for their capability as plant growth promoter through some mechanisms, directly and indirectly. The purpose of this research to screen rhizobacteria of *Bacillus* sp. and *Pseudomonas* sp. drought tolerance as plant growth promoter of maize (*Zea mays* L.). Screening of rhizobacteria as growth promoter of 47 *Bacillus* CR and 34 *Pseudomonas* CRB resulted 24 and 9 isolates were able to stimulate the growth of maize sprouts, respectively. Further screening of those growth promoter of the rhizobacterial isolates to drought tolerance resulted 7 isolates of *Bacillus* CR and 6 isolates of *Pseudomonas* CRB that were able to grow on medium with osmotic pressure -1 MPa and -2 Mpa, respectively. Potential rhizobacterial isolates of growth promoter and drought tolerance were tested for antagonist mechanisms which aims to determine ability to live together in one carrier medium if to be made formulation. Both non antagonist rhizobacterial isolates were evaluated for their potential in producing exopolysaccharide (EPS) revealing that CRB 19 and CR 90 exhibited the highest activity of EPS production up to 0.346 mg ml^{-1} on medium with -2.0 Mpa and 0.107 mg ml^{-1} on medium with -0.73 MPa, respectively. Based on 16S rRNA sequence analysis revealed CRB 19 and CR 90 belonged to *Pseudomonas aeruginosa* strain B2 and *Brevibacillus brevis* B33, respectively. Those growth promoter and drought tolerant of *Bacillus* CR and *Pseudomonas* CRB had potency to be developed as inoculants of maize planted in dry land agriculture.

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Inventory of Shore Birds Species and It's Food Distribution on The Coastal Area at Peukan Bada, Aceh Besar District

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The research about "Inventory of Shore Birds Species and Food Disstribution on the coastal area at Peukan Bada Beach, Aceh Besar District" has done from September 9th to october 7th, 2012. The aim of this reseacrh was to know about species number of shore birds and its food distribution on the coastal area at Lam Guron village, Peukan Bada, Aceh Besar District. Shore birds species observation used point count method. Observation point was determited by purposive sampling, with the distance between points was 100 meters to 200 meters around observation location and each point had 10 minutes to 15 minutes observation. Shore birds food distribution observation used quadrate method with sampling technique. Quadrate compartment was putted on location that shore birds looked for food, with the distance between each station was 150 meters and sample was taken for each 10 meters for 3 stasions and 3 plots. The observation result about shore bird species had founded 3 spesies, that were *Caradrius alexandrinus, Caradrius leschenaultii,* and *Tringa totanus.* The shore bird's food that had founded on coastral area at Lam Guron village were 5 species, that were *Telescopium telescopium, Palola maenicus*, Polychaeta sp, *Uca* sp, *Scopimera* sp. Morishita distribution average was between 0,032 to 0,432, it included the same creteria. We found 3 shore bird species and shore bird's food distribution pattern on coastal area at Lam Guron viilage, Peukan Bada, Aceh Besar. The index is < 1 that meaning was shore bird's food distribution pattern was same.

Keywords : inventory, shore birds, food distribution

INTRODUCTION

The costral area is an area that has interaction between 3 kind of nature element, that is land, sea, and air. This area has function as buffer zone for many migration animal (fish, shrimp or birds) to find food, reproduction and grawing the child (Fachrul, 2007:121). Shore birds has meaning as a group of water bird ecologically depend on coastal area to fullfill their life needed (Storer, 1961, in Sativani, 2010:4). Shore bird's life is depend on coastal area. The kind of coastal area that they like is mangrove and its mud. They use those place to find food and they use vegetation that grow there as place to rest and reproduction (Petroleum, 1996:126).

Commonly, shore bird presence is appropriated by its pleasure livinghood. polichaeta and others. Crustacea is one of the most prey for shore bird (Gosztonyi & Kuba, 1998 *in* Arbi, 2008:2). Morfologically, shore bird has beak model that is appropriated to exploitate the different food sources, include at the shore area, shallow waterworks and swamp. It has long doot and there is no membrane, so it can walk on shallow waterworks and soft mud (Buchanan, 2000 *in* Sanir, 2009). Food has nutrition that must be fullfilled by every life creature to defend their life. Food could came from its livinghood, the bird that live around the river and lake get water insect, fist and crab as its food (Fachrul, 2007:61).

MATERIAL AND METHOD

This reseach taked place on food coatal area at the Peukan Bada Beach, on September 9th, 2012 until October 7th, 2012. Instrument that was used were alcohol 70%, monokuler tetescope, binokuler, telescope, stereo microscope, stopwatch, Mckinnon book, shovel, paralon pipe, sampling plastic, label piper and filter.

This research used point count method to observe shore bird species, according to Bibby determining point count is made by random according to sampling area distribution with quadrate line for wide area. Point count used view distance 100-200 meters circumference, while to take ovar the sampling shore bird food distribution used quadrate sampling with sampling techniqve with 3 station that was accorded by shore bird's place to find food. For each station is made 3 plots with size 1 x 1 meters, the plot is putted on each 10 meters, advance shore bird's foo sampling analyze that had found done at Laboratorium Biology, Syiah Kuala University.

Sampling of shore bird's food distribution data analyze is did by descriptif statistic using Morishita index Krebs (1989) *in* Gundo (2010:139) :

$$\mathrm{Id} = \frac{\mathrm{ni}\ \sum(\mathrm{Xi}\ (\mathrm{Xi} - 1))}{\mathrm{N}\ (\mathrm{N} - 1)}$$

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Criteria of distribution index of Morishita:
Id>1 = pattern is cluster
Id=1 = pattern is random
Id<1 = pattern is flat

RESULT AND DISCUSSION

Result of shore bird species that had found is presented in Table 1, shows there are 3 kind of shore bird that had found and it come from 2 family, those are Charadridae and Scolopacidae. From 3 shore bird species, the most shown is *Caradrius leschenaultii.* There are many kind of this bird for each species because Scolopacidae group and Charadridae group like mud coastal area, open wet area and near to sea. It can couse shore bird species, like Scolopacidae, often come to mud area because there are many invertebrata there. It can prove that coastal area at Lam Guron village with half of it is mud area with mangrove is strategic area that has enough food for shore bird, so it has many shore bird there. Nature condition around coastal area influence to shore bird presence, for example, sea water condition is rise, this can cause shore bird can't find food.

Table 1. Shore Bird's species found in coastal area at Lam Guron village, Peukan Bada, Aceh Besar.

No	Shore Birds	Family	Individu Numbers		
			I	II	II
1.	*Caradrius leschenaultii*	Charadridae	119	117	143
2.	*Caradrius alexandrinus*	Charadridae	58	73	64
3.	*Tringa totanus*	Scolopacidae	133	141	57
		Amount individual	310	331	264

Table 2. Showed distribution value of morishita (Id) each species from shore bird food. Rate value distribution of morishita the about 0,032 until 0,432. It showed the distribution pattern of morishita from species of shore bird with the same criterion or flatten. According criterion of distribution besade on Breds (1989) *in* Gundo (2010:139) distribution criterion of morishita if Id>1 leaflet pattern sort of individu character to gather in a group, Id=1 leaflet pattern Id<1 the leaflet pattern variety individu is same or flatten. Sukardjo (1986) *in* Widiastuti (1998:7) told that sort of resistance to lost water is one of barir factor for fauna of mangrove, the prtection from sunshine and level of water of soil surface influence to mangrove's fauna distribution look for.

Table 2. Distribution of Shore Bird Food Finded the Coastal Area at Lam Guron Village, Peukan Bada, the great Aceh.

No.	Species	Morishita index (Stasiun)			Mean of distribution	Criterion distribution pattern
		I	II	III		
1.	*Telescopium telescopium*	0,263	0,918	0,004	0,395	Flat
2.	*Palola maenicus*	0,031	0,001	0,247	0,093	Flat
3.	Polychaeta sp	0,496	0,050	-	0,182	Flat
4.	*Uca sp*	0,041	0,013	1,243	0,432	Flat
5.	*Scopimera sp*	0,006	0,093	-	0,032	Flat
Amount total		0,837	1,074	1,494		
Mean		0,167	0,214	0,298		

Shore bird food when water decrease because one of causes of food existance is water. Whe water decrease, animal is like *Uca* sp species could be seen io d analyzeatan all of coastal area according to data analyze food distribution could be known that from thee station are seen food distribution that looks alike. Between there of it, it means in coastal area of Lam Guron had distribution food forethemore they have possibelity gone the round of in rea wich they like to looking for food.

Muddy land is aart of ebb area thatis commonly found at beach area with (calm water) or in area estuary river, the main substrat is mud, and usually it can't be planted, but there are much invertebrata animal here, for example mollusca, crustacea and other (Davies, 1996, in Elfidasari, 2006:189).

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CONCLUSION

1. Shore birds species that has found at the coastral area at Peukan Bada, Aceh Besar are 3 species (*Caradrius leschenaultii, Caradrius alexandrinus,* and *Tringa totanus)* with total of its individu are 905 individu.
2. Food species of shore bird that has found at Peukan Bada, Aceh are 5 species (*Telescopium telescopium, Uca* sp, *Scopimera* sp, Polycaeta sp, and *Palola maenicus*).
3. Shore bird food distribution pattern at Lam Guron Viilage, Peukan Bada less than 1 that means individual kind of shore bird distribution pattern is flat.

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Biodiversity in Logged Forest of Tesso Nilo, Riau Province, Sumatra

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Tesso Nilo is located at Riau province which is the largest area in Sumatera island. Previously, it has good lowland forest, however the forest has been threatened by logging and land conversion. Tesso Nilo area covers 188,000 ha and includes four districts: Inderagiri Hulu, Kuantan Sengingi, Pelalawan and Kampar. In order to provide scientific justification for conserving Tesso Nilo area, the field survey was conducted in June 2003 to assess the diversity of flora, medicinal plants, birds, mammals, reptiles, amphibians, fishes, insects and small mammal parasites. The survey was conducted in logged forests around Segati river, Toro river, Mamahan river, tributary Mamahan river and Sawan river. Although the area has been disturbed, the remaining forest contains very rich plant species. The high richness of plant species was shown by the high value of Mennhenick index. Records from the 1 ha studied plot identified a total of 360 species included in 165 genera and 57 families with 215 tree species and 305 sapling species. The local community has utilized 83 species of medicinal plants and 4 species of toxic plants for fishing. However, the most important medicinal plants which have economical value were 'pagago' *Centella asiatica* and 'patalo bumi' *Eurycoma longifolia*. The diversity of bird species reached 114 species represented 29% of the total Sumatran bird species (397 species). The most important species was the Sumatran Hill mynah (*Gracula religiosa*) which is almost extinct and the Wrinkled Hornbill (*Aceros corrugatus*). The other vulnerable bird species were Crestless Fireback (*Lophura erythrophthalma*), Crestless Fireback (*Lophura ignita*), and Hook-billed Bulbul (*Setornis criniger*). A total of 34 species or 16.5% of 206 species of Sumatran mammals was recorded in the area. The important mammal species included Sumatran tiger (*Panthera tigris sumatrae*), Sumatran elephants (*Elephas maximus sumatrensis*), the Sun bear (*Helarctos malayanus*) and three species of primates. The diversity of herpetofauna contained 15 reptile species and 18 species of amphibians. The most important herpetofauna was the critically endangered False Gharial (*Tomistoma schlegelii*). The diversity index (Simpson index) of fish species was high (0.833) with the number of species: 50 represented 18% of the total Sumatran fish species (272 species). The important fish species were *Breinsteinea* sp. and *Chaca bankanensis* which were unique and rare. Since insects are the largest group of animal, this survey focused only on beetles. The identified beetles were classified into 644 species and 34 families. The Longhorn beetles (Cerambycidae) and the Scarab beetles (Scarabaeidae) indicated the highest species diversity. The diversity of small mammal parasites was high. The ectoparasites were categorized into 14 species and the endoparasites were categorized into 2 orders (Cestodes and *Acantocephala*) and 3 species.

Keywords: biodiversity, logged forest, richness, Sumatran tiger, Sumatran elephants.

INTRODUCTION

Background

The rate of deforestation in Sunda lowland forest especially on Sumatera island increased with 'immense speed' (Lambert and Collar 2002). It was affected by massive forest destruction because of extensive logging and land conversion for housing, agriculture, development of other infrastructure and forest fragmentation. Riau is the largest province in Sumatera and possessed good quality of lowland forest which has been threatened by legal concession companies and illegal logging. A part of those lowland forest is Tesso Nilo forest (previously called Air Sawan forest) which is located in four districts: Indragiri Hulu, Kuantan Sengingi, Pelalawan and Kampar.

Tesso Nilo forest covered an area of 188.000 ha, previously this area was stated by the government for production forest to supply logged wood and other wood products. However, since 1980 there were many conflicts between elephant and people. Therefore, the area was planned to be nature reserve for conserving Sumatran elephants. As a primary requirement of being proposed and declared for conservation area, the biodiversity in the area should be assessed.

Main Objective

To provide scientific data on the biodiversity of Tesso Nilo such as: plant diversity, medicinal plants, diversity of birds, mammals, reptiles, amphibians, fishes, insects and parasites of small mammals.

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METHODS

Tesso Nilo area covers four districts: Inderagiri Hulu, Kuantan Sengingi, Pelalawan and Kampar. It is located between 0º0′5,1″ dan 0º14′56″ South and between 101º31′14,6″ and 101º52′1,9″ East. In the east, the area is bordered with Kerumutan Wildlife Preservation and in the west is bordered with Bukit Rimbang Nature Reserve. Selected survey locations were logged forest near Segati river, Mamahan river, Toro river, tributary of Mamahan river and Sawan river.

Flora, medicinal plant, insect and parasites of small mammal surveys were conducted in one location i.e. logged forest around Segati river (Figure 1). Bird and mammal surveys were carried out in two locations i.e. logged forest around Segati and Mamahan rivers. However, fish and herpetofauna surveys were conducted in five locations.

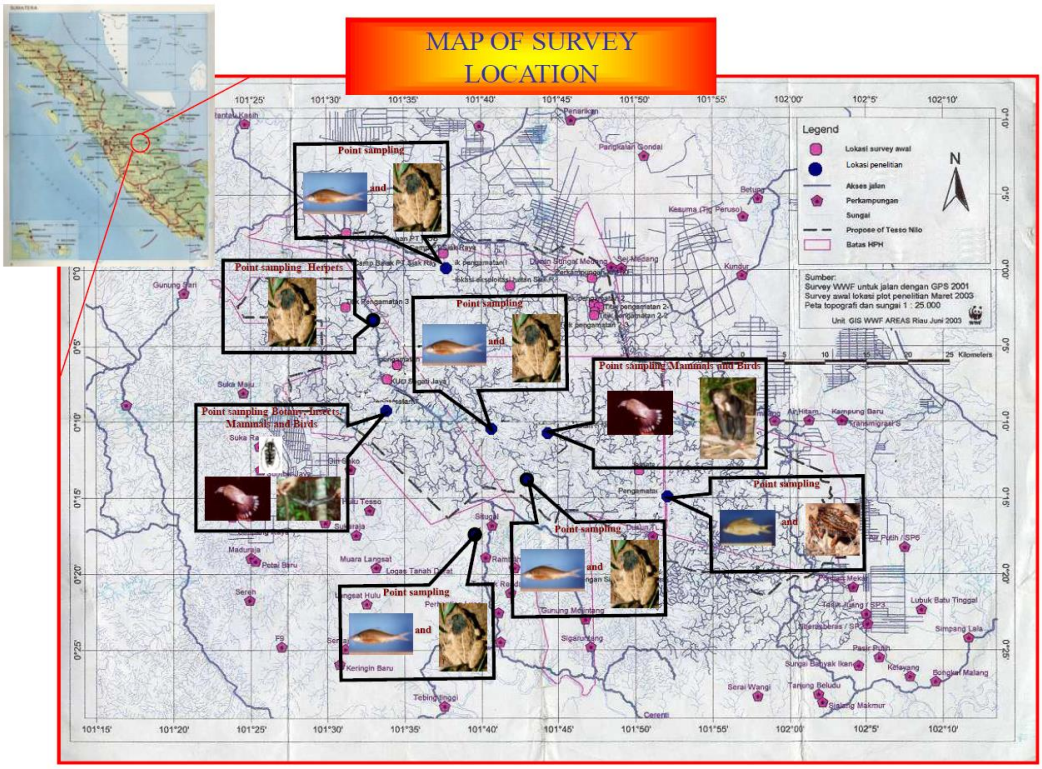

Figure 1. Survey location at Tesso Nilo forest.

RESULTS AND DISCUSSION

Diversity of plant

The result of plant survey on 1 ha (100 x 100 m2) plot indicated that the richness of Tesso Nilo lowland forest was very high. Although the forest suffered from heavy disturbances by logging, the remnant forest contained high diversity of flora which was confirmed by Gillison (2001) as the top rank on lowland forest biodiversity in the world. The identification of plant species in 1 ha sampling plot recorded 360 species from 165 genus and 57 families consisting of 215 tree species and 305 sapling species.

Interms of plant diversity, Tesso Nilo forest had the highest diversity (Mennhenick) index than the other lowland forest in Sumatera (Table 1). The population of protected but threatened species such as *Irvingia malayana*, *Koompasia malaccensis*, *Dyera polyphylla*, *Sindora sumatrana*, *Sindora brugemanni*, *Sindora leiocarpa*, *Sindora velutina*, *Scorodocarpus borneensis*, *Fagraea fragrans* was very poor. The other species included in the **Red List of IUCN** such as 'gaharu' *Aquilaria malaccensis*, 'ramin' *Gonystylus bancanus*, *Dialium* spp., *Shorea* spp., *Dipterocarpus* spp. and durian *Durio* spp. were only remnant saplings.

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Table 1. Comparison of plant diversity in field studies of Sumateran lowland forests.

Research Location	Size (ha)	No of species	Density per ha	Diversity (Mennhenick) index	Sources
Tesso Nilo forest	1	215	557	9.11	This study
Edge of Alas river, South-east Aceh	1	81	542	3.48	Sambas, 1999
Lowland forest at Ketambe Research station	1.6	132	480	4.76	Abdulhadi et al. 1996 cited by Purwaningsih & Ismail 2003
Bukit Tiga Puluh forest , Jambi	0.09	30	610	4.04	Partomihardjo et al. 1996 cited by Purwaningsih & Ismail 2003
Rimbo Panti (800 m asl.)	1	145	429	7.00	Purwaningsih (unpubl.data.)
Rimbo Panti (200 m.asl.)	1	80	451	3.76	Purwaningsih (unpubl.data.)

Diversity of medicinal plant

Local people around Tesso Nilo have utilized at least 82 medicinal plant species and 4 plant species for fishing. The medicinal plant species were categorized into 78 genus or 46 families. Those were used to cure 38 diseases. The important medicinal plant species were 'pagago' (*Centella asiatica*) and 'patalo bumi' (*Eurycoma longifolia*). The community already planted 'pagago' in their backyard. Although 'patalo bumi' was often being used and has economical value, they have not planted in their backyard so they always collected it from the forest.

Diversity of birds

Bird survey was conducted in three sites in two locations. Two sites were located in the forest near Segati river (0008.898′ LS, 101034.281′ BT, 133 m asl) with 0.5 km distant and one site was near Mamahan river (0010.227′ LS, 101040.725′ BT, 133.3 m dpl). The survey recorded 107 bird species from 28 families: Ardeidae, Accipitridae, Phasianidae, Turnicidae, Columbidae, Psittacidae, Strigidae, Cuculidae, Strigidae, Trogonidae, Alcedinidae, Bucerotidae, Capitonidae, Picidae, Eurylaimidae, Pittidae, Apodidae, Pycnonotidae, Aegithalidae, Timaliidae, Sylviidae, Rhipiduridae, Dicaeidae, Nectariniidae, Ploceidae, Sturnidae, Dicruridae and Corvidae. If the results of this survey were combined with the preliminary survey by Rasfianto in 1992 (for a review see Gillison 2001), the diversity of Tesso Nilo area would reach 114 species or 29% of the total number of 397 bird species in Sumatera (MacKinnon et al. 1992).

The important bird species was Sumatran Hill mynah (*Gracula religiosa*) which is almost extinct. Other vulnerable species which are not protected yet, included *Lophura erythropthalma*, *Lophura ignita*, *Aceros corrugatus* and *Setornis criniger*. The presence of *Rhipidura albicollis* at Tesso Nilo forest was a new record for its distribution.

Diversity of mammal

Like bird survey, mammal survey was conducted in two locations i.e. at the logged forests around Segati river and Mamahan river. A total of 34 mammal species or 16.5% of 206 mammal species in Sumatera were recorded by *reconnaissance survey* (RS) and capture and recapture method using traps and mistnets. Based on *reconnaissance survey*, the diversity index of both locations was 3,696 which means that the diversity of mammal was high if we assumed that the diversity < 1 very low, 1 - 2 low, 2 - 3 medium, 3 - 4 high, > 4 very high (maximum Shannon-Wienner index = 5).

The results showed that the density of small mammal population in the studied plot was low only 10 individuals/ha. This indicated that the forest of Tesso Nilo was relatively good because in heavily disturbed forest, the density of small mammals could reach 20 individuals/ha. Instead of density, other indicator of disturbed habitat was an increase in the number of

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commensal or semi commensal of small mammals such as house rat (*Rattus tanezumi*), wood rat (*R. tiomanicus*) and short-nosed fruit-bat (*Cynopterus brachyotis*). However, the survey did not record those rats and only 1 individual of Short-nosed fruit-bat was recorded.

The presence of three primate species i.e. *Hylobates agilis, Presbytis femoralis* and *Macaca nemestrina* in Tesso Nilo area indicated that the quality of the forest was still good. Primates usually selected to forage in the middle and top of canopy. The canopy is very important for dispersion of mammal species. Since there were some protected large mammals such as the Sumatran tiger (*Panthera tigris sumatrae*), the Sumatran elephant (*Elephas maximus sumatranus*), Sunbear (*Helarctos malayanus*) and Tapir (*Tapirus indicus*) Tesso Nilo area indicated high conservation value. Indeed, the presence of Sumatran tiger (*Panthera tigris sumatrae*) as the top predator and its prey such as Wild boar (*Sus scrofa*), Sambar deer (*Cervus unicolor*) and Barking deer (*Muntiacus muntjak*) was a good indicator for the quality of the forest.

Diversity of herpetofauna

Herpetofauna surveys were conducted in some locations which were forests and rivers including Air Sawan, Sengkalalo, Toro and its tributaries, Segati and Mamahan rivers. The survey recorded 33 species of Herpetofauna consisting of 15 species of reptiles and 18 species of amphibians. The reptiles were 8 species of snake, 2 species of agamids, 1 species of flying lizard, 1 species of skink, 1 species of varanid, 1 species of crocodile and 1 species of fresh water turtle. The important reptile was the critically endangered false gharial (*Tomistoma schlegelii*) which has been protected by Indonesian law (*PP No. 7/1999*). The amphibians were 1 species of litter frog, 2 species of toads, 1 species of narrow-mouthed/chorus frog, 1 species of sticky frog, 12 species of frogs and 1 species of tree frog. Among those amphibians there was one species which could be used as bio-indicator of good forest i.e. Spotted stream frog (*Rana signata*).

Diversity of fish

Fish surveys were conducted at Sawan , Sangkalalo, Toro, Segati and Mamahan rivers. There were 50 fish species or 18% of the total Sumatran fish species (272 species) recorded from the area which represented 31 genera, 16 families and 4 orders. None of the recorded species was categorized as threatened species by IUCN in 2001 (Wargasasmita 2002). The large number of collected fish was categorized into Cyprinidae (18 species or 37.50%), Bagridae (5 species or 10.42%), Belontidae (4 species or 8.33%) and Siluridae (4 species or 8.33%). Toro river had the highest species (32 fish species) and the highest diversity index (0.914). It was not suprising because previously the vegetation of the bank in this area was dense primary forest.

Important fishes for swamp ecosystem i.e. 9 species of *Rasbora* spp., *Luciocephalus pulcer*, *Hemirhamphus pogonognathus* and *Spaerichthys osphromenoides* were collected from Toro and Sawan rivers. Edible fishes which have high economical value were *Hemibagrus nemurus*, *Channa lucidus*, C. *striata*, *Clarias* sp., *Ompok hypopthalmus*, *Cyclocheilichthys armatus* and Barau fish (*Hampala macrolepidota*). Ornamental fishes included *Epalzeorhynchus kallopterus*, *Rasbora heteromorpha* and *Betia fusca*. However, unique fishes because of their form and very rare were *Breinsteinea* sp. and *Chaca bankanensis*, recorded at Toro river and Mamahan river. The threatened Arowana fish *Sclerophagus formosus*, which was already included in CITES Appendix 1 was known to be at Segati river of Gunungsahilan village. However, this survey did not record it.

Diversity of insect

Insect survey, in particular beetles (Coleoptera) was conducted at the logged forest of Segati river. The survey recorded 644 beetle species which were grouped into 34 families. Two beetle families which had high diversity were Cerambycidae (81 species) followed by Scarabaeidae, 76 species. These two families are important as they indicated the good quality of the forest. Most of Cerambycidae larvae are wood borer, so the high diversity of Cerambycidae showed the high diversity of vegetation (Noerdjito et al. 2003). In addition, the high diversity of Scarabaeidae especially subfamily of Coprinae (Scarabaeinae) which feed on the faeces of various mammal species indicated the richness of mammal species in the Tesso Nilo forest.

Diversity of parasites of small mammal

Parasites are good indicator of high diversity of wildlife especially wild small mammals in a forest, because each parasite species needs a specific host for its life cycle (Saim & Suyanto personal communication). Survey on parasites of small mammals was carried out at the logged

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forest around Segati river. A total of 14 species of ectoparasites and 3 species of endoparasites were recorded from 4 species of rats (*Maxomys surifer*, *M. whiteheadi*, *M. rajah* and *Sundamys muelleri*), Common tree shrew (*Tupaia glis*) and Spotted-winged fruit-bat (*Balionycteris maculata*).

The ectoparasites consisted of 4 species of ticks (Acarina: Ixodidae), 6 species of mites (Acarina other ticks), 1 species of lice (Anoplura: Hoplopleuridae) and 2 species of fleas (Siphonaptera). The Red spiny rat (*Maxomys surifer*) was a dominant ectoparasite host since it carried 3 species of ticks (*Amblyomma* sp., *Dermacentor* sp, *Ixodes* sp.), 6 species of mites (*Demodex* sp., *Echinolaelaps* sp., two species of *Laelaps* spp., 2 species of Trombiculidae) and 1 species of lice (*Polyplax* sp.). The endoparasites consisted of 2 species of Cestodes (*Hydatigera taeniaeformis* and *Hymenolepsis* sp.) and 1 species of Acantocephala (*Moniliformis* sp.).

CONCLUSIONS

1. Tesso Nilo area is a remnant lowland forest in Sumatera. Although it has been heavily logged, the remnant forest still possesses:
   - high diversity of plants especially high economical value, protected and threatened species
   - richness of lowland forest birds including almost extinct Sumatran Hill mynah
   - rare large mammals such as elephant, tiger, tapir, sun bear and gibbon
   - habitat of reptile dan amphibian including the threatened False Gharial
   - high fish diversity including fish consumption for animal protein sources and recreation fish
   - high diversity of insects especially beetles
2. Since the area is accessible, its biodiversity has been suffered from extinction.

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Economic Contribution of Bats Species, *Macroglossus minimus* Schinz, 1824, to Durian (*Durio zibethinus*) Pollination in Kokap, Kulon Progo

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The new concept of biodiversity management is community-based anagement. Unfortunately, the participation of local people to manage biodiversity is still very low since the people do not realise the direct and indirect economic values for them. The objective of the study is a economic valuation of the existence of bats species, *Macroglossus minimus* Schinz, 1824 in Kokap, Kulon Progo. After proving *M. minimus* as the durian pollinator, the economic contribution which expressed indirect use value of *M. minimus* was analyzed using simple multiplication i.e. the production of durian each year (kg/year) x average price of durian in local market (Rp/kg). For more information, the economic contribution of direct use value of *M. minimus* was examined based on survei; whereas its option value, bequest value and existence value was analysed based on the information both from survey and liturate study. The economic value of *M. minimus* in Kokap was not able to be fully converted into rupiah. The only value on which converted into rupiah in this research is indirect use value of *M. minimus* as durian pollinator i.e. Rp. 110,176,000.00 per year; which excluded its function on production of others *chiropterophilus* plants such as kapok and petai. The direct use value is as foods and medicines. The option value such as for education and research on biomedical, biomechanical, and biochemical is not significant for local people yet. The bequest value, which reflected value such as the willingness of people to invest money to conserve the species, is low, or negative, because of the misperception of *M. minimus* as pest. The existence value of this species is low, because this species is not unique in this area and the people is not interested to care and come to the area to see its existence. The total economic value of *M. minims* at least Rp.110,176,000.00 per year.

Keywords: biodiversity, economic valuation, bats, chiropterophilus, durian

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Biodiversity of Phosphate Solubilizing Bacterial at Red-Yellow Podzolic Soil in South Sumatera

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Research on Biodiversity of Phosphate Solubilizing Bacterial (PSB) at Red-Yellow Podzolic (RYP) Soil in South Sumatera has been done. The purpose of this study to determine the species diversity of phosphate solubiizing bacterial at RYP soil in South Sumatra. Samples of RYP soil were collected from five sites located in the South Sumatra namely Abab, Benakat, Limes, King, and Talangjimar. Determination of PSB was done by selecting the phosphate solubilizing ability of phosphate bound by Fe and Al oxide in the Picovskaya's medium modified phospahte sources insoluble form $FePO_4$ and $AlPO_4$. The result showed eleven isolates of PSB consisting of eight genera are *Arthrobacter, Azotobacter, Bacillus, Corynobacterium, Micrococcus, Mycobacterium, Pseudomonas, and Steptococcus*. The PSB in the form of $FePO_4$ has the Shannon-Weaver diversity index (H ') of 2.12 while the PSB in the form of $AlPO_4$ has a value of H' by 1.64. Based on each of the Shannon-Weaver index of the PSB in the form of $FePO_4$ and $AlPO_4$ still has a relatively high diversity.

Keywords : Phosphate Solubilizing Bacterial, Red-Yellow Podzolic, Biodiversity, South Sumatera

INTRODUCTION

One type of land in South Sumatra is dominated Red Yellow Podsolic (RYP) soil which has the characteristics of low pH, many containing oxides of Al and Fe, has a high P holding capacity thus causing non-soluble P in the soil (Deubel and Merbach, 2005; Hasanuddin, 2006). According Prihatini (2009), the main problem of phosphate fertilization on RYP soil is a low efficiency because of the binding process of phosphate fertilizers by the soil. On acidic soil, phosphate fertilizer will be bound by the iron (Fe) or alumnium (Al) to form a bond Fe-P or Al-P which is insoluble phosphate so it is not available in the soil. According to Rao (1994), on the soil there are many bacteria that have the ability to release phosphate from the bonds of Fe, Al, Ca and Mg to be available, for example, bacteria of the genera Pseudomonas and Bacillus. According Kolwzan et al., (2006) Soil bacteria, including phosphate solubilizing bacteria has been there on the soil since the process of soil formation.

PSB contribution to the availability of phosphate in soils is determined by the diversity and population. Growing number of species are found and the higher the population of each species, the higher the potential contribution to the solubilization of phosphate. Based on the above information, it is necessary to investigate the diversity of phosphate solubilizing bacteria on red-yellow podzolic (RYP) soil in South Sumatra. The purpose of this study was to determine the types of phosphate solubilizing bacteria in the RYP soil and determine diversity based on the ability of solubilizing phosphate bound by Al and Fe.

MATERIAL AND METHODS

The main material used is a source of insoluble phosphates: $AlPO_4$ and $FePO_4$, reagents and media for characterization: Indol test medium, MR-VP medium, starch agar medium, sugar fermentation medium, medium of Pikovskaya, semi-solid medium, Mannitol Agar meium, medium of Nutrient Agar (NA), medium of Nutrient Broth (NB), medium of Nutrient Gelatin, medium of Simmon`s Citrate (SC), medium of Skim Milk Agar (SMA), reagent characterization (Barrit`s A, Barrit`s, Gram A, Gram B, Gram C, Gram D, $H_2O_2$ 3%, iodine, Kovac`s, methyl red), Phenol Red, safranin, and samples of RYP soil.

Isolation dan Selection PSB

Ten grams of soil sample RYP put 250 ml erlenmeyer containing 90 ml physiological saline solution and homogenized. Culture diluted to 10^{-8}, each dilution taken 1 ml was inoculated on medium Pikovskaya using spread plate method. The cultures were incubated and observed to indicate the presence of bacterial colonies growing. Colonies that show different characteristics and surrounded by clear zones were calculated and refined, to obtain pure isolates (Munawar, 2012).

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Pure isolates that show a clear zone with a confirmation test with inoculate to Pikovskaya medium by a source of phosphate $FePO_4$ and $AlPO_4$. Each isolate purely made suspension in 10 ml physiological saline, homogenized with a vortex. Density is calculated using the counting chamber until it reaches 10^6 cells / ml. Then the paper discs dipped into the suspension isolates in a test tube, then the paper disc that has been dipped in each bacterial isolate aseptically placed on the surface of the Pikovskaya medium by a source of phosphate $FePO_4$ and $AlPO_4$. The cultures were incubated at room temperature for 7x24 hours. The phosphate solubilizing bacteria potentially marked the formation of a clear zone around the colony (Premono (1994).

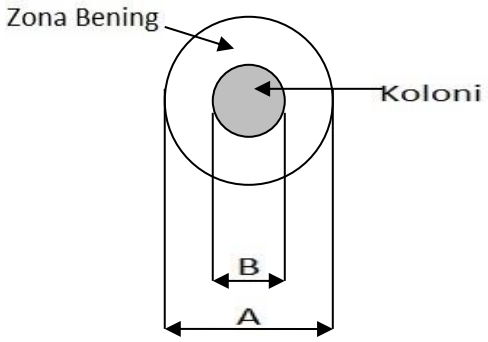

Figure 1. Scheme of the clear zone formed by colonies of phosphate solubilizing bacteria isolates

Characterization and Identification of PSB

Bacterial isolates obtained were characterized by colony morphology, cell morphology and biochemical tests (Benson, 2001), making the phylogenetic dendogram and identification using Bergey `s book Mannual of Determinative Bacteriology, 8th Edition (Buchanan and Gibbons, 1974), Bergey`s Mannual of Determinative Bacteriology, 9th Edition and Bergey`s Manual of Systematic Bacteriology, 2nd Edition (Holt, et al., 2000)

Determination of the Shannon-Weaver diversity index

Shannon-Weaver diversity index is determined by the ability of solubilizing the phosphate in the form of $AlPO_4$ and $FePO_4$. Shannon-Weaver index calculation using the equation of Kaplan and Kitts, (2004).

$$H' = -\sum \left(\frac{ni}{N}\right) + \ln\left(\frac{ni}{N}\right)$$

Remarks:
H'= diversity index,
ni = Number of individuals of each species,
N = number of individuals of all species.

HASIL DAN PEMBAHASAN

Isolat bakteri pelarut fosfat yang berjumlah 22 isolat, diseleksi berdasarkan jumlah kelimpahan bakteri dalam setiap gram tanah, setiap isolat BPF dipilih yang mempunyai kelimpahan > 8,0 x 10^5 sel/gram tanah. Menurut Zobell dan Prokop (1966), jenis-jenis bakteri yang mempunyai kelimpahan tinggi yaitu > 8,0 x 10^5 sel gram tanah yang mampu memberikan kontribusi signifikan dalam aktifitasnya, termasuk melarutkan fosfat. Keseluruhan isolat BPF yang diperoleh sebanyak 22 isolat terbagi dalam delapan genera yaitu *Arthrobacter, Azotobacter, Bacillus, Corynobacterium, Micrococcus, Mycobacterium, Pseudomonas, dan Steptococcus*.

Isolat bakteri yang mempunyai kemampuan untuk melarutkan fosfat ditandai dengan adanya zona bening (gambar 1 dan 2) disekitar koloni bakteri karena adanya pelarutan fosfat yang ada pada medium pikovskaya, baik yang mengandung fosfat dalam bentuk $AlPO_4$ dan $FePO_4$. Menurut Suliasih & Rahmat (2007), bakteri yang mempunyai kemampuan untuk melarutkan fosfat pada medium pikovskaya padat ditandai oleh daerah bening (*holozone*) yang mengelilingi koloni bakteri tersebut.

Terbentuknya zona bening disekeliling koloni bakteri disebabkan oleh sekresi asam organik oleh koloni bakteri ke dalam medium sehingga menyebabkan pH medium menjadi menurun dan terjadi khelasi dengan kation pengikat fosfat yang efektif melarutkan fosfat. Menurut Rao (1994),

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mekanisme pelarutan fosfat secara kimia oleh bakteri pelarut fosfat yaitu dengan cara mereduksi pH substrat melalui sekresi sejumlah asam organik seperti asam-asam format, asetat, propionat, laktonat, glikolat, fumarat dan suksinat.

Shannon-Weaver diversity index (H') of PSB in the form of $FePO_4$ of 2.12 while the PSB in the form of $AlPO_4$ has a value of H' by 1.64. Based on each of the Shannon-Weaver index of the PSB in the form of $FePO_4$ and $AlPO_4$ still has a relatively high diversity index (Dahuri 1994). Dendogram phylogeny form presented in Figure 3.

Dendogram phylogeny shows that the type of PSB in the genus are generally clustered together, except there is one type of the genus Micrococcus namely *Micrococcus* sp (T3H3D3-3) is not a group, it is likely due to the added capabilities of the characters used in the form of phosphate dissolving $AlPO_4$ and $FePO_4$, this character does not exist in the book of identification used.

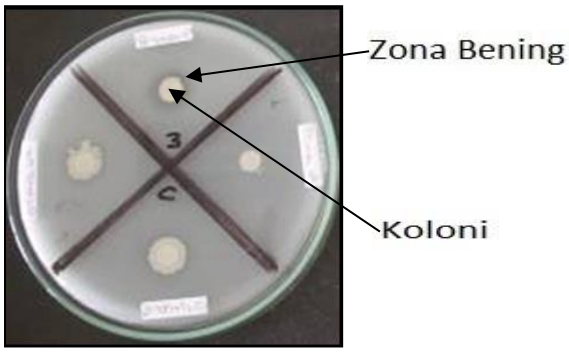

Figure 2. Colony of phosphate solubilizing bacteria

pH of pikovskaya medium used in the selection of phosphate solubilizing with a phosphate source $AlPO_4$ has pH 6.0 and pH of pikovskaya medium with a source of phosphate $FePO_4$ has a pH of 5.0. Pikovkaya medium with a source of phosphate $AlPO_4$ and $FePO_4$ has a low pH (<7), according to Rao (1994) by phosphate solubilizing bacteria is influenced by the pH, the higher the decrease in pH of the medium shows a growing number of organic acids are produced, so that the higher the power of solubilizing phosphate.

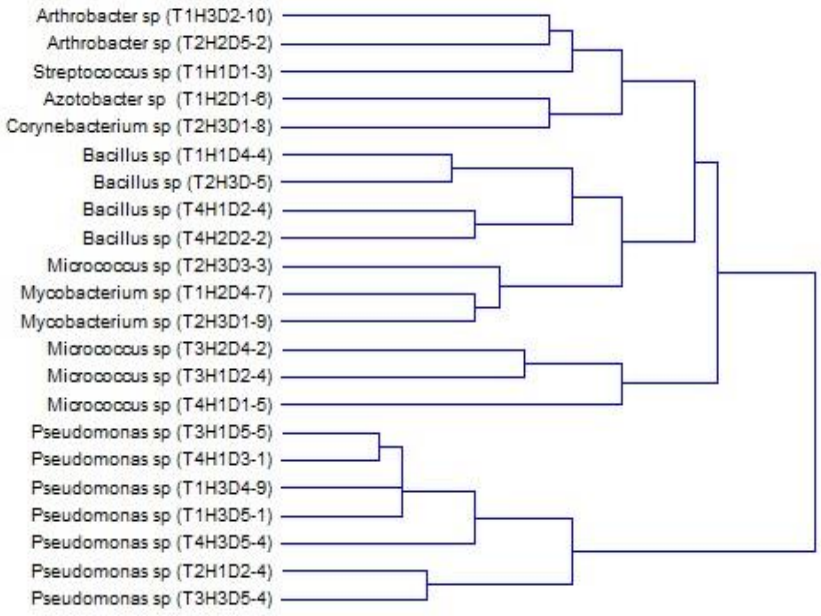

Figure 3. Dendogram phylogeny of 22 isolates of the BPF based on morphological and physiological characters

Availability of phosphate will become available not affected by pH which causes phosphate bonded with metals in the soil, according to Hanafi (2005) available phosphate will become

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unavailable due to tied by Al and Fe in acidic conditions. Unavailability caused by phosphate pH below 5.6 Fe solubility (toxic micro nutrients) and Al (toxic elements) increased to form Al-P and Fe-P were then subjected to crystallization. The Fe-P into variscit ($AlPO_4.2H_2O$) faster than Al-P into strengit ($FePO_4.2H_2O$).

CONCLUSION

Obtained eight genera of phosphate solubilizing bacterial namely Arthrobacter, Azotobacter, Bacillus, Corynobacterium, Micrococcus, Mycobacterium, Pseudomonas, and Steptococcus. PSB diversity index in the form of $AlPO_4$ and $FePO_4$ are relatively high.

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Azospirillum spp. isolated from iron sand and their ability of phosphate solubilization

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Azospirillum species are free-living nitrogen-fixing bacteria commonly inhabit soil and in association with a broad range of root plants. They play important roles in the promotion of plant growth and are capable of growing under stress conditions. This study was done to observe *Azospirillum* occurrence in iron sand at Munggangsari coast, Purworejo, Indonesia, and their ability to solubilize phosphate *in vitro*. There were totally 32 strains of *Azospirillum* spp. isolated from iron sand habitat with population numbers ranging from 1.2 x 10^3 to 1.1 x 10^6 CFU g^{-1} sand. Out of the 32 isolates there was only one isolate that was not able to solubilize inorganic phosphate *in vitro*. Nine isolates showed high capability in solubilizing phosphate with solubilization efficiency values ranging from 150 to 190.

Keywords; Azospirillum, iron sand, N2-fixing bacterium, phosphate solubilization

INTRODUCTION

The iron sand of Indonesia coasts contains Fe with concentrations of 14.6-56.75% (Yasir 2011), in which their physical and chemical properties are dominated by sand texture, low cation exchange capacity and organic matters (Djajakirana *et al.*, 2009). This marginal environment is occupied by few natural plants, where it might also limit the growth and development of soil microorganisms.

Azospirillum spp. are associative nitrogen-fixing bacteria that have no specific plant hosts (Bashan and Holguin, 1997) and able to promote plant growth of various plants (Siddiqui, 2010; Tilak *et al.*, 2010). The bacteria are widely distributed in soil and associated with the roots of *Gramineae*, cereals, annual and perennial plants (Bashan and Holguin, 1997; Saharan and Nehra, 2011), as well as mangroves (Ravikumar *et al.*, 2002). They inhabit temperate and tropical regions, freshwater and high salt environments (Baldani *et al.*, 2005), acidic and alkaline soils (Syarifudin, 2002; Konimozhi and Panneerselvam, 2011), and lowland to a mountain up to 4,000 m (Bano, 2006). These azospirilla have the ability to grow in extreme environments through physiological mechanisms, for instance, cyst formation, melanine and polysaccharide productions (Bashan, 1999).

Plant growth promoted by *Azospirillum* are, particularly, due to N_2 fixation, phytohormone production, and inorganic phosphate solubilization (Hayat *et al.*, 2010; Saharan and Nehra, 2011). Thus, availability of phosphate in soils is mostly due to the activities of inorganic phosphate solubilizing bacteria such as *Azospirillum.* Suliasih and Widawati (2005) observed several isolates of *Azospirillum* from soil in Wamena Biological Garden Papua ($5.0x10^3$-$7.5x10^5$ cells g^{-1} soil) which had the ability to solubilize phosphate. In addition to high capability of nitrogen fixation and indole acetic acid (IAA) production, some strains of *A. zeae* Gr24 and Gr35 were reported to be able to solubilize phosphate in Pikovskaya medium. Solubilization of inorganic phosphates by strains of *A. halopraeferans* was due to secretion of organic acids of the bacteria and cation dissociation process (Seshadri *et al.*, 2000). The aims of this study were to isolate *Azospirillum* spp. from iron sand soil and to assess their ability in solubilizing inorganic phosphate *in vitro.*

MATERIALS AND METHODS

The coast of Munggangsari is one of the iron sand mining areas, where it is located in Purworejo regency, Central Java province, Indonesia (7°50'37" S longitude; 109°52'34" E latitude). There were several plant species grown in iron sand soil such as *Spinifex littorius* Merr., *Calotropis gigantea* (L.) R.Br., *Calopogonium mucunoides* Desv., *Premna serratifolia* L., *Sebastiana chamaelea*, *Pandanus* sp., *Crotalaria pumila*, *Tilia cordifolia*, *Heliotropium ovalifolium*, *Richardia scabra* L., *Althernanthera maritima* (Mart.), *Alysicarpus monilifer* (L.), and *Ipomoea pres-caprae* (L.) R.Br. The iron sand soil samples were collected randomly from five different sites. They were taken from surface soil of 0 to 15 cm depth. All samples were packed in plastic bags and brought to the laboratory and stored in a refrigerator prior to analyze. Measurements of water content, pH, carbon and nitrogen contents were also conducted.

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Azospirillum was isolated using surface culture plating. A weight of 10 g of iron sand soil was suspended in 90 mL sterile distilled water in Erlenmeyer flask and mixed thoroughly on a magnetic stirrer. Amount of 1 mL of aliquot was then transferred to 9 mL of sterile distilled water in a test tube and made serial dilutions up to 10^{-5}. Serial dilutions (0.1 mL) were spread onto Congo red medium (*DL*-Malic acid 5 g, K_2HPO_4 0.5 g, $MgSO_4$ 0.2 g, NaCl 0.1 g, *Yeast extract* 0.5 g, $FeCl_3.6H_2O$ 0.015 g, KOH 4.8 g, agar 18 g, Congo red 15 mL of 1:400 aqueous solution of Congo red, sterilized separately and added into the medium before platting; pH 7.0) (Caceres, 1982). After incubation for three days at 30 ^oC, colonies appearing pink or scarlet colour were transferred onto the fresh mediums. The medium for isolation of *Azospirillum* was semi selective, which basically nitrogen-free bromthymol blue (NFb) medium supplemented with Congo red (Bashan *et al.,* 1993).

Azospirillum population was estimated by total plate count method. The total number of bacterial isolates was expressed as colony forming unit (CFU) per gram of iron sand soil. Isolates grown separatelly on Congo red medium was identified as members of *Azospirillum.* Identification based on morphological, biochemical, and physiological characteristics was carried using Bergey's Manual of Determinative Bacteriology 9th Edition (Holt *et al.,* 1994).

Phosphate solubilization test was conducted in Pikovskaya medium (Rao, 1982). A loopfull 24-hour bacteria cultures on nutrient agar medium was inoculated onto the Pikovskaya medium ($Ca_3(PO_4)_2$ 5 g, glucose 10 g, $(NH_4)_2SO_4$ 0.5 g, KCl 0,2 g, $MgSO_4.7H_2O$ 0.1 g, yeast extract 0.5 g, $MnSO_4.7H_2O$ 0.025 g, $FeSO_4.7H_2O$ 0.025 g, agar 15 g, distilled water 1000 mL, pH 7.0), and the plates were incubated at 30^oC until six-days. The formation of clear zone around the colony was measured. Analysis of the phosphorous solubilization was made by measuring the solubilization efficiency (E) based on this formula (Nguyen *et al.,* 1992),

$$E = \frac{Solubilization\ diameters\ (S)}{Growth\ diameter\ (G)}\ x\ 100$$

RESULTS AND DISCUSSION

The physical properties of the iron sand of Munggangsari coast were 31-33 ^oC for air temperature and 29 – 39 ^oC for sand temperature, and water contents ranging from 2.20 – 6.14%. The chemical properties included soil pH ranged from 5.75 to 6.37, carbon and nitrogen contents were 0.39% and 0.07%, respectively. It was shown that C/N ratio of the sand soil of Munggangsari coast was very low (5.57), indicating poor in nutrient content. Most of coastal area was characterized by low organic matters and fertility (Verplancke, 1992), and low cation exchange capacity causing the content of micro and macro nutrients to decrease (Massoud, 1975). One of the important factors for growth of *Azospirillum* in the sand soil was pH tend to neutral. Dobereiner and Day (1976) reported that *Azospirillum* requires near neutral pH for its abundace.

Thirty two strains of *Azospirillum* had been successfully isolated from the sand soil. Their population densities were varied depended on environmental factors, ranging from 1.2 x 10^3 to 1.1 x 10^6 CFU g^{-1} sand soil (Table 1). Muthezhilan *et al.* (2012) reported that the occurence of plant growth promoting rhizobacteria of sand dune in Chennai coast, India ranged from 4.4 x 10^6-7.5 x 10^7 CFU g^{-1} soil.

Table 1. The population of *Azospirillum* isolates from iron sand soil

No.	Sampling sites	Isolate codes	CFU g^{-1} sand
1	Iron sand soil sample 1	KP11	1.2 x 10^3
		KP12	7.0 x 10^3
		KP13	2.0 x 10^4
		KP14	6.0 x 10^5
		HP11	1.8 x 10^5
		HP12	1.0 x 10^4
		HP13	3.0 x 10^5
2	Iron sand soil sample 2	KP21	1.8 x 10^4
		KP22	8.0 x 10^4
		KP23	3.0 x 10^4
		KP24	1.3 x 10^5
		KP25	2.4 x 10^5
		KP26	1.2 x 10^5

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No.	Sampling sites	Isolate codes	CFU g^{-1} sand
		KP27	1.6×10^5
		HP21	6.0×10^5
		HP22	1.0×10^5
3	Iron sand soil sample 3	KP31	2.2×10^4
		KP32	9.0×10^3
		KP33	3.0×10^4
		KP34	6.0×10^4
		KP35	1.8×10^5
		KP36	1.4×10^5
		KP37	7.0×10^4
		HP31	2.3×10^5
		HP32	5.0×10^5
		HP33	1.1×10^6
4	Iron sand soil sample 4	HP41	7.0×10^4
		HP42	1.6×10^5
5	Iron sand soil sample 5	KP53	1.1×10^4
		HP51	4.0×10^4
		HP52	1.0×10^5
		HP53	1.0×10^5

Table 2. Morphological characteristics of the bacterial isolates

No	Isolate codes	Colony colors	Cell shapes	Gram	Motility	Pellicle formation	Flagella arrangement	Pleomorphism
1	KP11	Pink	Vibroid	-	+	+	Single polar	+
2	KP12	Pink	Rod	-	+	+	Single polar	+
3	KP13	Pink	Vibroid	-	+	+	Single polar	+
4	KP14	Pink	Rod	-	+	+	Single polar	+
5	KP21	Pink	Rod	-	+	+	Single polar	+
6	KP22	Pink	Rod	-	+	+	Single polar	+
7	KP23	Pink	Rod	-	+	+	Single polar	+
8	KP24	Pink	Rod	-	+	+	Single polar	+
9	KP25	Pink	Rod	-	+	+	Single polar	+
10	KP26	Pink	Vibroid	-	+	+	Single polar	+
11	KP27	Pink	Rod	-	+	+	Single polar	+
12	KP31	Pink	Vibroid	-	+	+	Single polar	+
13	KP32	Pink	Vibroid	-	+	+	Single polar	+
14	KP33	Pink	Rod	-	+	+	Single polar	+
15	KP34	Pink	Vibroid	-	+	+	Single polar	+
16	KP35	Pink	Vibroid	-	+	+	Single polar	+
17	KP36	Pink	Rod	-	+	+	Single polar	+
18	KP37	Scarlet	Rod	-	+	+	Single polar	+
19	KP53	Pink	Rod	-	+	+	Single polar	+
20	HP11	Pink	Vibroid	-	+	+	Single polar	+
21	HP12	Pink	Rod	-	+	+	Single polar	+
22	HP13	Pink	Rod	-	+	+	Single polar	+
23	HP21	Pink	Vibroid	-	+	+	Single polar	+
24	HP22	Pink	Vibroid	-	+	+	Single polar	+
25	HP31	Pink	Vibroid	-	+	+	Single polar	+
26	HP32	Pink	Rod	-	+	+	Single polar	+
27	HP33	Pink	Vibroid	-	+	+	Single polar	+
28	HP41	Pink	Rod	-	+	+	Single polar	+
29	HP42	Pink	Rod	-	+	+	Single polar	+
30	HP51	Pink	Vibroid	-	+	+	Single polar	+
31	HP52	Pink	Vibroid	-	+	+	Single polar	+
32	HP53	Pink	Vibroid	-	+	+	Single polar	+

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Population of sand soil bacteria was dominated by pink colonies while only one had scarlet colony grown on Congo red medium (Table 2). The isolates were assumed as *Azospirillum.* Further characterization resulted in 17 strains having rod shape cells, and 15 isolates were vibroid cells. All the isolates were Gram negative and motile with single polar flagellum, form pellicle in NfB semisolid, and pleomorphic cells. Biochemical tests revealed that all the strains were positive in oxidase, catalase, and nitrate reduction. Similarly, nutritional tests showed that all isolates could utilize malate, succinate, pyruvate, or lactate as sole carbon sources (Table 3). According to Begey's Manual of Determinative Bacteriology 9th edition (Holt *et al.,* 1994), these isolates belonged to *Azospirillum.*

Those isolates were capable of solubilizing inorganic phosphate to an available phosphate with various solubilization efficiencies (E) after six-days incubation. Table 4 showed that nine isolates (KP14, KP23, KP24, KP27, KP31, KP34, KP36, KP37, HP150) were having high E values ranging from 150 to 190; although KP34 strain did not produce clear zone around the colony. These results indicated that most of *Azospirillum* isolates promising to play a role in the availability of phosphorous in the iron sand habitat. In tropical soil, phosphorous was the most limiting nutrient, only 0.1% of the total P present was available to the plants because of its chemical bonding and low solubility (Tilak *et al.,* 2005). The present isolates were similar to 46 bacterial strains isolated by Muthezhilan *at al.* (2012) from sand dunes of Chennai coast, India and six strains had high phosphate solubilizing activity. Several researchs had proven that strains of *Azospirillum* spp. isolated from different environments, including coastal areas were able to solubilize phosphate with E values ranging from 100 to 160 (Widawati and Muharam, 2012).

Table 3. Biochemical and nutritional characteristics of the isolates

No	Isolate codes	Oxidase	Catalase	Nitrate reduction	Sole carbon source utilization			
					Malate	Succinate	Pyruvate	Lactate
1	KP11	+	+	+	+	+	+	+
2	KP12	+	+	+	+	+	+	+
3	KP13	+	+	+	+	+	+	+
4	KP14	+	+	+	+	+	+	+
5	KP21	+	+	+	+	+	+	+
6	KP22	+	+	+	+	+	+	+
7	KP23	+	+	+	+	+	+	+
8	KP24	+	+	+	+	+	+	+
9	KP25	+	+	+	+	+	+	+
10	KP26	+	+	+	+	+	+	+
11	KP27	+	+	+	+	+	+	+
12	KP31	+	+	+	+	+	+	+
13	KP32	+	+	+	+	+	+	+
14	KP33	+	+	+	+	+	+	+
15	KP34	+	+	+	+	+	+	+
16	KP35	+	+	+	+	+	+	+
17	KP36	+	+	+	+	+	+	+
18	KP37	+	+	+	+	+	+	+
19	KP53	+	+	+	+	+	+	+
20	HP11	+	+	+	+	+	+	+
21	HP12	+	+	+	+	+	+	+
22	HP13	+	+	+	+	+	+	+
23	HP21	+	+	+	+	+	+	+
24	HP22	+	+	+	+	+	+	+
25	HP31	+	+	+	+	+	+	+
26	HP32	+	+	+	+	+	+	+
27	HP33	+	+	+	+	+	+	+
28	HP41	+	+	+	+	+	+	+
29	HP42	+	+	+	+	+	+	+
30	HP51	+	+	+	+	+	+	+
31	HP52	+	+	+	+	+	+	+
32	HP53	+	+	+	+	+	+	+

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Table 4. Solubilization efficiency (E) of *Azospirillum* isolates on Pikovskaya medium

No.	Isolate codes	E	No.	Isolate codes	E
1	KP11	133	17	KP36	158
2	KP12	119	18	KP37	164
3	KP13	104	19	KP53	121
4	KP14	173	20	HP11	136
5	KP21	125	21	HP12	123
6	KP22	105	22	HP13	136
7	KP23	155	23	HP21	150
8	KP24	158	24	HP22	145
9	KP25	145	25	HP31	140
10	KP26	145	26	HP32	130
11	KP27	190	27	HP33	120
12	KP31	158	28	HP41	118
13	KP32	142	29	HP42	125
14	KP33	127	30	HP51	133
15	KP34	170	31	HP52	0
16	KP35	109	32	HP53	129

CONCLUSION

It was concluded that 32 strains of *Azospirillum* spp. were successfully isolated from iron sand soil with the population ranged from 1.2 x 10^3 to 6.0 x 10^5 CFU g^{-1} sand. Nine isolates from iron sand soil had high capability in solubilizing phosphate *in vitro* with solubilization efficiency values ranging from 150 to 190.

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Screening of Nitrogen Fixer from Rhizospheric Bacteria Isolates with High Ammonium Excretion Ability

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The aims of study is to determine the characteristics of nitrogen fixer from rhizospheric bacteria which was isolated from rhizophere of maize (*Zea mays* L.) and rice (*Oryza sativa*) in Takalar district of South Sulawesi. The studies were consisted isolation of bacteria fixing bacteria by using nitrogen free media of Ashby and Burk. Characterization of bacteria isolates which potentially high ammonium excretion had done by catalase, gelatin, nitrate reduction, starch hydrolysis, casein hydrolysis, methyl red, and gram staining methods. We have isolated twenty of nitrogen fixing bacteria growth on nitrogen free medium, however among the isolates only six isolates have capability to release ammonium which detected by nessler reagent, namely ATP1.4; ATJ2.4.6; BTJ1.1.1; BTJ2.2.1; BTJ2.2, and BTP1.2.2 with an average of ammonium release were 95 □g/L, 5.0 □g/L, 315 □g/L, 256 □g/L, 192 □g/L, and 261 □g/L, respectively. The six isolates have different physiological characteristics in the ability to reduce nitrate, cell wall appearance (gram staining), catalase reaction, and the ability of the hydrolysis of starch and gelatin.

Key words; ammonium excretion, nitrogen fixer, rhizophere bacteria

INTRODUCTION

Rhizobacteria are dominant microorganism prefer inhabit in surface around the roots and have important role in mineral cycle, soil fertility and plant growth. The involvement of these bacteria in the soil is essential for biochemical processes which bind free nitrogen in the atmosphere and then conversion to ammonia (Gothwal, 2008). Nitrogen is one of the essential elements which require by all living organisms. Although nearly 80% of the atmosphere consists of nitrogen molecules, very few forms of life can use it in the free state. In fact, most organisms can use it only when combined with other elements such as oxygen and hydrogen (Benson, 2001).

Plants acquire nitrogen in the common form of nitrate (NO_3^-), ammonia (NH_3), and as well as urea $(NH_2)_2CO$ oxidation. Biological nitrogen fixation is an important process in which nitrogen fixed from atmosphere into soil ecosystems and many species of free living microbes have role in this primary of nitrogen cycle (Avinash, 2009). Several bacterial that able to fixed nitrogen have been studied which is isolated from rhizophere of some crops such as genus of Acetobacter, Arthrobacter, Azoarcus, Azospirillum, Azotobacter, Bacillus, Beijerinckia, Derxia, Enterobacter, Herbaspirillum, Klebsiella, Pseudomonas, and Zoogloea (Barraquio et al., 2000; James et al. , 2000; within Myoungsu, 2004).

A biological fixation bacterium has a specific of the enzyme complex known as nitrogenase. Which include the molybdenum nitrogenase, ATP hydrolysis, redox-active, oxygen-sensitive protein complex of two components: dinitrogenase (NifDK heterotetramer) and dinitrogenase reductase (NifH homodimer). Dinitrogenase containing iron and molybdenum cofactor (FeMo-co) which is active for the reduction of N2 (Juan, 2012). Ammonia (NH_3) is the primary product of nitrogen fixation and generates to assimilations of amino acid and nucleate acid, but if it was high concentrations can be toxic to cells. However, bacteria nitrogen fixation has a very effective mechanism for preventing the accumulation of NH_3 in the cell i.e. by converting to ammonium (NH_4^+) which also could be as precursor for the feedback mechanism of fixing nitrogen. Therefore, NH_4^+ in the cell plays a role in regulating the synthesis of nitrogenase (Gordon, 1981).

Biological nitrogen fixation is gaining importance in crop ecosystem because of current concern on the environmental and soil health quality reduction that are caused by the continuous using the nitrogen fertilizers synthetics, meanwhile we need for improving sustainable crop productivity. Thus, biological fixation of atmospheric N, especially non-symbiotic N-fixation in the soil has been subject of continuing interest in recent decades especially for low input of nitrogen in field. Weniger and Veen (1991) have been reported that the ability of *Azospirillum brasilense* to release NH_4^+ in wheat only 1 to 2% of the shoot nitrogen originated from atmospheric sources. The reason for the limited nitrogen supply from associative nitrogen fixation is probably that *Azospirillum* spp., like other free-living Diazotrophs generally does not release fixed nitrogen to its environment but it's in contrast to symbiotically living Rhizobia. Therefore,

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the future research need to search bacteria which have ability to release nitrogen fixed as NH_4^+ in the soil.

The objectives of current work are isolation and characterization of indigenous soil bacteria from rhizophere of maize (*Zea mays* L.) and rice (*Oryza sativa*) in Takalar district of South Sulawesi which have ability to release ammonium into environment.

MATERIALS AND METHODS

Isolation of nitrogen-fixing bacteria

Nitrogen-fixing bacteria were isolated from 4 locations rhizophere of maize (*Zea mays* L.) and rice (*Oryza sativa*) in Takalar district of South Sulawesi. Rhizophere of soil samples were collected carefully by uprooting the root and sieved through 2.00 mm mesh. One gram of the each crops soil sample was added into 250 ml Erlenmeyer flask containing 100 ml of Burks and Ashby-nitrogen free media as preliminary screening as well as enrichments growth of nitrogen fixation bacteria, and each of soil samples was inoculated triplicate. The flasks were incubated for 14 days at 27°C. One ml of those growth medium was collected and transferred into glasses dilution vial which containing 9 ml sterile distilled water, shaken gently for homogeneity. Serial dilutions were made as 0.1 ml of aliquots from dilution 10^{-1} until 10^{-3} were inoculated by spread method on Burk's and Ashby nitrogen-free solid media. The plates were incubated for 7 days at 27 ºC. Pure colonies were obtained by repeated streaking three times on both nitrogen free solid media. Morphologically different colonies were isolated and subculture for further analysis.

Bacterial strain storage medium

The nitrogen-fixing bacteria were stored in Burk's N-free medium with following composition in l^{-1}, Sucrose, 20.0 g; $MgSO_4.7H_2O$, 0.2 g; K_2HPO_4, 0.8 g; KH_2PO_4, 0.25 g; $CaSO_4$, 0.13 g; $FeCl_3$, 1.45 mg; Na_2MoO_3, 0.253 mg. While, Ashby medium in l^{-1}as mannitol, 15 g; $CuCl_2.2H_2O$, 0.2 g; K_2HPO_4, 0.2 g; $MgSO_4.7H_2O$, 0.2 g; MoO_3 (10%), 0.1 ml; $FeCl_3$ (10%), 0.1 ml. The pH was adjusted to 7.0 and autoclaved at 121ºC for 15 min (Atlas, 2009). Those media composition were used as same as previous media at enrichment and isolation stage above.

Quantification of ammonium release in medium

To quantify the ability of all bacteria isolate to release ammonium, one ml of well grown pure bacterial strains with different morphology were inoculated in 30 ml of Burks and Ashby broth at 100 ml Erlenmeyer flasks and incubated in an orbital shaker at 100 rpm in 27ºC for 24h. This incubation condition was an exponential growth phase of the bacteria strain isolates. After 24h incubation samples were taken and centrifuged at 13,000 rpm speed for 15 minutes. Three ml of the supernatant was taken and the pH set to 11 with the addition of NaOH 1N. Then added 0.07 ml EDTA, 0.07 ml sodium potassium tartrate, and 0.13 ml Nessler reagent were homogenized and incubated for 30 min at 25ºC. The absorbance determined at a wavelength of 435 nm by spectrophotometer (Yuen and Pollard, 1952; Leonard, 1961).

Phenotypic characterization of bacterial isolates

Physiological and biochemical characteristics of the nitrogen fixation bacteria isolates were examined according to methods described by John et al. (1994). The isolates were characterized for the following traits: colour pigment, colony shape, elevation, colony edge (margin), and surface. The Gram reaction was performed as per standard procedure. Catalase test, carbohydrate and casein source utilization, gelatinase test, reduction of nitrate and metal-red test were performed according to standard methods (Seely et al, 1991; Gothwal et al, 2008).

RESULTS AND DISCUSSION

Isolation and Purification of ammonium excreting bacteria

Four crop soil samples were taken in Takalar district which namely maize soil 1st, maize soil 2nd, rice soil 1st and rice soil 2nd. Twenty nitrogen fixing-bacteria have been isolated from four samples of soil around the roots of maize and rice. All of bacteria isolates are assumed have capacities to fix nitrogen from atmosphere as they were isolated through enrichment nitrogen free media to enhance biological nitrogen fixation while inhibit other microorganism. The macroscopic morphology of colony bacteria isolates was summarized in table 1.

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Table 1. The Macroscopic appearance of nitrogen fixing bacteria isolated around plant roots of maize and rice soils of Takalar district.

Isolate codes	Macroscopic appearances				
	Colony Shape	Elevation	Surface	Colony edge	Colour
ATJ1.1.1	Circular	Convex	Glistening	Smooth	White
ATJ1.2.1	Irregular	Convex	Glistening	Smooth	White
ATJ1.2.2	Circular	Convex	Glistening	Smooth	water soluble
ATJ1.3	Circular	Convex	Glistening	Smooth	White
ATJ1.4	Circular	Convex	Glistening	Smooth	White
ATJ2.2	Circular	Convex	Glistening	Smooth	White
ATJ2.3.2	Circular	Convex	Rough	Smooth	White
ATJ2.4.6	Circular	Convex	Glistening	Smooth	White
ATP1.2	Circular	Raised	Glistening	Smooth	water soluble
ATP1.3	Circular	Raised	Glistening	Smooth	White
ATP1.4	Circular	Raised	Glistening	Smooth	water soluble
ATP2.1.1	Circular	Raised	Glistening	Smooth	water soluble
ATP2.3.3	Irregular	Flat	Rough	Lobate	White
ATP2.4.1	Circular	Convex	Glistening	Smooth	water soluble
ATP2.4.2	Irregular	Raised	Glistening	Undulate	Red
ATP2.4.3	Circular	Raised	Rough	Undulate	Red
BTJ.1.1.1	Circular	Convex	Glistening	Smooth	White
BTJ2.2.1	Circular	Convex	Glistening	Smooth	water soluble
BTJ2.2	Circular	Convex	Glistening	Smooth	water soluble
BTP1.2.2	Circular	Raised	Glistening	Smooth	water soluble

The macroscopic appearance of 20 nitrogen fixation bacteria isolated showed morphological different, although there are some isolates had same morphological appearance, but those isolates from another crop rhizophere, hence we assume tentatively that bacteria isolates as different strains, i.e. macroscopic observations of isolates ATJ1.4 and ATJ2.2 codes are seems same, but they were obtained from maize 1st ATJ1.4 1, while ATJ2.2 obtained from maize 2nd. There were 11 isolates from maize which its 6 isolate from maize 1st and 5 isolates from maize 2nd. In the rice rhizophere found 9 isolates, which 4 isolates from rice 1st and 5 isolates from rice 2nd.

The ability of bacteria isolates to release ammonium

Twenty isolates were grown in nitrogen free medium broth for 24h and then centrifuged to separate cell bacteria and the supernatant, the result showed among 20 isolates, only 6 isolates that have ability to excrete ammonium into medium (Table 2).

Table 2. The amount of ammonium release by 6 isolate

No	Isolate code	Ammonium (µg/l)	Average (µg/l)
1.	ATP1.4	99,92 84,55 99,92	95
2.	ATJ2.4.6	30,74 15,37 -30,74	5
3.	BTJ1.1.1	937,69 553,39 -545,71	315
4.	BTJ2.2.1	322,81 -46,12 491,90	256
5.	BTJ2.2	69,17 399,67 107,60	192
6.	BTP1.2.2	391,99 361,24 30,74	261

Ammonium excretions for all isolates have collected at 24h incubation. Figure 1 shows a graph of the averages growth of isolates which pointed average time of exponential growth of

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isolates at 24h. Previous studies suggested that ammonium excretion began in exponential growth phase to the stationary phase (Narulaet al., 1981 and Bali et al., 1992 in Hartono, 2009) and this phase of bacteria also stated that the amount of ammonium in medium was not from cell bacteria lyses.

Table 2 showed the amount of ammonium that can be excrete by the bacteria isolates in exponential growth phase and from 20 isolates were examined for their ability to release ammonium, only 6 isolates that have affinity to excrete ammonium into medium. The six isolates that have potential to produce ammonium are namely ATP1.4; ATJ2.4.6; BTJ1.1.1; BTJ2.2.1; BTJ2.2, and BTP1.2.2. The average value for 6 isolates to release amount of ammonium were 95 □g/l, 5 □g/l, 315 □g/l, 256 □g/l, 192 □g/l, and 261 □g/l, respectively. However, when compared in both medium used, the isolates were grown in Burk's medium has a highest ammonium content value compared with the isolates were grown on medium Ashby's. This result might be due to the Ashby's medium containing mannitol a complex polysaccharide which would lead medium to be salty and possible repressed the activities of nitrogenase complex as well as the affinities of cell membrane tended less to diffuse ammonium into medium.

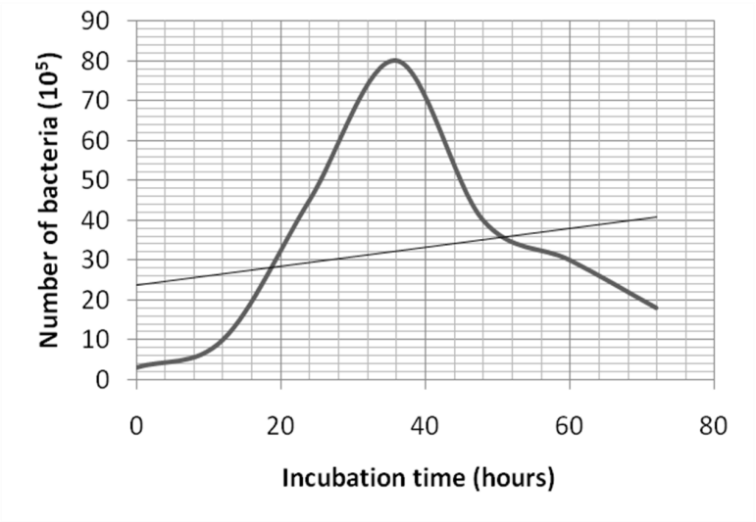

Figure 1. Exponential growth of Bacteria Selected (average)

Characteristics of nitrogen fixing bacterial isolates

Nitrogen fixation bacteria isolates which have been tested and showed significant values for release ammonium were characterized. Various different biochemical properties of the isolates are shown in table 3. The isolate strains were found mostly coccus (rod) form and have different type of cell wall. The catalase activity and gelatin hydrolysis were present in all isolates, whereas no isolate was found to perform **positive of metyl-red test which also explain that the isolate did not ferment carbohydrate to produce organic acid as *Escherchia coli* biochemical pathway**. Starch degrading capacities were detected in isolates code of ATP1.4, ATJ2.4.6, BTJ1.1.1, BTJ2.2.1, and BTP1.2.2. Among isolates, ATP1.4 and BTP1.2.2 have capacity degraded casein. While, only Isolates BTJ1.1.1 expressed the negative nitrate reduction.

Table 3. Characteristics of nitrogen fixing bacterial isolates from the rhizophere of maize and rice crops

Isolate code	Microscopic		Biochemical					
	Shape	gram	katalase	Gelatin Hydrolysis	Nitrate reduction	Starch hydrolysis	Casein Hydrolysis	Methyl red
ATP1.4	Coccus	-	+	+	+	+	+	-
ATJ2.4.6	Coccus	+	+	+	+	+	-	-
BTJ1.1.1	Coccus	+	+	+	-	+	-	-
BTJ2.2.1	Coccus	-	+	+	+	+	-	-
BTJ2.2	Coccus	-	+	+	+	-	-	-
BTP1.2.2	Coccus	-	+	+	+	+	+	-

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CONCLUSION

The studies reported that there were 20 nitrogen fixation bacteria successfully isolated from rhizophere of maize and rice crop in Takalar district. However, only 6 isolates those have significant value levels to excrete ammonium and have different in morphology and biochemical characteristics. The isolate namely BTJ1.1.1 has highest capacity to excrete ammonium to media. Further studies need to identify the bacteria isolates by independent methods and their effect to plant growth.

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Early Ontogenesis of Small Nypa Palm Worm *Namalycastis abiuma* (Polychaeta: Nereididae)

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The purposes of research to find the best condition for fertilisation and production of Nypa palm worm larvae (*Namalycastis abiuma*). Gamete samples were collected using a tube glass capilarry injected to the middle part of body segment of the matured worm. Artificial fertilisation was done by mixing the sperms and oocytes in the same container with sterilised sea water as the media. The initial development process prior to fertilisation was observed until benthic phase larvae (3-setigers). Fertilisation was done under salinity of 10-15‰ and water temperature of 27-29°C. The cleavage was achieved within 15-25 minutes and larval stage up to 260 hours after fertilization. The fertilisation and larval development of *N. abiuma* were highly influenced by the water salinity and temperature, room temperature and culture media.

Keywords : *Namalycastis*, Ontogenesis, Nypa palm worm, Larval, Polychaeta

INTRODUCTION

The supply of polychaete worms has been suggested to alleviate the problems of feed for broodstocks in aquaculture industry (Olive, 1999). This potential role was also doubled with the higher nutrient profiles of polychete worms than other natural feed sources to induce the development and maturation of shrimp (Murugesan *et al*., 2011) and fish (Parthiban *et al*., 2006). It was also found that polychaetes were the best maturation diet for shrimp broodstock which possesed the greatest variation of prostaglandin E_2 (PGE_2) when compared with other live feeds (Meunpol *et al*., 2010).

The small nypa palm worm (*Namalycastis abiuma*) is one of Nereidid species that has been used as fishing bait for fish and shrimp in West Kalimantan. Nypa palm worm was known as potential feed source, however, worms for commercial purposes were still collected from natural populations in an unsustainable way. In addition, the scientific knowledge needed for mass production and stock sustainability was not fully available. In the other hand, reproductive data and larval characteristic are crucial information for mass production and key to success in palm worm's aquaculture. In order that, the purpose of our study was to know the artificial fertilization procedure for larval mass production in laboratory and to determine the characteristics of Nypa palm worm larvae.

MATERIALS AND METHODS

Nypa palm worms were collected from mangrove area of Kapuas estuarine in West Kalimantan on May until July 2012. Worms were taken to the laboratory using a container that also contained sediment samples. The worms were then removed from the sediment sample and the sorting was done to separate mature and immature individuals. Selected mature worms were then placed in a different container. Matured individuals were differed by the immatures based on body coloration. Female matured individuals showed redish body color, meanwhile, male showed greenish body color.

Gametes from each individual were collected with a tube glass capillary injected to the ventro-lateral part of body segments and observed under the microscope. Matured egg was determined by its diameter and lipid droplets distributed in the egg. The size of egg diameter was measured by eyepiece micrometer. The diameter of matured eggs were up to 120μm with full lipid droplets. Matured eggs and spermatozoa were then mixed in a container of sea water with 2:1 ratio.

Fertilization was conducted based on environmental laboratory setting using sea water as media and filter paper to minimize the dissolved contaminants. Initially, the experiment with serial sea water concentration was performed to find optimum salinity ranging from 1‰ to 33‰, i.e. 1-3‰, 4-6‰, 7-9‰, 10-12‰, 13-15‰, 16-18‰, 19-21‰, 22-24‰, 25-27‰, 28-30‰, and 31-33‰. The salinity was obtained by mixing sea water with distilled water. Optimum salinity was determined by the successful of fertilization, cell division and larval development.

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Fertilization was observed under the microscope with 100x magnification. Successful fertilization was determined by the formation of fertilization membrane and the initiation of egg cell division. Eggs were then observed every 5 to 10 minutes. Unfertilized eggs were counted and separated from the fertilized ones. The rate of conception was calculated based on the percentage of fertilized eggs to the total number of eggs.

The larval characteristics determination is important to know the stages of development. The process of development included the cleavage stages from 2, 4, 8, 16 and 32-cell division until 3-setiger larval stage. Larval morphology and development were also observed and described descriptively at each stage of development. Larval morphological characteristics were referred to other general larvae of Nereididae according to Rouse (2000), *Hediste* (Sato, 1999) and *Namanereis littoralis* (Ezhova, 2011).

RESULT AND DISCUSSION

The maturity of Nypa palm worm was characterized by the changes in body color, but non swarming epitokous. Unlike other Nereidid species such as *Platynereis dumerilii* and *Nereis virens*, the Nypa palm worms do not perform modified parapodia, chaeta and eyes for spawning processes in the water. Nypa palm worm maturity characteristics were more similar to *Nereis diversicolor* (Scaps, 2002). Matured male individuals were characterized by the changing of body color from pink to dark red with greenish side part of the body, meanwhile, the matured female individuals showed dark red side part (Junardi *et al*., 2012).

An overview of all developmental stages of Nypa palm worm is showed in Table 1. The optimum salinity was ranged from 10 to 15‰ and the water temperature was between 25-29ºC. Unfertilized eggs were indicated by lipid droplets accumulated in the center of the egg, meanwhile fertilized eggs were indicated by the formation of fertilization membrane and cortical migration of lipid droplet. Fertilization was achieved in the period of 15 to 70 minutes (n = 30). The characteristics of cell division was described by the spiral cleavage, holoblastic, meridional and unequal. The first cleavage occured 40 minutes after conception with the formation of dividing two blastomeres, i.e. macromeres and micromeres (non homogenous).

Table 1. Oocytes cleavage and development in range of salinity 10-15‰ and temperature 27-29ºC.

No	Time (minutes)	Cells division
1	0	Mixed oocytes and sperm
2	15	Fertilized membrane
3	40	2-Cells
4	70	4-cells
5	140	8-cells
6	180	16-cells
7	220	32-cells
8	260	morula

Early trochophore larvae was indicated by slow rotation driven by multiciliated equatorial prototroch and followed by the formation of two eyes. The next stage was late trochophore and metatrochophore which indicated by the development of chaetae and chaetigerous segment. Developmentally, segmented larvae formation of the three first segments (3-setiger) or nectochaeta early stages and the last stage of nectochaeta were formed within three days after fertilization.

The fertilization of *N. abiuma* can occur in a wide range of salinity. In this study, we found that the optimum salinity for fertilization was ranged between 10-15‰. It showed that the reproduction characteristic of *N. abiuma* is well suited to the conditions with fluctuating salinity in the estuarine environment. The range of salinity was also suitable for larval development. Optimum range of salinity was also found in *Namanereis littoralis* (6-21‰) (Ezhova, 2011). In the other hand, *Hediste diadroma* can still adapt to higher salinity of 27.5-30‰ which is usually infavorable for fertilization in other Namanereidinae species (Junardi *et al*., 2012). Meanwhile, water temperature of 25-29°C is assumed to be favorable for most tropical species.

Fertilized eggs showed lipid droplets migration to the peripheral part of the egg. This characteristic is also found in other Nereidid species i.e. *Platynereis dumerilii* (Fischer, *et al*., 2010). The cleavage characteristic of N. abiuma is also similar to *Platynereis dumerilii* (Subfamily Nereidinae) (Fischer *et al*., 2010), *Sabellastarte spectabilis* (Family Sabellidae) (Bybee *et al*., 2006) or other Polychaeta worms. After 32-cell division, the next stage is a morulla rotation which

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is initially moving at the bottom until its planktonic phase. The free-swimming phase of morulla is called the early stage of larval trochophore. Lipid droplets still exist in the larval stage. This characteristic is generally described as lechitotropic larvae (Tosuji and Sato, 2006). Lechitotropic larvae is a short-period growing larvae with yolk as food source for metamorphosis. However, the characteristics of the Nypa palm worm's larvae could be very different at the subfamily level. For example, late-stage *Namanereis littoralis* larvae are coated inside a capsule rather than free-swimming larvae (Ezhova, *et al*., 2011).

The trochophore larvae is also characterized with the enlargement of its diameter from 120 μm into 200 μm with body surrounding cilia (equatorial cilia) and two-eye development. Free-swimming larvae was developed at the second day after fertilization; it was gradually turned into oval and ciliated body at the latero-dorsalis part. This free-swimming larvae has high mortality rate when the salinity of the culture media is higher than 21‰. We assumed that the optimum salinity for fertilization and larval development was ranged between 10-21‰. Based on this result, we conclude that larval survival is strongly influenced by water salinity.

At the third day after fertilization, the larvae is becoming spherical but non parapodia. Yet, the chetae has developed at certain parts of the body. The above characteristics are described as final stage of larval trochophore or metatrochophore. At the next stage, the larvae was supplemented by the development of chetigerous parapodia (chetiger). This nectochaeta stage was characterized by the formation of chetiger and antenna at the prostomium part of the larval body. The final stage of larval development is called nectochaeta which takes six days. The morphological characteristic of this final stage is a formation of a pair of tentacle cirri in anterior and anal part of the body. The third-day formation of 3-chetiger larva in *N. abiuma* takes longer time than the one in *Nereis* sp. (Yuwono, *et al*., 2002).

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Pseudomonas cocovenenans as a Positive Control Bacteria for True-Lipase Activity Assay

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Activity of true lipase enzyme (lipase EC 3113) can be detected qualitatively through orange fluorescence performance around bacteria colony grown on Rhodamine B medium. The orange fluorescence observed under UV light exposure on wave length 366 nm. The research conducted using 3 isolates namely *Pseudomonas cocovenenans*, *Pseudomonas fluorescens*, and *Pseudomonas aureuginosa*. The result showed that rod Gram negative bacteria *Pseudomonas cocovenenans* could function as positive control bacteria for true lipase assay since capable showed strongly performance of orange fluorescence after incubated for 13 days compared to the two others bacteria.

Key words: *Pseudomonas cocovenenans,* true-lipase enzyme, rhodamine B medium, orange fluorescence.

INTRODUCTION

Lipase enzyme consists of glycerol ester hydrolase that works at long chain of acylglycerol (true lipase, EC 3.1.1.3) and that works at short chain of acylglycerol (esterase, EC3.1.1.1) producing fatty acid and glycerol (Jaeger *et al*., 1999). True-lipase (EC 3.1.1.3) is important in industry, used for lipid hydrolysis or as catalist in synthetic organic chemical. Therefore, research in lipase enzyme is propectous, includes research in gene responsible in synthesis lipases and exploration isolates producing lipases. Involved in this prospect was selection of thermophilic bacteria capable producing thermophilic lipases.

Mechanism assay for selecting isolate capable in producing true-lipase enzyme EC 3113 is conducted by growing isolates on medium agar containing oil as substrate and Rhodamine B as an indicator. Positive result is indicated by pink zone surrounding growth colony as a result of reaction between enzyme excreted and oil substrat in medium. The reaction produces fatty acid that then react with rhodamine B forming pink color. Reaction with rhodamine B performes an orange fluorescence when it observed under UV rays exposure at λ 350 nm (Kouker dan Jaeger, 1987).

Assay mechanism requires isolate functions as positive and negative control isolate to ensure the observation by comparing performance result. Based on that specific character, the observation was aimed to obtain lipolitic bacteria that capable as positive control in true lipase enzyme activity assay.

MATERI AND METHOD

Research material

Bacteria isolates (*Pseudomonas cocovenenans*, *Pseudomonas fluorescens*, *Pseudomonas aureuginosa*, *Eschechia coli*), medium NB, medium NA, olive oil, rhodamine B, UV cabinet λ 366 nm.

Induction Culture

0,4 mL (2%) of bacteria broth culture in NB medium was pippeted and inoculated into 20 mL new NB medium supplemented with 0,2 ml olive oil (concentration 0,1%). Incubation in temperature 53^{o}C for 24 – 48 hr in shaker incubator, regarding as induction culture.

Lipolitic Assay (Kouker and Jaeger, 1987)

Serial suspension dilution was made from induction culture until 10^{-}3 using sterile aquadest. The last suspension was inoculated into medium NA containing olive oil and rhodamine B pointly or streakly method. The culture was then incubated in temperature 53^{o}C for 24 hr or until isolate showed fluorescence performance. fluorescence performance was observed in the UV cabinet using wave length 366 nm. Isolate that possess lipolitic activity showed orange fluorescence halo around growth colony. For comparation, it was used *Eschericia coli* known as negative true-lipase bacteria.

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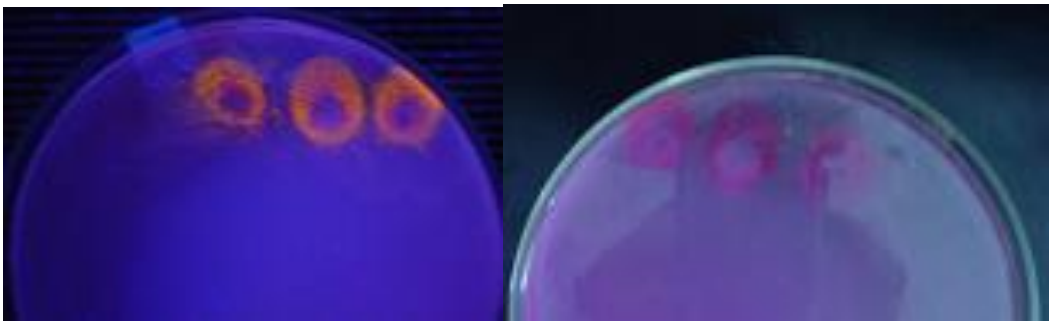
RESULT AND DISCUSSION

Induction Culture

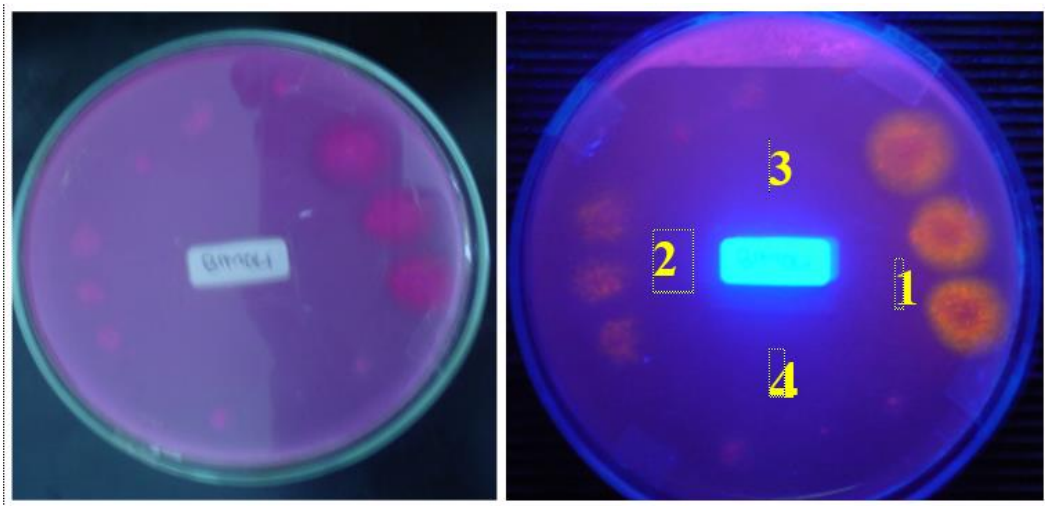
All bacteria isolates grew in NB medium, indicated by turbid broth. Isolates also developed a pink growth colony when grown on NA medium.

Lipolitic EC 3113 Assay

Bacterial growth on assay medium consist of NA + olive oil + rhodamine B yield pink growth colony. fluorescence observation of true-lipase bacterial activity in UV cabinet at λ 366 nm showed different performance of orange fluorescence around bacterial colony after 13 days incubation. *P. cocovenenans* showed the stongest fluorescence, while the others indicated weak fluorescence (picture 1 and 2). Furthermore observation showed that the longer incubation period the wider fluorescence zone. While *E.coli* colony did not show fluorescence performance. Below is fluorescence picture of *P. cocovenenans* in UV cabinet observation (left) and directly observation (right) :



Picture 1. *Pseudomonas cocovenenans*, left : under UV λ 366 nm observation, right : no UV observation.



Picture 2. Performance of agar plate assay of true-lipase activity, left : no UV observation, right : under UV observation. Assay isolates : 1.*P. cocovenenans*,2.*P. fluorescens*, 3. *P. aureuginosa*, *4. E. Coli*

Table below is fluorescence assay and cell morphology observations of *P. cocovenenans* and other bacteria :

Isolate	Fluorescence	Cell morphology	Gram character
P. cocove nenans	+++	rod	Gram -
P. fluorescens	+	rod	Gram -
P. aureuginosa	+	rod	Gram -
E. coli	-	rod	Gram -

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Information :
+++ strongly orange fluorescence
+ weak orange fluorescence
- no orange fluorescence

After incubation, isolate *P. cocovenenans* showed the most strong orange fluorescence. Furthermore, after 13 days, fluorescence zone developed stronger array and wider zone. Fluorescence might be influenced by bacterial growth. The longer incubation time the more enzyme produced and more fatty acid yielded. When fatty acid meet rhodamine B, they develop orane fluorescence.

For comparation, bacteria that only produce esterase enzyme but not lipase EC 3113 do not form orange fluorescence zone. Those isolate only form clear zone in medium containing oil. Bacterial colony color is pink like medium color but not form reaction zone with oil substrat and rhodamine B (Kouker dan Jaeger, 1987).

Lipolitic bacteria is actually easy isolated using pH indicator Nile-blue or Victorian-blue. However, both indicators get reaction with pH exchange and inhibit some bacterial growth. Plate assay method of Kouker and Jaeger (1987) eliminates pH exchange sensitivity and enable to reisolate the test organism.

Lipase enzyme sinthesis is inducible characteristic (Moat dan Foster, 1984), means that the existance of substrat in the growth medium inducing enzyme sinthesis. Ruiz et al. (2005) use emulsified CeNAN-olive oil agar medium with rhodamine B as indicator for lipolitic assay, successfully obtain 48 strain fluoresency isolates. One isolate, *Bacillus* CR179 showed the strongest fluoresency performance.

CONCLUSION

Regarding the research result can be concluded that *Pseudomonas cocovenenans* capable as positive control isolate in true lipase enzyme assay.

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Bacterial Diversity in Buffaloes Meat and Bowel from Traditional Market Based on Its Initial Contamination

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An experiment has been conducted to know bacteria diversity in domestic buffalo meat and bowel from Pandeglang Banten traditional market in order to ascertain its safety based on its initial contamination. The total bacterial was assessed by total plate count method as index of quality. The buffalo meat and bowel samples were taken from liver, intestine, lymph, lung and tripe. Results showed that the contaminated bacterial were aerobic bacteria, coliform, *Escherichia coli*, and *Staphylococcus* spp. in buffalo meat and bowel. The amount of aerobic bacteria was in the range 1.7 x 10^5 up to 2.3 x 10^6 CFU/g(colony-forming units per gram), while total coliform bacteria was in the range 2.0 x 10^3 up to 6.8 x 10^4 CFU/g. Total number of *E. coli* was in the range 2.0 x 10^3 up to 6.0 x 10^4 CFU/g and for S*taphylococcus*, spp. was in the range 2.0 x 10^4 up to 2.7 x 10^5 CFU/g. No *Salmonella* was detected in all samples observed. The total coliform bacteria, *E. coli* and *Staphylococcus*, spp in all buffalo meat and bowel samples have exceeded the standard microbial number from the Indonesia National Standard (SNI). It is necessary to further evaluate the quality assurance and security management. Proper sanitation and hygiene in slaughterhouse must be achieved to minimize pathogenic bacteria contamination and improve domestic meat quality. Improvement of the quality is needed to shelter meat domestic production from import competition, as a preparation for Indonesia to achieve Indonesia beef self-sufficiency in 2014.

Keywords: bacteria, buffalo, initial contamination

INTRODUCTION

The total of Buffaloes population in Indonesia in 2012 are 1.37 million, it is increasing 5.5% compared to its population in 2011 (Ditjennak, 2012). Buffaloes have been in Indonesia for a long time, so they are well adapted to the environment and become the part of the lives of the majority of Indonesia society. The increasing of human activity caused in decreasing of buffalo population (Sulaeman, 2011). Buffaloes are in the second rank based on its population less than beef (Santoso, and Tuhwerkih, 2003). It is known that buffaloes have more advantages than other ruminants such as cows and goats. The buffaloes have high natural adaptability (low mortality and more resist to pathogen) and can live with low quality feed (Fadhilah, 2011 and Nurani, 2011). In addition to the meat and milk used, energy is also used to plow fields, pull carts and complement traditional ceremonies. Buffalo meat has a unique taste compared to beef, while the buffalo skin can be used to craft especially for drum (Fadillah, 2011). In Tana Toraja, South Sulawesi, the buffalo has an important role in ritual ceremonies (Sariubang et al., 2003).

On buffalo meat and bowel which is consumed there is probability to have bacterial contamination that could be harmful for consumers. Generally buffaloes are not slaughtered in slaughterhouse with good animal health control facility where the cows usually are slaughtered with good facility. Quality assurance and security management may not be noticed by the slaughterer, so the presence of microbial contamination in meat and bowel derived from microbes that entering the blood circulation at the time of slaughtering. Then subsequent contamination can occur during preparation as the division process carcass, meat or bowel prior to distribution (Soeparno, 1994).

Bacteria which are not expected to exist in meat and bowel are coli groups like Escherichia coli, especially E. coli 0157: H7, it can cause hemorrhagic colitis which is often found in polluted water by human waste (Harsojo, 2011). Meat is a good medium for bacteria to grow. According the Food and Agriculture Organization (FAO), quoted from Badwater (2003) and Sri Poernomo (1995), more than 80% of food poisoning is caused by pathogen bacteria. According to Sri Poernomo (1995) and Keeraptipibul (2005), the case of food poisoning can occur due to the cross contamination of bacteria. It will happen when the bacteria from one contaminated food sources will transfer to another source that has not been polluted yet and usually are freshly cooked food. The contamination may occur due to either food location storage or food that has not been polluted then become polluted because the cross contamination.

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The purpose of this experiment is to analyze the initial contamination that can affect the quality of buffalo meat and bowel and compare the common result with the results of previous studies.

MATERIALS AND METHODS

Materials

The samples were used in this research were buffalo meat and boewl which were purchased from traditional market in Pandeglang, Banten. Each sample was bought from some butchers with replication for three times.

Determination of total aerobic bacteria

Determination of total aerobic bacteria was done by weighing a sample of 25 g, and then mixed with sterile peptone water (225 ml) and subsequently performed graded dilution. A total of 0.1 ml suspension grown on plate media for Petri dishes which were containing nutrient agar (Oxoid) and stored at room temperature for 24-48 hours for the next calculated the number of bacterial colonies.

Determination of total coliform bacteria

Determination of the number of coliform bacteria was done as in determining the number of aerobic bacteria, except the used of media material. The used medium was selective for Mac Conkey media (Oxoid) and stored at a temperature of 37^0 C for 24-48 hours for the next calculated the number of bacterial colonies.

Determination of the number of bacteria E. coli

Determination of the number of bacteria *E. coli* were performed using EMB medium (Oxoid) according to the method of Fardiaz (1989) and Harsojo (2011).

Determination of the number of Staphylococcus spp

Determination of the number of *Staphylococcus*, spp. was done by weighing a sample of 25 g, and then mixed with sterile peptone water (225 ml) and subsequently performed garded dilution. A total of 0,1 ml suspension grown on plate media in Petri dishes which were containing that Baird Parker (Oxoid) and stored at a temperature of 37^0 C for 24-48 hours. After that the number of bacteria that had grown was calculated.

Detection of Salmonella

Detection of *Salmonella* was done by using enrichment medium Tetrathionate Broth Base. a sample was weighed 25 g, then it was put into 225 ml enrichment medium and incubated at 37^o C for 24 hours. After 24 hours incubation one loop suspension was cultivated in selective media (XLD). The colonies were examined at 37^o C after 24-48 hours. The colonies were identified using biochemistry and serology test (Harsojo, 2011).

RESULTS AND DISCUSSIONS

The total of bacteria in buffalo meat and bowel showed in Table 1. In the table 1 showed the total of aerobic bacteria in buffalo meat and bowel were found in the ranged from 1,7 x 10^5 and 3,3 x 10^6 CFU/g. The results of research of Harsojo (2010) showed the number of aerobic bacteria in the meat was 1,7 x 10^6 CFU/g, the result is greater than the results obtained now and have passed the limit that allowed by SNI (2009). According to the Indonesian National Standard (SNI) (2009), Limit of Microbial contamination (BMCM) of animal origin are permitted is 1 x 10^6 CFU/g.

The total number of aerobic bacteria in the bowel such as liver and buffalo are also lower compared with the results of previous studies. In all bowel samples, the number of aerobic bacteria is still below the limit that allowed by SNI (2009). These results demonstrate an increase in knowledge of hygiene so that the results obtained are now on average lower 1 decimal.

In this research also carried out observations for coliform bacteria contamination in buffalo meat and bowel. Coliform bacteria as one type of bacteria are often used as indicator of sanitation (Suriawiria, 2003). The use of coliform bacteria as indicator have advantage in identifying any contamination in food and other materials, because they are more resistant than other bacteria

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during processing and storage process (Harsojo, 2011). Therefore, the use of detection techniques coli bacteria in the material is very important, it will be known whether the material is able for consumption proficiency or not. The presence of coliform in food is undesirable because it means the material has been contaminated by the human feces and other possibilities were also contains other harmful pathogenic bacteria (Suriawiria, 2003 and Harsojo, 2011). The total of coliform bacteria in buffalo meat and bowel showed in Table 2. In the table 2 showed that the number of coliform bacteria were found in the range 2,0 x 10^3 and 6,8 x 10^4 CFU / g. Based on the SNI (2009), allowable limit of the permissible concentration of coli bacteria which is allowed 1,0 x 10^2 CFU / g. Thus all the materials tested samples have passed the allowed threshold of SNI. Compared with the previous results of the Harsojo study (2010), it showed that the number of coli bacteria in meat, liver and intestine in this study there is a difference of 1 decimal lower.

Table 1. The Number of Aerobic Bacteria in Samples of Buffalo Meat and Bowel (CFU/g)

Sample	Total Aerobic Bacteria	
Outer Carcass Meat	4,2 x 10^5	1,7 x 10^7 *
Inner Carcass Meat	3,3 x 10^6	
Liver	1,7 x 10^5	1,2 x 10^6 *
Intestine	2,0 x 10^5	2,3 x 10^6 *
Lymph	4,2 x 10^5	-
Lung	3,4 x 10^5	-
Tripe	2,7 x 10^5	-
SNI (2009)	1,0 x 10^6	

Note : - = no data; * Harsojo (2011)

Table 2. The Number of Coliform in Samples of Buffalo Meat and Bowel (CFU/g)

Sample	Total Coliform Bacteria	
Outer Carcass Meat	5,0 x 10^3	4,2 x 10^5 *
Inner Carcass Meat	6,8 x 10^4	
Liver	2,1 x 10^4	2,3 x 10^5 *
Intestine	1,8 x 10^4	7,7 x 10^5 *
Lymph	2,2 x 10^4	-
Lung	2,0 x 10^3	-
Tripe	2,2 x 10^4	-
SNI (2009)	1,0 x 10^2	

Note : - = no data; * Harsojo (2011)

It is indicating that the slaughterers have more attention to hygiene compared with previous results. The number of coli bacteria in buffalo meat, liver and intestines are lower than previously obtained results. However, the results of the number of coliform bacteria in meat and buffalo intestine was in the range 2.0 x 10^3 up to 6.8 x 10^4 CFU / g and has exceeded the standard coliform number from the SNI (2009) which is allowed, 1.0 x 10^2 CFU / g.

In the table 3 showed the number of *E. Coli* were found in buffalo meat and bowel. *E. coli* in the presence of meat and the bowel are not expected because showed that the materials may have been contaminated with human feces.

Table 3. The Number of E. Coli bacteria in Samples of Buffalo Meat and Bowel (CFU/g)

Sample	Total E. Coli Bacteria	
Outer Carcass Meat	4,0 x 103	2,0 x 104 *
Inner Carcass Meat	6,0 x 104	
Liver	1,6 x 104	1,2 x 105 *
Intestine	1,5 x 104	4,0 x 105 *
Lymph	2,2 x 104	-
Lung	2,0 x 103	-
Tripe	1,3 x 104	-
SNI (2009)	1,0 x 10	

Note : - = no data; * Harsojo (2011)

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In the last foodborne illness in Germany which is caused by the *E. Coli* bacteria attacked in more than 3000 people and 14 people die. Technological developments lead changes in microorganisms, eating habits, and climate change has led to create new strains such as E. coli that are pathogenic (E. coli 0157: H7). This strain is known can cause bleeding and had horrendous world (Winarno, 2003).

In Table 3 showed *E. coli* bacteria were found at all the samples. It is indicate the low hygiene management during cutting and splitting process surrounding slaughter house, thus increasing the risk of *E. coli* contamination. The number of *E. coli* bacteria in buffalo meat and bowel was in the range 2.0 x 10^3 up to 6.0 x 10^4 CFU / g. The highest number of bacteria *E. coli* contamination was found in inner carcass meat. This is indicating unhygienic process while meat and bowel combined in one container and causing contamination.

In this experiment also observed the presence of *Staphylococcus* spp contamination in buffalo meat and bowel. Although *Staphylococcus* spp is not as dangerous as Salmonella, this bacteria can cause intoxication. It can causes foodborne illness if present in food. In the United States has been reported Staphylococcus spp poisoning which is a symptom of intoxication. According SUPARDI and SUKAMTO (1999), in each year 20% up to 50% of all poisoning occurance caused by foodborne. In addition, *Staphylococcus* infections can cause symptoms such as boils, meningitis, osteomyelitis, pneumonia and mastitis in humans and animals.

In this study no *Salmonella* was found in all the samples. However, it does not mean the buffalo meat and bowel product are safe for consumption because of coliform bacteria in the control with 0-week storage and the presence of contaminant *Staphylococcus* that above the requirements of SNI (2009).

Table 4. The Number of Staphylococcus spp. in Samples of Buffalo Meat and Bowel (CFU/g)

Sample	Total E. Coli Bacteria	
Outer Carcass Meat	2,7 x 105	2,0 x 104 *
Inner Carcass Meat	2,5 x 105	
Liver	1,2 x 105	1,9 x 104 *
Intestine	3,0 x 104	2,0 x 104 *
Lymph	2,0 x 104	-
Lung	3,9 x 103	-
Tripe	2,4 x 105	-
SNI (2009)	1,0 x 102	

Note : - = no data; * Harsojo (2011)

The high initial contamination on processed foods indicates that manufacturer hygiene less attention to sanitation and food are sold. Another possibility is that at the time of the transportation and retail seller's place they are not concerned about food safety or are not familiar with the Hazard Analysis Critical Control Point (HACCP).

Salmonella was not found in all the samples studied. However, it does not mean that meat and bowel have safely consumed because the presence of coliform bacteria, *E. coli* and *Stahylococcus* spp. in the control with 0-week storage are above the requirements of SNI (2009). Extraordinary Events (KLB) which caused by *Salmonella* and *Staphylococcus* foodborn illness are rarely reported in Indonesia. Percentage of the reported issues is still too small comparing with outbreaks that coomonly occured.

CONCLUSION

Results showed that the contaminated bacterial were aerobic bacteria, coliform, *Escherichia coli*, and *Staphylococcus* spp. in buffalo meat and bowel. Total number of aerobic bacteria, coliform, *Escherichia coli*, and *Staphylococcus* spp. was obtained in lower number with previous experiment results. The total coliform bacteria, *E. coli* and *Staphylococcus*, spp in all buffalo meat and bowel samples have exceeded the standard microbial number from the Indonesia National Standard (SNI). *Salmonella* was not found in all samples studied. It is necessary to further evaluate the quality assurance and security management. Proper sanitation and hygiene in slaughterhouse must be achieved to minimize pathogenic bacteria contamination and improve domestic meat quality to achieve Indonesia beef self-sufficiency.

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The Potentiality of Forest Litter Fungi as IAA (Indole-3-Acetic Acid) Producer

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Many industrial forest in Indonesia are replanted under intensive silviculture program. This program includes utilizing of soil microorganism for promoting better plant growth. Preliminary study was done on the exploration of forest litter fungi that are able to produce IAA. Six thermotolerant fungi (*Aspergillus* sp B.10, *Aspergillus* sp 20.2, *Penicillium* sp 4.2, *Aspergillus* sp 9.2, *Penicillium* sp 1.2 and *Trichoderma* sp B.2) have been isolated from forest litter. All isolates produced IAA with various concentration, ranges from 15.56 ppm to 76.81 ppm. Two isolates (*Penicillium* sp 1.2 and *Trichoderma* sp B.2) which produced the high IAA contents were further examined. Bioassay of 10^{-2} ppm, 10^{-4} ppm and 10^{-6} ppm of crude extract IAA using mung bean (*Vigna radiata*) seedling indicated that the extract only significantly effected on the number of lateral root. Filtrate from those isolates induced lateral root formation more than those of IAA standard on the same concentration.

Keywords: *IAA, fungi, plant growth*

INTRODUCTION

Intensive silviculture program implemented to achieve Indonesia's forests healthy, sustainable and prospective. This program was developed in the Industrial Plantation Forest (HTI). Procurement of quality seeds are given priority in this program. One of the seeds that interest to developed is meranti (*Shorea*). Intensive silviculture component that are being developed is the utilization of forest plants and litter microbe to support the growth of plant seedlings. This could be supported by examining the potential of microbes to enhance growth of seed plants. Hindersah and Tualar (2004) states that the microbes capable as agents that influence on the plant growth increase (plant growth promoting agents). These microbes produce a variety of growth hormone (GH), vitamins and organic acids. Some fungi capable to produce GH. Several species of *Fusarium* (Hasan 2002) and *Sclerotium* (Sarma et al. 2002) has been known capable to produce auksin (IAA). IAA produced by fungi as secondary metabolites (Ünyanyar 2000). IAA is a common product of the metabolism of L-tryptophan in several microbes (Ahmad et al. 2004).

Most fungi from the litter exist as decomposers that require capability to growth on high temperature conditions (thermotolerant), grow at thermophylic temperatures 60 ^{0}C or more (Maheswari et al. 2000), at decomposition process (Sylvia et al. 2005). Fungi play a role in the decomposition of litter organic matter (Kurzatkowski et al. 2004). Litter from planting area in some plantation in East Kalimantan and Central Kalimantan is often used in Dipterocarpaceae breeding process. Plants that were given litter from the planting area grow better than plants that were not given (Tjitrosoemitro S in December 2009, personal communication). The nutrients from leaf litter can improve seedling growth of Dipterocarpaceae (Brearly et al. 2003). The study of the fungi that have potentiality to produce IAA not much done. Therefore, research of the fungi from plant litter in producing IAA need to be conducted.

MATERIAL AND METHOD

Fungal Isolation

In 250 ml Erlenmeyer 0.1 grams litter added into sterile aquades of 99 ml. Heat treated of the suspension was done in the water bath at 60°C for 15 minutes. 1 ml sample was plated on PDA medium in Petri dishes and incubated at 60°C. Colonies that grew then purified, and identified to genus according to Burnet and Hunter (1972).

IAA Assay

Crude Extract IAA Production

Stock cultures of fungi (7-10 days) on PDA medium used as working cultures. Three pieces of culture works inoculated into 100 mL of Czapek Dox liquid medium with 1% pepton. Cultures

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incubated at shaker machine for 7 days. Filtrate separated from the biomass fungi by centrifugation at 4500 rpm for 30 minutes. The filtrate used as a source of ZPT (IAA crude extract).

IAA content was detected by adding 4 ml Salkowsky reagent (400 ml H_2SO_4, 20 ml 0.1 $FeCl_3$, sterile aquadest 580 ml) into 2 ml of the filtrate (Hasan, 2002). The change of the color into pink indicates the IAA. OD read using spectrophotometer with 500nm wavelength. IAA concentration (ppm) obtained through the conversion of IAA standard curve (Ahmad et al., 2005). Two isolates that produce the highest concentrations of IAA to be further investigated its influence on the growth of mung bean sprouts (*Vigna radiata*) as the plant test.

Bioassay IAA

Sterilized seeds of mung bean with chlorox 3% for 3 minutes and rinsed wuth sterile water. Mung bean seed was grown until the first leaves appear (approximately 7 days) and treated with crude extract IAA. Seeds grown in liquid culture in a sterile jar containing the crude extract IAA with diferent concentrations (10^{-2} ppm, 10^{-4} and 10^{-6} ppm). Growth of seedlingsin aquadest used as negative control.

RESULTS

Six types of thermotolerance fungi *Aspergillus* sp B.10, *Aspergillus* sp 20.2, *Penicillium* sp 4.2, *Aspergillus* sp 9.2, *Penicillium* sp 1.2 and *Trichoderma* sp B.2 isolated from forest plant litter.Those six isolates able to produce IAA (from 15.56 ppm to 76.81 ppm, table 1) when grown in Czapex Dox medium with 1% pepton.

Table 1. IAA content of crude extract of some isolates

Isolate	IAA Crude extract (ppm)
Aspergillus sp. B.10	15.56
Aspergillus sp. 20.2	19.21
Penicillium sp. 4.2	19.52
Aspergillus sp. 9.2	24.10
Penicillium sp. 1.2	32.23
Trichoderma sp. B.2	76.81

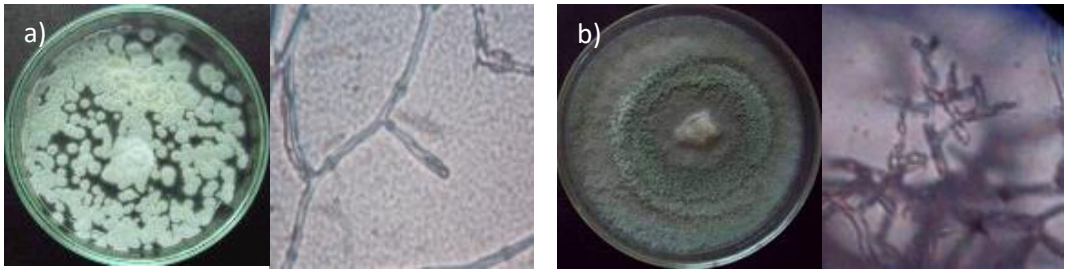

Figure 1. Colony isolates on PDA medium and microscopic cross-section Penicillium sp 1000x magnification of 1.2 (a) and Trichoderma sp B.2 (b).

Table 2. The influence of IAA on lateral root number of mung bean seedling 3 weeks after **planting**

Isolate (source)	IAA (ppm)	Average of lateral root number
Penicillium sp 1.2	10^{-2}	60 f
	10^{-4}	73.17 g
	10^{-6}	53.67def
Trichoderma sp B.2	10^{-2}	57.17ef
	10^{-4}	60.83 f
	10^{-6}	49.33de
standar IAA	10^{-2}	32.14ab
	10^{-4}	40.00bc
	10^{-6}	46.00c
Sterile aquadest	0	29.50a

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The numbers which followed the same letter is not significantly different according to Duncan's test results on P <0.05.

Isolates that produce filtrate with the highest IAA contents (*Trichoderma* sp B.2 (Fig. 1a) and *Penicillium* sp 1.2 (Fig. 1b)) are used for bioassay. Crude extracts from the fungus only affects in the number of lateral roots (Table 2). Standard IAA was also only affects the number of lateral roots. Crude extracts IAA from the filtrate in general gives a better effect on the number of lateral roots compared with standard IAA. The number of lateral roots varied from 29.5 to 73.17.

The influence of the IAA content on fungal filtrate (crude extracts IAA) on lateral root number varies by fungal tipe. Crude extracts IAA from *Penicillium* sp 1.2 stimulate lateral root growth better than crude extracts IAA from *Trichoderma* sp B.2. 10^{-4} ppm of crude extract IAA of *Penicillium* sp 1.2 is the optimum concentration that encouraging lateral roots growth. Generaly, the number of lateral roots produced by treatment with crude extract of the fungus filtrate IAA better when compared with standard IAA.

DISCUSSION

Isolation of forest litter fungi were performed at temperature of 40 ^{0}C and 60 ^{0}C to obtain the fungus that survive at high temperatures. The six fungi survive when grown at high temperatures, these fungi are thermotolerant (Maheswari et al., 2000). The ability of this fungus lives in the high temperature will facilitate adaptation to the thermophilic phase in composting process.

IAA Production Capabilities

IAA concentrations of crude extracts from six termotolerat fungi varied between 15.56 ppm to 76.81 ppm. The isolates potential to be developed as IAA producer. 10^{-4} ppm crude extract IAA and 10^{-6} ppm IAA standard provide optimum effect in the number of lateral roots, at each treatment. Auxin encourage the growth of lateral roots and root hair development (Casimiro et al., 2001) and increase the number of lateral roots (Woodward and Bartel, 2005).

Treatment with crude extracts of all IAA concentrations from *Penicillium* sp 1.2 and *Trichoderma* sp B.2 generally give better results when compared with standard IAA at the same concentration. Possibility this is occur because the filtrate contain amount of IAA and also other organic compounds that affect the growth of lateral roots, whereas IAA standard consisted of pure IAA components.

CONCLUSIONS AND RECOMMENDATIONS

The six fungi isolated from forest plant litter (*Aspergillus* sp B.10, *Aspergillus* sp 20.2, *Penicillium* sp 4.2, *Aspergillus* sp 9.2, *Penicillium* sp 1.2 and *Trichoderma* sp B.2) is termotoleran and capable to producing IAA in crude extract of various concentrations (15.56 ppm -76.81 ppm). *Penicillium* sp 1.2 and *Trichoderma* sp B.2 filtrate produce the highest concentration of IAA . 10^{-4} ppm crude extract IAA from *Penicillium* sp 1.2 significantly affected to increase the number of lateral roots. Further research on the extraction and utilization of fungal isolates for plant growth needs to be done

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Preliminary Study of Alkalitolerant Xylanolytic Soil Fungi from South Sumatra, Riau and D.I. Yogyakarta

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Fungi, typically have a high level of tolerance for acidity. However, there are some fungi that are capable of growing in alkaline conditions. Xylanolytic enzymes, that have optimum activity on alkaline condition, can be used in the pulp and paper industry. Alkaline xylanolytic enzymes can help the process of bleaching pulp thus reducing the use of chlorine compounds. The aim of this research was to study about alkaline xylanolytic fungi that isolated from neutral-acidic soil. The soil samples that used as fungal source was taken from some areas in South Sumatra, Riau and D.I. Yogyakarta. Isolation of fungi was be done on alkaline media (pH 9). Screening of enzyme production were performed on basal media xylanolytic with xylan birchwood as the sole source of carbon. As many as 80 isolate alkalitolerant fungi successfully found in all soil samples. The number of isolate from each sample was influenced by its soil acidity. The result showed that isolate 10WNGM, 7JT and 11ME have high alkaline xylanolytic enzyme activity in order to compared with another isolates. Alkalitolerant fungi that found in the soil of some areas in Indonesia could be produce the alkaline xylanolytic enzyme and potentially to develop in the industry.

Keywords : alkalitolerance fungi, xylanolytic enzymes, soil

INTRODUCTION

Fungi has been studied extensively for their biotechnology potential. Fungi are organisms with a high level of tolerance to acidity. Nevertheless, many fungi that can grow in alkaline conditions. The term of alkaliphile is used for microorganism that grow optimally or very well at pH values above 9, often between 10 - 12, but cannot grow or grow only slowly at the near neutral pH (Horikoshi, 2004).

Alkaliphiles have been isolated from neutral environment (pH ± 6,5), sometimes even form acidic soil samples and feces. Alkaliphiles fungi can be found in a variety of habitats with a wide pH range, although more numerous in the alkaline environment. As those species were not isolated on acidic media, the use of an alkaline medium. Alkaliphiles have made a great impact in industrial applications, so the research about alkaliphiles especially alkaliphiles enzyme for industry is still wide open (Horikoshi, 2004; Rele, 2004).

One of enzyme that have a huge benefit in industry was xylanase. Xylanase can help the pulp bleaching process. Alkaline xylanase well-liked in pulp dan paper industry, because of the unbleach pulp has a high pH and temperature (Saul et al., 2008). Alkaline xylanase can produce by fungi that grow in alkaline condition. Therefore screening thermostable xylanase and active at high pH should be done, considering the generally optimum pH for xylanase activity is under acidic conditions (at pH 4-6) (Bajpai, 1998).

Fungi is the major producer of xylanase because their enzymes products are extracellular. Xylanase fungi was complete enzymes with the enzymes for chain branches of xylan (Pang & Che-Omar, 2005). Although xylanases from eubacteria and archaebacteria have considerable higher temperature optima and stability than those of fungi, but the amount of enzyme produced by these bacteria is comparatively lower than that produced by fungi. In general, the level of xylanase in fungal culture is typically much higher than those from yeasts or bacteria (Kar et al., 2006). The use of fungal xylanase in the pulp bleaching process has been tested in the laboratory. The enzyme was used at the beginning of the process of bleaching and followed by the application of chemicals.

Xylanase from *A. indicus, A. niveus, A. flavus* have been used on hardwood Kraft pulp before alkali extraction process and following to chlorine compounds addition. Enzymatic treatment resulted in decline in Kappa number and enhance paper brightness (Angayarkanni et al., 2006). Xylanolytic crude enzyme from *A. niger* decreased the Kappa number of the pulp baggasse. The use of chlorine compounds in this process also decreased by 30% (Raghukumar et al., 2004).

Xylanase produced by certain fungi will be different from the others. Therefore the discovery of local fungal strains with high xylanase activity is an important point that must be

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done. This research was initiated to screen alkalotolerant xylanolytic fungi from neutral environment with the objective of exploring their biotechnological potential.

MATERIAL AND METHODS

Sample collection

Sample that used as source of xylanolytic fungal isolates was upper soil. Soil in the area surrounding the pulp and paper industry PT. Tanjung Enim Lestari (TEL) South Sumatra, PT IKPP Mandau Riau, PT RAPP Kerinci Riau, *Acacia* plantation in Muara Enim South Sumatra and Wanagama and Bunder forest Yogyakarta, sawmills in Palembang and Yogyakarta, and landfills Karyajaya Palembang was used as source of fungal isolates. pH of all soil samples were measured.

Soil fungi isolation

All fungal species were isolated on alkaline cornmeal agar (ACMA) containing 100mg/L chloramphenicol by the dilution plate method (Nagai et al., 1998). The plates were incubated at room temperature for 7 days. The fungal strains were transferred to fresh agar media until pure culture were obtained.

Screening for xylanase production on basal agar media

All fungal isolates were screened for xylanolytic potency on two stage. First, screening were performed on alkaline basal agar media containing 1% birchwood xylan as sole carbon source. The composition media was followed Kar et al. (2006). The inoculated plates were incubated for 7 days at room temperature. The clearing zones formed around the fungal growth were more visible when the plates were flooded with 0.1% (w/v) Congo Red. After 30 min of incubation, plates were washed with 1 M NaCl. The fungal isolates that formed clearing zones around their colonies, were selected. Diameter of the clearing zones was measured and compared with the diameter of the fungal colony to calculate the index xylanolytic.

Screening for xylanase production on basal liqiud media

The second screening were done in liquid media. The composition of mineral salts medium was the same as that of the first screening with birch wood xylan as the carbon source. However, the agar was not added. The culture of fungal isolates were incubated on room temperature in rotary shaker (150 rpm) for 7 days. After incubation, the medium was filtered through Whatman No.1 filter paper and the filtrate was centrifuged at 3.000 rpm for 20 minutes at 4ºC. The clear supernatant was used as source of xylanase activity (Lemos et al., 2000).

Xylanase assays

Xylanase activity was determined by mixing 0.9 ml of 1% (w/v) birch wood xylan (prepared in 50 mM phosphate buffer, pH 7) with 0.1 ml of suitably diluted enzyme and the mixture was incubated at 50 C for 30 minutes (Bailey et al., 1992). The reaction was stopped by addition of 1.5 ml of 3,5-dinitrosalicylic acid (DNS) and the contents was boiled for 5 minutes (Miller, 1959). After cooling, the colour developed was read at 540 nm. The amount of reducing sugar liberated was quantified using xylose as standard. One unit of xylanase is defined as the amount of enzyme that liberates 1 mmol of xylose equivalents per minute under the assay conditions. The protein concentration was measured following the method Bradford with BSA as standard.

The capability of excellent fungal isolates to cellulose and lignin

The excellent xylanolytic fungal isolates were observed their cellulolytic and lignolytic capabilities and lignolitik using basal media containing carboxymethylcellulose (CMC) and tanic acid. CMC and tanic acid concentration used in basal media was respectively 1% and 0.5%

RESULT AND DISCUSSION

Isolation of fungi from the soil around the pulp and paper industry PT. TEL South Sumatra, PT. IKPP Riau, PT. RAPP Riau, *Acacia* plantation in Muara Enim South Sumatra, Wanagama and Bunder forest Gunungkidul Yogyakarta, sawmills and landfills in Palembang has been done. The results of isolation obtained 80 alkaline fungal isolates (Table 1).

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Soil of the area above was used as source of fungal isolates because it is assumed that the soil contains a lot of plant debris which is the main source of xylan. As heterotrophic organisms, fungi like environment with high organic matter as their habitat.

Table 1. The number of alkalitoleran fungal isolates were found in each sample

No	Origin	Code	Soil pH	Number
1	*Acacia* plantation Muara Enim, Sum-Sel	ME	4,79	11
2	Around PT. TEL Tanjung Enim, Sum-Sel	TEL	3,06	3
3	Sawmill in Riverside of Keramasan, Palembang	KRMS	5,81	4
4	Landfills Karyajaya, Palembang	TPA	7,37	6
5	Sawmill of teak, Palembang	JT	6,03	7
6	Sawmill of acacia, Palembang	AKS	5,52	12
7	Around PT. IKPP Mandau, Riau	IKPP	5,14	7
8	Around PT. RAPP Kerinci, Riau	RAPP	4,88	4
9	Wanagama Forest, Gunung Kidul, D.I. Yogyakarta	WNGM	6,56	12
10	Bunder Forest, Gunung Kidul, D.I. Yogyakarta	BDSM	6.37	14
	TOTAL			80

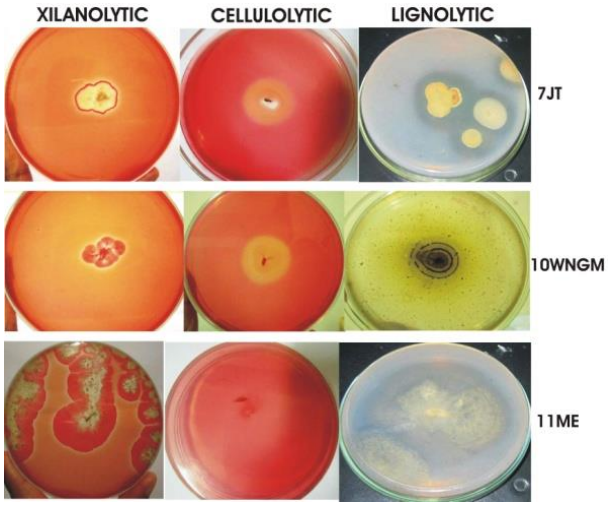

Figure 1. The capability of selected alkalitolerance fungal isolates to xylan, cellulose and lignin.

Isolation was used the media that have high pH (pH 9). Nevertheless, all soil samples have a neutral to acidic pH (Table 1). Therefore, it is expected these isolates have considerable tolerance to pH. The amount of alkalitolerance can be found on broad pH range, though more numerous when isolated from alkaline soils (Horikoshi, 2004).

Table 2.

No	Isolate of fungi	Xylanolytic index	Specific activity of xylanase (U/mL)	Celulolytic index	Lignolytic index
1	10WNGM	1,4483	203,7214	1,7797	1,8505
2	7JT	1,2119	112,7260	1,9636	1,6067
3	11ME	1,7215	52,6341	0,0000	1,9234

Formation of a clear zone on agar medium containing 1% birchwood xylan as the sole carbon source indicates the occurrence of xylan hydrolysis by alkalitolerance fungal isolates (Figure 1). Xylan hydrolysis is due to the fungus is able to produce xylanase enzyme (Nair et al., 2008). Almost all fungal isolates were able to hydrolyze xylan and form a clear zone, but only 23 alkalitolerance fungal isolates that more capable among another isolates. The second screening in liquid medium also containing 1% birchwood xylan fungal isolates showed varying ability to produce xylanase. A total of 23 fungal isolates tested xilanasenya production capability in liquid media. Based on the xylanase activity in liquid media has found that 10WNGM (203,7214 U/mL) was the highest xylanolytic alkalitolerance fungal isolate. Another alkalitolerance fungal isolates

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(7JT and 11ME) have high xylanolytic activity too (Table 2). The ability of xylanase production of selected alkalitolerance fungal isolates alkalitoleran must be assessed in a variety of conditions to obtain optimum enzyme production.

Birchwood xylan is the major component of xylan in hardwoods (hardwood) that widely used as raw material for pulp (Sunna & Antranikian, 1997). Therefore, xylanase produced by 10WNGM, 7JT and 11ME potentially be used in the early stages of pulp bleaching process. Especially if the cellulase was not produced such as on 11ME isolate. Higher capabilities of lignolitic was support the pulp bleaching process. Selected alkalitolerance fungal isolates that have high capabilities to hydrolysis birchwood xylan potentially to be further studied, especially production of alkaline xylanase

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Wild Edible Mushrooms Sold in Baliem Valley Traditional Market Papua

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Inhabitants of the Baliem Valley depend on the forest resources of the area for their livelihood, including the use of wild edible mushrooms as food source.. This study has aim to documenting the basic data on wild edible mushrooms sold in Baliem Valley traditional market. The survey took place in four traditional markets: pasar Misi, pasar Sinakma, pasar Baru and pasar Jayapura. The result showed that there were 2 species of wild edible mushrooms commonly sold by indigenous people, which were locally called: “lilake” and “ekiyapi”. They sold mushrooms one day after they were gathered from the forest surrounding their village during rainy days. The seller not specifically sold mushrooms; there was also commercialization of other cultivated vegetal products. The basic composition (total carbohydrates, crude fat, and protein) of those wild edible mushrooms were also determined in this study.

Keywords: wild edible mushrooms, Baliem Valley, traditional market, indigenous people

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Nematode Diversity: Food Web Condition of Decomposing Mangrove Leaf Litter (Familia Rhizophoraceae)

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Nematodes are important component of the soil ecosystem due to their roles in the soil food web. Their diversity reflects the food web condition as they inhabit each trophic level. The main objective of this research was to investigate the food web condition of three decomposing mangrove species based on nematode diversity. The results showed that 25 nematode families were observed from *R. apiculata* litter, 30 of *B. gymnorrhiza*, and 30 were from *C. tagal*. Food web indices indicated that structure, enrichment, and channel indices of *R. apiculata* ranged between 97.83 and 99.14, 44.83 and 80.95, 23.53 and 100.00 respectively. As those for *B. gymnorrhiza* were 96.74 and 99.11 (SI), 45.00 and 82.90 (EI), 19.29 and 100.00 (CI), whereas in *C. tagal* they were between 97.23 and 99.50 (SI), 47.37 and 79.17 (EI), 22.81 and 100.00 (CI). Decay rate of the leaf litter seemed to have no significant difference (0.18, 0.12, and 0.12% day^{-1}), It was concluded that connectance within the food web was complex and appeared to be dominated by fungal pathway as litter decomposition progress. Nutrients, moreover, were moderately to highly available in the ecosystem

Keywords : Nematodes, food web condition, mangrove plant litter

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Inventory of Orchids Species in Borme District, Pegunungan Bintang

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Orchids are the flowering plants that have aroused most widespread interest among scientists for the study since years. It is well known that the biodiversity of orchid species is being lost globally partly because most of them only survive in certain habitats. Papua is a part of the New Guinea Island which is rich with various indigenous orchid species. Unfortunately the orchid diversity in Papua is being threatened for reasons such as uncontrolled commercial exploitation of forest wealth. Borme is one of the twelve districts in PegununganBintang Regency which is planned to become a new regency name Katembang. In connection with it there are many forest areas will be converted into infrastructure development. Those conversions have adverse impact on the presence of orchids species in the areas. Inventory of orchids species in Hutan Sau Matan Ail Boul, District Borme was done to identify and create an inventory of all orchids species that exist in that area. Standard floristic method was used to find orchids species with randomly exploration. A total of 27 species of orchids from 15 genera were identified, and among those 5 species are terrestrial, 14 species epiphytes, and 6 species lithophytes.

Keywords: Inventory, Orchids, District Borme, Papua.

INTRODUCTION

Orchidaceae are cosmopolitan family, occurring in almost every habitat apart from glaciers. The richest concentration of orchid varieties is found in the tropics, mostly Asia, South America and Central America with important area in northern Andes (Columbia, Ecuador, Peru). Base on scientific and economic (horticulture) reasons, tropical orchids have aroused widespread interest among scientist for study since some years ago include in New Guinea. About 3200 species of orchids are found in New Guinea whereas two genera namely *Bulbophyllum* and *Dendrobium* known as the two biggest genera 569 species and 512 species separately (Millar, 1978). The number placed New Guinea as an important orchids area after northern Andes (Schuiteman and de Vogel 2001 *cit*. Sri Nurani Kartika Sari et. al. 2012). However the known orchids of New Guinea are mostly from eastern part (Papua New Guinea).

So far, no comprehensive report on the study of orchids from the western part of New Guinea namely Papua (Sihombing dan Lestari, 2002; Ungirwalu, dkk. 2007; Agustini, dkk. 2008; Lugrayasa, dkk. 2009 ; Agustini, dkk. 2012; Agustini, dkk. 2013) Unfortunately, the orchids diversity of Papua as a whole is being threatened for reasons such as biotic influences, socio economic development and also uncontrolled commercial exploitation of forest wealth. Recently, the establishment of some new regencies in Papua cause the rapid pace of development of infrastructures everywhere included Borme area.

Borme is one of the twelve districts in Pegunungan Bintang Regency which is planned to become a new regency name Katembang.This region rich in orchids but the rapid anthropogenic changes to habitats are surely threat to their diversity and survival. The exploration of orchids in Pegunungan Bintang still remains uncompleted due to a complicated geographic mosaic. Therefore, concerted efforts have to be made to study orchids in nature and document these species before we lost it.

The study was conducted to identify and create an inventory of orchids species in Hutan Sau Matan Ail Boul, District Borme,Pegunungan Bintang.

MATERIALS AND METHOD

This research was done in January-March 2013 by exploration in Hutan Sau Matan Ail Boul. Collecting of information and random explore method on part of the forest were done to find orchids species. Morphological inscription of found orchids were made descriptively. Morphological characters were note including root (length, hairy/hairless, colour); stem (sympodial, monopodial), node (shape, length, and number of node(s) each stem); leave (length, wide, colour, lamina shape (apex and margin), leaves arrangement and venation; inflorescence type; flower (colour and size of sepal/tepal); fruit (shape and number of fruit's node).

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Identification of orchids species using books namely Orchids of Papua New Guinea. An Introduction (Millar,1978); Mengenal Anggrek Alam Papua Seri Pertama (Dinas Kehutanan, PAI Papua, WWF Region Sahul, 2003); Flora Malesiana: Orchids of New Guinea CD Roms series Vol. I-VI (Schuiteman& de Vogel, 2009); Lowland orchids of Papua New Guinea (O'Byrne,1994).

RESULT AND DISCUSSION

Hutan Sau Matan Ail Boul is a forest with a pristine condition so it supports the growth of orchids in the area. Orchids in the forest area were found at an altitude of 878 to 1980 m above sea level. A total of 27 different taxa of orchids were found in this study. 14 of them are identified to species level, 12 to genus level and 1 taxon are unidentified (Table 1).

Various types of orchids were found occupying a variety of different habitat types. Among them are epiphytic (60%) followed by lithophyte/semi terrestrial orchids (22%) and the rest is terrestrial (18%). Genus *Dendrobium* is found more frequently than other genera (Table 1). The number of orchids genera in Borme is similar with the genera found in forest surround Oksibil District, 15 and 14 genera respectively. It does not mean that we will find the same genera or even species in other region of Pegunungan Bintang Regency, since many species of orchids require unique climate or altitude to survive.

Table 1. Orchids species in Hutan Sau Matan Ail Boul, Borme District, Pegunungan Bintang

Num.	Genus	Species	Habitat
1.	*Agrostophyllum*	*Agrostophyllum*sp	Epiphyte
2.	*Bulbophyllum*	*B. orbiculare*	Epiphyte
		*Bulbophyllum*sp	Epiphyte
3.	*Cadetia*	*Cadetia*sp	Epiphyte
4.	*Calanthe*	*C. rodochila*	Terrestrial
5.	*Coelogyne*	*C. asperata*	Epiphyte
		C. beccari	Epiphyte
6.	*Dendrobium*	*D. brasii*	Terrestrial
		D. branderhorstii	Epiphyte
		D. bifalce	Lithophyte
		D. chrysopterum	Epiphyte
		D. dionaeoides	Lithophyte
		D. erosum	Epiphyte
		D. hellerianum	Epiphyte
		D. macrophyllum	Lithophyte
		*Dendrobium*sp.1	Epiphyte
		*Dendrobium*sp.2	Lithophyte
		*Dendrobium*sp.3	Lithophyte
7.	*Dipodium*	*Dipodium*sp	Epiphyte
8.	*Dendrochilum*	*Dendrochilum*sp	Lithophyte
9.	*Malaxis*	*Malaxis*sp	Terrestrial
10.	*Mediocalcar*	*Mediocalcar*sp	Epiphyte
11.	*Oberania*	*Oberonia*sp	Epiphyte
12.	*Phaius*	*P. tankervilleae*	Terrestrial
13.	*Spathoglottis*	*S. plicata* Bl.	Terrestrial
14.	*Trichotosia*	*Trichotosia*sp	Epiphyte
15.	----------	Unidentified	Epiphyte

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Figure 1. Some identified species were found in this study. A. *Dendrobium branderhorstii*. B. *Dendrobium erosum*, C. *Dendrobium polycema*, D. *Glomera montana*.

CONCLUSION

Fifteen genera and twenty seven species were collected from Hutan Sau Matan Ail Boul, Borme. There are still numerous undiscovered species in the wild at Borme areas.

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Diversity of Gastropods (Mollusca) in Kumu River, Pasir Pengaraian, Rokan Hulu Regency, Riau Province, Indonesia

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Kumu River is one of the river in Rokan Hulu Regency, Riau Province, Indonesia. Many people use it as water resources like bathing, washing and transportation. But human activities has caused impact to this river even pollution that caused by changing into agriculture and people settlement that have affected many organisms around that can be used as indicator, one of them is gastropods. This study was conducted to determine the diversity of gastropods in Kumu river from April to June 2013. The samples were taken from three different stations, those are upper stream, middle stream and down stream. And from every station, samples collecting was done about 10 times by using square 1 x 1 metre randomly. Everything in the square was putting into a bucket and will be filtered at the edge of the river. Samples were obtained, inserted into sample bottles that contains alcohol 70% and marked. Samples that have been preserved, and then sorted, identified and counted the number of individuals of each species, and data analysis. Data analysis, among others, to calculate the diversity index, evenness index, and dominance index in the Laboratory. From the analysis, we found 3 families, 4 spesies and 181 individual total of gastropods. The diversity index ranged from 0.41 to 1.359, evenness index ranged from 0.592 to 0.981 and the dominance index ranged from 0.264 to 0.755. *Pomacea canaliculata* was the most dominant gastropods from all stations. And can be concluded that the diversity of gastropods in Kumu River is at low category.

Key words : Kumu river, diversity, Gastropods

INTRODUCTION

River is one of the flowing fresh-water ecosystem and having complete water mass mixing pattern (Goldman and Horne, 1983). In Rokan Hulu Regency, there are 2 main rivers those are Rokan Kanan river and Rokan Kiri river. Both of them separate into small river, those are Apung river, Dantau river, Ngaso river, Batang Sosa river, and Batang Kumu/Kumu river. This river still used by people as water resources, like bathing, washing, transportation and fishery (Dinas Kesehatan Rokan Hulu, 2011).

One of the benthic organism that always live in the river ecosystem is gas-tropods. It can be used as indicator in the river environment, because it is able to tolerate every environment condition, sedentary life, grouping and their abudant number (Parker dan Haswell, 1972; Purnama, Nastiti, Agustin dan Affandi, 2011 *in* Halawell, 1986; Rosyadi, Nasutin dan Thamrin, 2009). Commonly herbivore by consuming mosses, algae and detritus. Some can be used for human as food and animal food (Mar-woto, Isnaningsih, Mujiono, Heryanto, Alfiah dan Riena, 2011).

Human activities such as dredging, silting up, fragmentation into agriculture and settlement even pollution from household waste, have made the river become polluted and will threat the gastropods. Setyobudiandi, Bengen and Damar (1996) reported, water pollution that happened continuously will threat the community of water organisms, in this case, gastropods.

Another threat come from invasive species *Pomacea canaliculata* that has reduced another gastropods population. Marwoto *et all* (2011) reported, population of *Pila ampullacea* (Linne, 1758), *P. polita* (Deshayes, 1830) and *P. scutata* (Mousson, 1848) has decreased and reduced because of the pest slugs *P. canaliculata*. Until now, this species can be found easily in every freshwater type. Based on introduction above, this study was conducted to determine the diversity of gastropods from kumu river.

MATERIALS AND METHODS

This study took place at three station: the upper, middle and lower stream of Kumu river, Rokan Hulu Regency, Province of Riau, Indonesia from April to June 2013.

Samples were taken by using square 1 x 1 metre about 10 times randomly from upper, middle and lower stream. Everything at the bottom of the river in the square were putting into a bucket and filtered at the edge. Samples were obtained, inserted into sample bottles that contains alcohol 70% and marked. Samples that have been preserved, and then sorted, identified and counted the number of individuals of each species. Samples were identified by using *Recent and Fossil Indonesian Shells* book (Dharma, Schwabe dan Schrödl, 2005) and leaflet *Keong air Tawar*

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Pulau Jawa (*Moluska*; *Gastropoda*) (Marwoto *et all*, 2011). Data analysis, among others, to calculate the diversity index Shannon and Weaver (Marrugan, 1987), evenness index Krebs (1989), and dominance index (Marrugan, 1987 in the Laboratory.

RESULTS

There were found 3 families, 4 spesies and total 181 individual numbers of gastropods in Kumu river. From analisys (table 1), the highest diversity index was collected from middle stream 1.359 and the lowest from down stream 0.41. From eveness index, the highest eveness was collected from middle stream 0.981 and the lowest from down stream 0.592. From dominance index, the highest come from the down stream 0.755 and the lowest from the middle stream 0.264.

According to Krebs (1989), diversity of the gastropods in this river was on the low category. This was caused by human activity by changing the habitat into agriculture and settlement, even the pollution from household waste. Even as evidence, the river has darkbrown colour. Community will has high diversity if it consist of many species with high abundant species. There will be interaction in that community that will cause energy flow like food chain, predation, competition and niche (Purnama *et all*, 2011 *in* Soegianto, 1994).

The eveness of the gastropods was located at the middle stream. This was caused at the middle stream has been changed by the people by damming the river. So the flow of the river from upper stream that contain organic materials and source of food for gastropods, will gather at the middle before reach the down stream. And the substrates also support for the gastropods to attracted to the rocks and limber in the river. Mean while at the down stream only muudy and only Filopaludina sumatrensis that high abudance at the location.

Table 1. Total individual number, diversity index, eveness index and dominance index from gastropods from Kumu River.

No	Famili	Species	Density of spesies from Kumu River (ind/m^2)		
			Upper stream	Middle stream	Lower stream
1	Viviparidae	*Filopaludina sumatrensis*	0	13	54
2	Ampullariidae	*Pomacea canaliculata*	4	19	9
3	Thiariidae	*Melanoides tuberculata*	36	23	0
		Thiara scabra	9	14	0
Number of Individual (N)			49	69	63
Number of spesies			3	4	2
Diversity index (H')			0,742	1,359	0,410
Eveness index (J)			0,676	0,981	0,592
Dominance index (C)			0,580	0,264	0,755

From dominance index, according to Maggurant (1987), there is a spesies that dominant in this river, unstable structure and community and also ecological pressure. *Pomacea canaliculata* is the dominant species in this river. This species is an invasive species and has a rapid development even now a days it is pest for human. This species will cause competition for food and it will survive from another species (Strong, Gargominy, Ponder dan Bouchet, 2008).

CONCLUSION

Kumu river has a very low gastropods species and from diversity index, it is in a low category. It was caused this river has polluted by human activities and also changing habitat into agriculture and settlement, even the invasive species *P. canaliculata* dominance in this river.

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Correlation Between Edge Effects and Diversity of Frugivorous Butterfly at Ciremai Mountain National Park Region 1 Kuningan

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The forest buffer zone of the Ciremai Mountain National Park (TNGC) had been fragmented due to anthropogenic disturbance. Fragmentation causes negative effect for environment, because it can increase local extinction and edge effects. Edge effects are impacts due to differences of environmental conditions between two fragmented habitats and affected animals are in. Insects such as frugivorous butterfly produces a specific response to deforestation, therefore it can be used as bioindicator to detect environmental changes. This research was conducted from December 2012 - January 2013 in the first Region of TNGC. Research locations were in the frontier of rehabilitation area, Lempong Bitung, Cigugur, Kuningan, West Java, perpendicular to the forest frontier along 200 m, which is at a distance of 0-50 m, 50-100 m, 100-150 m, and 150-200 m. The result showed that there were 11 species, which belong to family Nymphalidae. Edge effects significantly affect the diversity of frugivorous butterfly in the TNGC. Environmental measurement parameters have a low correlation to diversity frugivorous butterfly in the TNGC.

Keywords : edge effects, frugivorous butterfly, diversity, Ciremai Mountain National Park, fragmentation

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The Comparison Of Some Biological Parameters Of Freshwater Prawn, *Macrobrachium sintangense*, From Java, Sumatera And Kalimantan

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The group of Macrobrachium has spread widely in South East Asia and East Asia region. In Indonesia, Macrobrachium sintangense *may commonly be discovered in Java, Sumatera, and Kalimantan. Not only does* M. sintangense *play essential role in freshwater ecosystem, this species would also have contributed for locals as the source of protein as well as for commercial commodity. The changes of environment might have driven significant effects to the habitat of many* M. sintangense*. The aim of this study was to observe the morphometric variations occurred within* M. sintangense *species originally taken from Java (Central Java, West Java and East Java , Sumatera and Kalimantan island. The sample collection was completely done from March 2012 to May 2013 in some places representing Java, Sumatera and Kalimantan. The water quality and the physical conditions of the habitat were recorded to gain comprehensive depiction of the environment in which this species would likely be residing. Male* M. sintangense *collected from Central Java (Malahayu reservoir) had attributed highest value for morphometry including the carapace length and bodyweight. For female* M. sintangense *taken from Kalimantan, it is concluded that these females led the highest value for the measurement of carapace length. Female* M. sintangense *from Central Java (Malahayu reservoir) had attributed highest value for bodyweight.Whether the berried female from Central Java had the heaviest mean of bodyweight and the highest number of absolute fecundity. It is generally informed that* M. sintangense *from Sumatera (Way Sekampung river, Lampung) had the lowest attributes of all.*

Key words : M. sintangense, *morphometry, freshwater prawn*

INTRODUCTION

Macrobrachium is known as a large group of prawn, widely spread as well as easily found throughout South East Asia and East Asia region including Indonesian archipelago. *Macrobrachium sintangense*, unlike *Macrobrachium rosenbergii* or *Macrobrachium ohione* that spends most of larvae phase in rather salty water (Bauer and Delahoussaye, 2008), has likely adapted well in freshwater body such as river, reservoir and lakes. The island of Sumatera, Kalimantan, and Java has been recognized as the regions in which the group of *M. sintangense* preferably inhabits.

Since *M. sintangense* (Figure 1) can broadly populate in inland water, this group may confer many banefits to locals. Locals are used to consuming this prawns as the source of protein. In Malahayu reservoir, Brebes, Central Java, one kilogram of *M. sintangense* may cost approximately IDR 40,000, which is slightly cheaper than that of beef. Thus, *M. sintangense* shall be taken into account as the protein alternative for humans with relatively cheaper price. Additionally, another advantage taken from this commodity is that this can be used as the feed for ornamental fish. And it is believed by the locals that it could enhance the color of the ornamental fish after feeding regularly (Said *et al*., 2012). As similar to other members of Macrobrachium group, ecologically, *M. sintangense* has also been contributing for ecological balance. This prawn plays the role as macrozoobenthos which are the prey for other bigger organisms, and, as well, acts as natural controller for mosquitoes' larvae (Collins, 1998 *in* Said *et al*., 2012).

The decrease of *M. sintangense* in number at present is caused by numerous reasons. Said *et al.* (2012) assumed that the overcatchment, the damaged habitat, and the high level of predation might lead the unfavorable condition of *M. sintangense* population. Other possible causes the decline in abundance for many Macrobrachium group may include habitat loss, chemical pollution, river channelization and overfishing (Bauer and Delahoussaye, 2008). In order to restore the abundance of *M. sintangense*, there is a need to conduct more researches on its recent condition, especially its diversity within the population.

It is broadly known that the phenotypic expression may be influenced by the external environment. Since the island of Sumatera, Java and Kalimantan is separated by straits and oceans, there is an assumption that the morphometric variation may take place within *M. sintangense* taken

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from different island. This study observes the morphometric variations occurred within *M. sintangense* species originally obtained from Java, Sumatera and Kalimantan island.

(a) (b)

Figure 1. *Macrobrachium sintangense* (a) female ; (b) male

MATERIALS AND METHODS

The sample collection was completely done from March 2012 to May 2013 in some places representing Java, Sumatera and Kalimantan. The exploration to obtain prawns in Java covered three places : West Java (Bogor Botanical Garden), Central Java (Malahayu and reservoir) and East Java (Porong River in Kabupaten Sidoarjo and Mas River in Kabupaten Mojokerto) and was done in March 2012, April 2012 and April 2013. We spotted Way Sekampung river for sample collection representing Lampung region in June 2012. The exploration at Durian river (Kabupaten Sintang, West Kalimantan) to represent Kalimantan conducted in September 2012. Moreover, in May 2013 we spotted Kapuas River and Melawi River (Kabupaten Sintang, West Kalimantan) to add sample collection from Kalimantan. Table 1 addresses the latitude for the sampling sites.

Table 1. Latitude of sampling sites

No.	Locations	Latitude	Description
1	West Java (Bogor Botanical Garden)	-	small pool 2QE inside the garden
2	Central Java (Malahayu reservoir)	07° 02’ 54,5” S 108° 48’ 44,6” E	inlet of the reservoir (Cikabuyutan river)
3	Sumatera (Way Sekampung, Lampung)	5°19’54.8’’ S 104°57’6.0’’ E	river banks
4	Kalimantan (Durian River, Kapuas River and Melawi River, Kabupaten Sintang, West Kalimantan)	0° 04.911’ S 111° 29,075’ E	river banks
5	East Java (Porong River, Kab. Sidoarjo and Mas River, Kab. Mojokerto)	07°26’38.7”S dan 112°28’07.0”E 07°25’30.9”S dan 112°28’24.2”E	river banks

The technique to collect samples was different for each sampling spot. To collect the samples in small pool within the botanical garden, we used hand net since the depth of the pool was rather shallow. For catching prawns in Way Sekampung river, we relied on the local’s traditional net called *waring* (Figure 2). Cast net and *osom* (Figure 3) were used in Malahayu reservoir and during the sample collection in Kalimantan and East Java, we used hand net. The mesh size varied from ¼ to ¾ inch. All dead specimens were preserved in ethanol 70%. Whether living prawns were maintained alive and reared in the laboratory for further observations.

The *in situ* analyses covered the parameter of DO (*dissolved oxygen*), temperature, and pH using Water Quality Checker (Horriba U-10, Japan). Other analyses including N-NO_2, N-NO_3, N-NH_4, Alkalinity, TOM (*Total Organic Matter*), Total Nitrogen, Total Phosphate, Hardness were

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measured chemically in the laboratory (Research Center for Limnology, Cibinong-Bogor) soon after delivered.

Table 2. Chemical analyses and the methods used

No.	Parameter	Unit	Method
1	$N\text{-}NO_2^-$	mg/L	Sulfanilamide, Spectrophotometry
2	$N\text{-}NO_3^-$	mg/L	Brucine, Spectrophotometry
3	$N\text{-}NH_4^+$	mg/L	Phenat, Spectrophotometry
4	Total Nitrogen	mg/L	Brucine using K-persulfate oxydator, Spectrophotometry
5	Alkalinity	mg CaCO3/L	Titrimetry
6	Total Phosphate	mg/L	Ascorbic acid using K-persulfate oxydator, Spectrophotometry
7	TOM (Total Organic Matter)	mg/L	Permanganatometry
8	Hardness	mg/L	Titrimetry

Source : APHA (2005)

Figure 2. The *waring*

Figure 3. The cast net and *osom*

The measurement for morphometry was comprising carapace length, bodyweight, eggs diameter, and absolute fecundity. A calliper with the 0.01 mm sensitivity was used whether the wet weight was counted by electronic balance with the 0.01 grams sensitivity. The egg countings was done using manual counter. Egg diameter observation was complete under binocular microscope equipped with ocular micrometer, taking the 40X magnification.

All data were statistically analysed by Kruskal Wallis test because data homogeneity assumption is not fulfilled. Dunn test (α=0.05), subsequently, was applied to trace which group was different one to another (Dunn, 1964 *in* Hollander & Wolfe, 1973). Data processings were provided by utilizing SPSS 16.0 *for Windows.*

RESULTS AND DISCUSSIONS

It is revealed by the information gained from Table 3, that the male prawns from Central Java (Malahayu Reservoir) have been categorized in different subset by Dunn test (α=0.05) in which the value of carapace length are longer than others. It is known, then, that the male's carapace length from Sumatera is the shortest.

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Table 3. Male's morphometry

Parameters	Origin				
	West Java	Central Java	Sumatera	Kalimantan	East Java
Carapace length (cm)	1.41 ± 0.16 (1 – 1.6)^{abc}	1.75 ± 0.22 (1.4 – 2.2)^{d}	1.18 ± 0.08 (1.1 – 1.3)^{ac}	1.53 ± 0.25 (1.3 – 2.2)^{cd}	1.51 ± 0.21 (1.3 – 2)^{bcd}
Body Weight (gram)	2.53 ± 0.96 (0.91 – 5.25)^{b}	4.16 ± 1.09 (2.53 – 6.45)^{c}	0.99 ± 0.20 (0.73 – 1.33)^{a}	2.25 ± 1.70 (1.05 – 7.12)^{ab}	2.11 ± 0.80 (1.12 – 4.42)^{ab}

Similar subscripts means no significant difference at α=0.05 by Dunn test (further test after data analyzed with Kruskal Wallis test)

Alkalinity may be one factor influencing the growth of exoskeleton in prawns. Alkalinity above 50 mg/Liter will provide enough calcium, thus, help the prawns normally grow. In contrast, alkalinity below 50 mg/Liter restricts the growth (Hicks and Pierce, 2011). Through Table 6, it is showed that the water analysed from Central Java had the highest value of alkalinity. Thus, it is assumed that the alkalinity might have contributed to the longest carapace length which males *M. sintangense* from Central Java ascribe to. The range of carapace length in male *M. sintangense* varied from (1.18±0.08) cm to (1.75±0.22) cm. As the comparison, the maximum carapace length of *M. rosenbergii* found in Negombo Lagoon, Sri Lanka was 1.04 cm (Munasinghe and Thushari, 2010). Whether Cai *et al.* (2004), ever found the male *M. sintangense* with carapace length of approximately 1.96 cm in Mekong river at Udon Thani, North-East-Thailand in June 1998. The highest value compared to Cai's finding was the maximum value of male's carapace length discovered in Central Java (Malahayu Reservoir). It was 2.2 cm and the minimum value was recorded by 1.4 cm.

In the male's bodyweight parameter, we can draw information that male prawns from Central Java have the highest value. The range for male's bodyweight mean is from (0.99±0.20) grams to (4.16±1.09) grams. Maximum bodyweight found was 6.45 grams and the minimum valued 2.53 grams for *M. sintangense* from Central Java. As the comparisons to weight, male *M. nobilii* found in Cauvery river, India, ever reached 12.67 grams in weight and the minimum was 1.45 grams (Mariappan and Balasundaram, 2004).

Table 4. Female's morphometry

Parameter	Origin				
	West Java	Central Java	Sumatera	Kalimantan	East Java
Carapace length (cm)	1.13 ± 0.26 (0.7 – 1.4)^{b}	1.16 ± 0.08 (1 – 1.3)^{b}	1.02 ± 0.15 (0.7 – 1.3)^{a}	1.18 ± 0.12 (0.9 – 1.5)^{b}	1.13 ± 0.14 (0.8 – 1.4)^{b}
Body Weight (gram)	1.03 ± 0.45 (0.3 – 1.87)^{bc}	1.41 ± 0.28 (0.87 – 2.08)^{c}	0.59 ± 0.24 (0.2 – 1.33)^{a}	0.74 ± 0.25 (0.31 – 1.44)^{ab}	0.69 ± 0.34 (0.27 – 2.05)^{ab}

Similar subscripts means no significant difference at α=0.05 by Dunn test (further test after data analyzed with Kruskal Wallis test)

Female's morphometry (Table 4) shows less value than males's morphometry does. It is similar to *M. rosenbergii* (Kurian and Sebastian, 2001 *in* Sarkar *et al*., 2012), *M. dayanum* (Sarkar *et al*., 2012) and *M. lamarrei* (Sharma and Subba, 2005) which exhibit the appearance of males are larger than that of females. As from Table 4, the range varied from (1.02±0.15) cm to (1.18±0.12) cm for carapace length and (0.59±0.24) grams to (1.41±0.28) grams for bodyweight measurement. Females from Sumatera (Way Sekampung river, Lampung) have always been displaying smallest carapace length and bodyweight of all. As for comparison to female *M. sintangense* morphometry, female *Macrobrachium nobilii* preserved from Cauvery river, India, had bodyweight ranged from 3.36 g to 7.18 g (Mariappan and Balasundaram, 2004).

The value of carapace length of berried female (Table 5) varied from (1.02±0.13) cm to (1.38±0.12) cm. Whether the bodyweight set from (0.63±0.12) grams to (1.56±0.39) grams. There is slightly different value of bodyweight compared to unberried female's. As for eggs diameter, it laid from (0.98±0.10) mm to (1.42±0.30) mm. The value is considered less than the eggs diameter of *M. dayanum* set from 2.5 mm to 3.0 mm (Sarkar *et al*., 2012). The eggs color appeared dark green for *M. sintangense*. It is deep green (olive green) in *M. dayanum* (Sarkar *et al*., 2012), yellow in *M. rosenbergii* (Kurian and Sebastian , 2001 *in* Sarkar *et al*., 2012) and green in *M.*

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lamarrei (Sharma and Subba, 2005). The absolute fecundity from Table 5 ranged from (82.67±6.03) to (182.33±120.17). In contrary, the total eggs found in *M. dayanum* from Padma river, Bangladesh, had been rather wider in range. It was fluctuated from 43 to 195 in number (Bhuiyan *et al*., 2007).

Table 5. Berried female's morphometry

Parameters	Origin				
	West Java	Central Java	Sumatera	Kalimantan	East Java
Carapace length (cm)	1.17 ± 0.12 (1 – 1.4)^{ab}	1.34 ± 0.13 (1 – 1.5)^{cd}	1.02 ± 0.13 (0.8 – 1.3)^{a}	1.38 ± 0.12 (1.3 – 1.7)^{d}	1.23 ± 0.13 (1 – 1.5)^{bc}
Body Weight (gram)	1.48 ± 0.32 (0.87 - 2.09)^{cd}	1.56 ± 0.39 (0.9 - 2.34)^{d}	0.63 ± 0.12 (0.38 – 0.83)^{a}	0.86 ± 0.21 (0.51 – 1.36)^{ab}	1.16 ± 0.42 (0.41 – 2.12)^{bc}
Absolute Fecundity (eggs)	163.33 ± 31.94 (127 - 187)*	182.33 ± 120.17 (73 - 311)*	82.67 ± 6.03 (77 - 89)*	76.00 ± 28.28 (56 - 96)*	139.80 ± 62.80 (76 - 235)*
Diameter of eggs (mm)	1.42 ± 0.30 (1.00 - 1.97)^{b}	1.18 ± 0.04 (1.06 - 1.22)^{b}	0.98 ± 0.10 (0.75 - 1.11)^{a}	1.23 ± 0.03 (1.19 - 1.25)^{b}	n.a

Similar subscripts means no significant difference at α=0.05 by Dunn test (further test after data analyzed with Kruskal Wallis test)
*) not significantly different by one way ANOVA (p>0.05) ; n.a (not available)

Table 6 represents the water quality from the habitat where the prawns as samples, were taken. The value of inorganic nitrogen (N-NO_2, N-NO_3, N-NH_4) took place to be low (< 1.0 mg/L). Chapman (1992) summarized the allowable level of fisheries and aquatic life, for N-NH_4 was 0.5 mg/L and 40 mg/L of nitrate. In addition to this, growth and survival of the prawns will be affected by un-ionized ammonia (NH_3) levels as low as 0.26 mg/L (Hicks and Pierce, 2011). pH and temperature are factors directly affecting the ammonia toxicity. It is comprehended that the higher level of pH and temperature of the water will shift the ammonia equation to the toxic form (Hicks and Pierce, 2011). Thus, desirable pH is around 6.5 to 9.5. All data presented in table 6 indicate that pH level is ranging from 5.53 to 7.73 and desirable for aquatic life.

Table 6. Water quality profile of the sampling sites

Parameter	Region				
	West Java	Central Java	Sumatera	Kalimantan	East Java
N-NO_2 (mg/L)	0.02	0.001	0.01	0.005	0.013 - 0.090
N-NO_3 (mg/L)	0.82	0.69	0.31	0.021	0.028 - 0.342
N-NH_4 (mg/L)	0.24	0.01	0.07	0.012	0.004 - 0.178
Alkalinity (mg $CaCO_3$/L)	62.62	160.02	31.62	12.21	12.6 - 15.0
TOM (mg/L)	10.73	21.73	15.43	42.57	22.9 - 23.9
Total Nitrogen (mg/L)	6.39	2.59	2.40	0.40	5.37 - 8.75
Total Phosphate (mg/L)	0.06	0.02	0.04	0.11	0.09 0.53
Hardness (mg/L)	61.78	135.93	21.52	19.37	120.4 - 158.3
pH	6.16	7.73	6.74	5.53	6.79 – 6.80
DO (mg/L)	4.27	9.75	8.86	5.21	4.46 – 4.68
Temperature (^oC)	26.9	32.20	25.6	28.3	26.04 – 28.62

Dissolved oxygen (DO) is another important parameter for *M. sintangense* as water biota. Chapman (1992) concluded that the appropriate level for DO in fisheries and aquatic biota is

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approximately around 5.0-9.0 mg/L. It will jeopardize aquatic life if the DO value drops to below 5.0 mg/L. Otherwise those biota are adapted to such conditions.

CONCLUSION

Male *M. sintangense* collected from Central Java (Malahayu reservoir) had attributed highest value for morphometry including the carapace length and bodyweight. For female *M. sintangense* taken from Kalimantan, it is concluded that these females led the highest value for the measurement of carapace length. Female *M. sintangense* from Central Java (Malahayu reservoir) had attributed highest value for bodyweight. Whether the berried female from Central Java had the heaviest mean of bodyweight and the highest number of absolute fecundity. It is generally informed that *M. sintangense* from Sumatera (Way Sekampung river, Lampung) had the lowest attributes of all.

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The Differentiation of Students Learning Achievement and Critical Thinking Between The Using of Learning Video and Graphic as Media in Problem Based Learning (PBL) Model in SMA Negeri 3 Medan

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The purpose of this study is to differentiate students learning achievement and critical thinking and to investigate the relations between students' learning achievement and critical thinking between the using of learning video and graphic as media in Problem Based Learning (PBL) model in SMA Negeri 3 Medan. The study involves two classes as research samples. Both of the classes studied in PBL, however XI-6 class used learning video as media, meanwhile XI-5 class used graphic as media. Blooms cognitive test instrument was given to the students to measure students learning achievement, whereas the critical thinking assisted by using Cornell critical thinking test instrument. The study found that there is a significant difference of students learning achievement between the using of learning video and graphic in PBL model with level of significance 0,01 (t_{obs} = 10,03 > t_{table} = 2.3727). The mean of cognitive post test score in PBL with learning video is higher 10,0 points then the graphic does. The study also confirmed that there is a significant difference of students' critical thinking between the using of learning video and graphic in PBL (t_{obs} = 6,403 > t_{table} = 2.3727). PBL with learning video able to increase 27% of students' critical thinking, meanwhile the graphic only 24%. The study investigation indicates that there is a relation between student learning achievement and critical thinking in this case. The higher students Cornell critical thinking test score for their critical thinking level positively contributes to the higher cognitive test scores for student learning achievement. 60% of visual students in this research samples to be estimated has a great role for students learning achievement and critical thinking progress in this study.

Keywords : Problem Based Learning (PBL), Learning Video, Graphic, Learning Achievement, Critical Thinking

INTRODUCTION

Peraturan Menteri Pendidikan Nasional (Permendiknas) Republik Indonesia No. 22 year 2006 about the Content of Standardization for Senior High School Education asserted that student should be able to relate their knowledge comprehension into Science, Technology and Society (STS) as the goal of our learning. In order to achieve this expectation, the involvement of thinking ability for every learning activity should be concern (Sanjaya, 2006). One of this thinking ability Ashman and Conway (1997) advocated is the critical thinking.

Biology is a study of life which extends from the global scale of the entire living thing which has a close relation to the process inside of the living body with its surroundings (Kurniawati, 2010). One of the biology learning material which has a close relation to the organism is about the reproductive system. Reproduction is the organism characteristic that processes new organisms creation of the existing ones (Campbell, 2008). Unfortunately, introduction this topic to the students has several obtrains. Based on Sugiono (2008), Samodra (2009) and Kurniawati (2010) researches most of the teachers in the school tend to use conventional method and less utilize any media that suitable for the learning process.

The limitation on teaching media utilization that suitable to teach the human reproductive system also a problem in SMA Negeri 3 Medan. One of the Biology teacher of grade XI science class in the researcher personal talked has mentioned that there is a need of finding new innovative media that enable to engage and stimulate students' motivation and critical thinking in learning human reproductive system topic in SMA Negeri 3 Medan.

According to Tiwari, 1999 and Cook and Moyle, 2002, Problem Based Learning (PBL) model has been advocated as a promising strategy to promote students' critical thinking (include reasoning, communicating and connecting) in such a problem solving way. The components of PBL, using real world situations (problems), group learning, student-directed solutions for problems, and teacher serving as facilitators of learning has much promise for, and important applications in enhancing students critical thinking (Downing, 2013). Through a PBL model students' engagement and motivation can be construct by introducing them to the 'real world' problem which relevant to their study (Beasly and Ford, 2003). Moreover, A study that was conducted by the National Assessment of Educational Progress (NAEP) (U.S. Department of

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Education, 2001), shown that there is a link between critical thinking skills and the increasement of student achievement in the classroom through Problem Based Learning model. Harold Wenglinsky (2004) found that teaching critical thinking is associated with higher test scores. Therefore it can be concluded that instruction emphasizing advanced reasoning skills in classroom promotes high student performance in Problem Based Learning model (Wenglinsky, 2004; Schmidt, 1993). Therefore, the Problem Based Learning model was a good answer to solve low students achievement problem in human reproductive topic.

Rusman (2012) argued that there are several factors that can engage and motivate the students to be actively participating in the learning process. One of them is the availability of learning media that suitable to the study concept. This statement indicated that the role of media is very important in learning activity. Thus to study human reproductive system topic, the learning media involvement is necesarry.

According to Sugiono, 2008; Samodra, 2009; Kurniawati, 2010, the role of media is very useful to improve student understanding about the human reproductive system that contains with the abstract concept and difficult facts. Shankar (2007) on his book's: Methods of Teaching Educational Technology mentioned that the learning video as a media function is to stimulate the real world environments and creates an actual environment for experimentations.

Beyond of this expectation, a video learning also has a potential in bringing a better learning quality. Video instruction to support the learning method in classroom is a more effective teaching technique than conventional lectures (Felton, Keesee, Mattox, McCloskey and Medley, 2001). The findings of their research demonstrated that there was significant effect toward students learning achievement by using audio and video media aided than the conventional method. Through a video learning student comprehension will be increased due to their real illustrating experience, then it will engage them with the material and bring concrete understanding about the topic (Arsyad, 2004).

Research questions of this research are:

1. Is there any significant difference of students' learning achievement between the using of learning video and graphic as media in Problem Based Learning (PBL) model about the human reproductive system topic based on the Blooms' cognitive test result?
2. Is there any significant difference of students' critical thinking between the using of learning video and graphic as media in Problem Based Learning (PBL) model about the human reproductive system based on Cornell Critical Thinking Test calculation Level X result?
3. Is there any relations of students' critical thinking and learning achievement between the using of learning video and graphic as media in Problem Based Learning (PBL) model about the human reproductive system topic?

MATERIAL AND METHOD

Samples

The sample of this research consist of the whole population of science class students XI grade in SMAN Negeri 3 Medan Academic Year 2012/2013. They were selected by simple random sample to subsets of the frame given in an equal probability. XI-6 class chosen to be treated with Problem Based Learning (PBL) model class and utilized learning video as media. The class consisted of 42 students. XI-5 class chosen to be treated with Problem Based Learning (PBL) model class and utilized simple graphic as media. The class also consisted of 42 students.

Instrument

There were two types of research instruments in this research. The first is the learning style questionnaire that researcher gave in the beginning of the class to determine students learning style and the second was two different tests. They are multiple choice of Blooms' cognitive test instrument to measured students learning achievement and Cornell critical thinking test level X instrument to measured students critical thinking. These test was given in the beginning of treatment to see students intial conditions and the end of treatment to see the effect of learning model and media.

The learning style questionnaire test was taken from students learning style worksheet of Active Learning for Higher Education (ALFHE) module. It's consists of seven questiones about peoples' custom in several indicator activities. The questions answer would lead the partcipants in their learning model approach way. Blooms' Cognitive test is the instrument to measured students

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learning achievement advance. There was 25 multiple choice questions related to the human reproductive system competency. Each question has five possible answer: A, B, C, D and E. The test consisted of 6 cognitive competencies as follows: Knowledge (C1), Comprehension (C2), Application (C3), Analysis (C4), Synthesis (C5) and Creat (C6). Each correct answer given one (1) point and the incorrect given null (0) point. The Modified Cornell Critical Thinking Test (CCTT) Level X was the test instrument to measure students critical thinking in this study. There are three possible answer of this test: Yes, Maybe and No that indicated two critical thinking criteria, namely deduction and induction. The test was consist of 20 multiple choice questions. Each of questions was made by researcher and being intergrated with the Standar Competency about human reproductive system in 2006 curriculum.

Media Preparation

The learning video used in this research has been taken and modified mainly from two sources: Anatomy and Physiology – Revealed 2.0 CD interactive published by Mc Graw Hill Higher Education and The University of Toledo, United State and Savy Human Body System E-Learning CD Interactive published by Centrinova, Indonesia. Special technique also modified the learning video by researcher. Researcher use some video editing software, such as Camtasia and Video Maker.

Problem Based Learning (PBL) Model

There were two groups of classes in this study. The first group is PBL class that utilized learning video as media meanwhile the second is PBL class that utilized graphic as media. The role of media in this method is as the facilitator in the confirmation of PBL stages. The following figure described the implementation of PBL class procedures.

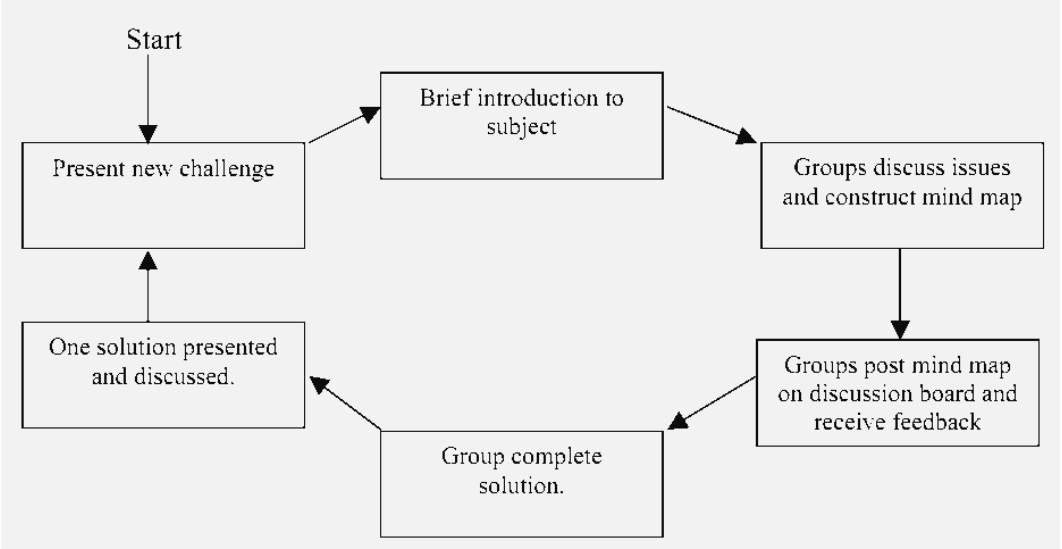

Figure 1. Problem Based Learning implementation step.

Research Design

The research aimed to find any significance differences of students learning achievement and critical thinking in Problem Based Learning (PBL) class by using learning video and graphics as the media. The research design drawn in the Table 1. below.

Table 1. Research design

Class	Pre-Test	Treatment	Post-Test
XI IA 6	T_1	X_1	T_2
XI IA 5	T_1	X_2	T_2

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RESULT AND DISCUSSION

Learning Achievement

The result of students cognitive post test average scores as the student learning achievement measurement in Problem Based Learning model that utilize learning video as media is higher 10, 0 points compared to students cognitive post test average score in Problem Based Learning model that utilize graphic as media, it can be seen in the Table 2 below. This result indicates that learning video utilization as media positively contributed increasing the students learning achievement.

Table 2. Bloom Cognitive instrument score

Class	Pre-Test	Post-Test
PBL with graphic	60,0	86,2
PBL with learning video	60,0	96,2

Hypothesis test also confirms this differentiation, where t_{obs} = 10,03 is higher than t_{table} = 2.3727 value. Therefore the study find that there is a significant difference of students learning achievement between the using of learning video and graphic in PBL model with level of significance 0,01.

However, both of the class has performed high increasement progress. Further investigation, investigates this phenomena by analyze students learning style observation result that has given in the beginning of the class. The data result indicates that aproximately 60% of the students in PBL model that utilize learning video and PBL model that utilize graphic is highly achieved their learning through visual learning style. It's mean, both graphic and learning video are a good learning media for visual learning style students. However, there are some others factors that might influence greater role of learning video than graphic as media to advance students learning achievement in this case.

The learning video as a motion picture has clarification function. It clarifying abstract concept in human reproductive system topic and providing it into the realistic picture that brings the students into a better comprehansion. This clarification function of learning video potentially influenced learning achievement advance compred with non motion media. Learning video also as an Audio Visual Aid played a great deal in retaining the information through hear sense and visual sense, compared with simple graphic that only emphasized in visual sense. As Edgar Dale theorized said learners retain more information by what they "do" as opposed to what is "heard", "read" or "observed", obviously learning video has more advantages than the graphic in enhancing learning achievement contribution.

Critical Thinking

Based on the Cornell critical thinking pre test instrument average score that was given to the students it was known that both of the classes are significance difference in initial critical thinking. It can be seen in Table. 3 that the average of pre test score in PBL class that utilize learning video as media is higher 6,0 points than the pre test score in PBL class that utilize graphic as media, that's mean the PBL class that utilize learning video as media sample population has higher initial critical thinking level than the PBL class that utilize graphic as media especially about human reproductive system topic.

The average score of critical thinking post test score in PBL class that utilize learning video as media population is also highly significance difference 12,0 points than the PBL class that utilize graphic as media in the end of the research treatment. It can be concludes that the critical thinking level in PBL class that utilize learning video as media increasing higher after being treated with the learning video media rather than graphic as seen in Figure 2 below.

Table 3. Cornell Critical Thinking instrument score

Class	Pre-Test	Post-Test
PBL with graphic	44,0	74,2
PBL with learning video	50,0	86,2

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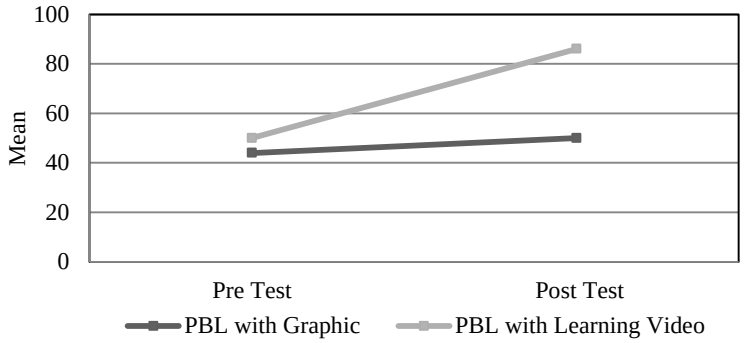

Figure 2. Graph of the differentiation of students critical thinking level increasement in PBL class that utilize graphic and PBL class that utilize learning video as media.

The hypothesis test also confirms this differentiation, where t_{obs} = 6,403 is higher than t_{table} = 2.3727 value. Therefore the study find that there is a significant difference of students critical thinking between the using of learning video and graphic in PBL model with level of significance 0,01.

In the other hand, based on the calculation of post test critical thinking instrument result, both Problem Based Learning model with learning video as media and Problem Based Learning model with graphic as media has higher critical thinking in induction criteria than the deduction criteria. The Cornell critical thinking post test score for deduction and induction criteria in Problem Based Learning model with learning video as media and Problem Based Learning model with graphic as media comparision result can be seen in Figure. 3 and Figure. 4 below.

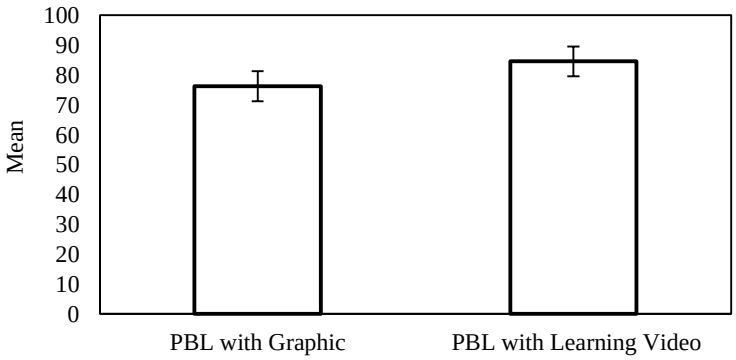

Figure 3. Deduction criteria of Cornell critical thinking post test result comparision between PBL model with learning video as media and PBL model with graphic as media.

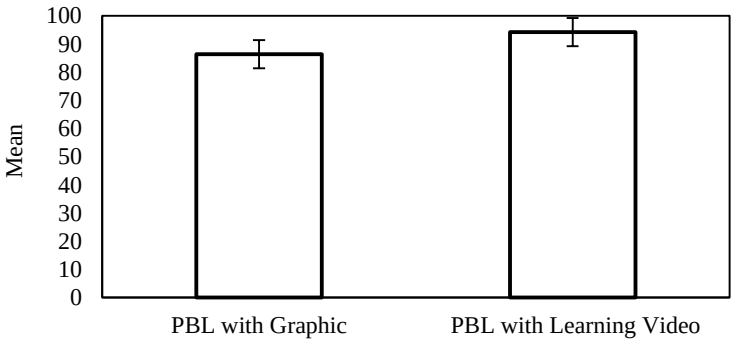

Figure 4. Induction criteria of Cornell critical thinking post test result comparision in PBL model with learning video as media and PBL model with graphic as media.

The utilization of learning video pottentially increasing the learning understanding, stimulating language ability, improving creativity/imagination, and also enhancing critical thinking

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and students motivation in the learning process. The role of learning video clarification in this case took a great responsible creating this causal effects as mentioned in the discussion earlier. Moreover, The learning video also help the students to understanding complexity and the dynamic of knowledge, concreting abstract concept and making the information stay longger in remembering phase, thus through this experience the learning video played a major factors enhancing students critical thinking day by day. In additional according to Dewey in Fitriyani (2009), problems that requires unique solution was a method to stimulated people to think. In order to creat problem situation at the class, Problem Based Learning methods was the best class model that stimulated the students to be a a good thinker that find out the problem's solution. The practice of this model believed would stimulating the students to thinking critically.

Relation of Students Critical Thinking and Learning Achievement

The data indicates that the higher students critical thinking score, the higher learning achievement students gathered. This relations has confirmed in National Assessment of Educational Progress (2001) findings that there is a link between critical thinking skills and the increasement of student achievement in the classroom.

Problem Based Learning (PBL) class model significantly increase students' critical thinking level. It proofes based on the increasement of Cornell critical thinking post test result. However, the improvement of students critical thinking between PBL class that utilize learning video as media with PBL class that utilize graphic as media in this study is not the same. The role of media function in this case influences the level significance diffrence of students learning achivement. Result of calculation of simple liniear regression formula has investigate that students critical thinking in PBL class that utilize learning video as media has 20,8 % higher influences the students learning achievement than the PBL class that utilize graphic did. Therefore it can be concludes that the effect of learning video utilization has significance higher increasing student critical thinking and learning achievement than the graphic media. Therefore it can be concluded that there is a good correlation between critical thinking of students from Problem Based Learning model with student's learning achievement. Teaching critical thinking through Problem Based Learning method is associated with higher students test scores in their learning achievement. Instruction emphasizing advanced reasoning skills in classroom promotes high student performance.

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