Asian Journal of Agriculture

| Asian J Agric | vol. 1 | no. 1 | June 2017|
| E-ISSN: 2580-4537 |

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Asian Journal of Agriculture

| Asian J Agric | vol. 1 | no. 1 | June 2017| E-ISSN: 2580-4537 |

Published semiannually **PRINTED IN INDONESIA**

E-ISSN: 2580-4537

9 772580 453012

Asian Journal of Agriculture

| Asian J Agric | vol. 1 | no. 1 | June 2017|

ONLINE
http://smujo.id/aja

e-ISSN
2580-4537

PUBLISHER
Society for Indonesian Biodiversity

CO-PUBLISHER
Universitas Mulawarman, Samarinda, Indonesia

OFFICE ADDRESS
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PERIOD OF ISSUANCE
June, December

Society for Indonesian Biodiversity

Universitas Mulawarman Samarinda, Indonesia

Published by Smujo International for The Society for Indonesian Biodiversity and Universitas Mulawarman Samarinda, Indonesia

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ASIAN JOURNAL OF AGRICULTURE
Volume 1, Number 1, June 2017
Pages: 1-5

E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g010101

Pathogenicity of entomopathogenic fungus *Metarhizium* spp. against predators *Menochilus sexmaculatus* Fabricius (Coleoptera: Coccinellidae)

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Manuscript received: 30 January 2017. Revision accepted: 17 March 2017.

Abstract. *Trizelia, Busniah M, Agung Permadi A. 2017. Pathogenicity of entomopathogenic fungus* Metarhizium *spp. against predators Menochilus sexmaculatus Fabricius (Coleoptera: Coccinellidae). Asian J Agric 1: 1-5. Metarhizium* spp is an entomopathogenic fungus that has wide host range. *Metarhizium* spp can not only infect insect pests, but can also infect beneficial insects such as predators. The pathogenicity of four isolates of *Metarhizium* spp. was investigated against *Menochilus sexmaculatus* predators. Isolates of *Metarhizium* spp were isolated from rhizosphere of cacao, cabbage, chili and rubber crops. The experiment was conducted by treating the fourth instar larvae with an appropriate conidial suspension of 10^8 conidia mL^{-1}. The results showed that all isolates of *Metarhizium* spp were pathogenic toward beetle predator *M. sexmaculatus*. Mortality of larvae within 7 days after application of conidial suspension varied between 27.50 to 67.50% and there were statistically significant differences among the tested isolates. *Metarhizium* spp. had also a significant effect in reducing pupation and adult emergence of *M. sexmaculatus* to below 30% and 3%, respectively. These studies indicate that entomopathogenic fungus *Metarhizium* spp was pathogenic to beetle predators, *M. sexmaculatus*

Keywords: Beetle predators, entomopathogenic fungus, *Menochilus sexmaculatus, Metarhizium*

INTRODUCTION

Entomopathogenic fungi have been used in control of several insect pests as alternative to chemical insecticides (Hajek and Leger, 1994). Among these entomopathogenic fungi, *Metarhizium* spp have great potential as biological control agents against insects pest and as a component within integrated pest management systems. Utilization of *Metarhizium* spp. for pest control has been widely reported. Trizelia et al (2011) reported that fungus *Metarhizium* spp can kill *Spodoptera litura* eggs. Mortality of *S. litura* eggs depends on the fungal isolates, ranging between 19.79%-75.70%. First instar larvae also die 3 days after eclosion. The maximum mortality of first instar larvae was 58.65%. Nunilahwati (2012) reported that *Metarhizium* spp can also infect *Plutella xylostella* larvae and the mortality of larvae up to 82% with LT_{50} value 2.26 days. Saranya et al (2010) reported that mortality of aphids, *Aphis craccivora* was 80.76% observed in 168 h, at the highest concentration (10^8 spores mL^{-1}) of *Metarhizium anisopliae*.

One of the most important aspects that should be considered in the use of *Metarhizium* spp as biological control agents, is the wide compatibility with other biological control agents such as insect predator *Menochilus sexmaculatus*. This is due to the fact that this fungus can infect many kinds of insects, both predators and pests. Although some entomopathogenic fungi are known to have narrow host ranges, especially some of those belonging to Deuteromycotina, they have a rather wide range of hosts from many insect orders, including natural enemies (Inglis et al. 2001). Compatibility between entomopathogenic fungi and predators is required to minimize risk to the environment.

Menochilus sexmaculatus beetle predators (Coleoptera: Coccinellidae) are one of the biological control agents. These predators feed on *Lipaphis erysimi* nymphs (Ali and Rizvi, 2009). Adults of *M. sexmaculatus* were able to prey on *Aphis gossypii* on an average rate of 44.50 individuals per hour (Nelly et al.2012). Solangi et al. (2007) reported that the mean number of prey consumed by *M. sexmaculatus* during entire adult life was 80.08, 69.95 and 68.96 of *R. maidis, A. gosypii* and *T. trifolii* respectively. This beetle has great potential to be a biological control of these three aphid species. Radiyanto et al (2011) reported that the maximum number of prey consumed by adult *M. sexmaculatus* females, was 300 individuals at various stages of *Rhopalosiphum maidis* Fitch (Homoptera: Aphididae) per 24 h. Ali et al (2012) found that the predatory potential of male *M. sexmaculatus* on *Rhopalosiphum padi* was 2294 to 2422 and female was 2912 to 3085 aphids.

An understanding of the interaction between entomopathogenic fungi *Metarhizium* spp and predator *M. sexmaculatus* in agricultural ecosystems is needed to predict the environmental impact of *Metarhizium* spp application to the development of predator populations. In addition to direct contact with entomopathogenic fungi, predators can also be threatened by consuming fungus infected prey. Er et al (2008) reported that *Metarhizium anisopliae* (Metschnikoff) Sorokin. was generally found to be pathogenic to *Coccinella septempunctata* and the mortality of adults was 47.61%. While Wu et al (2014) reported that *B. bassiana* strain SZ-26 showed high toxicity against *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) but not a detrimental effect to predatory mite *Neoseiulus (Amblyseius) barkeri* (Hughes) (Acarina: Phytoseiidae).

The purpose of this research was to study the pathogenicity of several isolates of *Metarhizium* spp against a predatory beetle *Menochilus sexmaculatus*.

MATERIALS AND METHODS

Fungal isolates

The isolates of *Metarhizium* spp. used was from the collection of the Laboratory of Biological Control, Department of Plant Pests and Diseases, Faculty of Agriculture, University of Andalas, Padang, Indonesia. Isolates were isolated from rhizosphere of cacao, rubber, pepper and coffee crops from various locations (Table 1). All isolates were grown on SDAY (*Sabouraud dextrose agar + yeast extract*) medium.

Aphids and *M. sexmaculatus* culture

Aphids (*Aphis maydis* and *A. craccivora*) and *M. sexmaculatus* predators were collected from the corn and long bean fields in the city of Padang and brought to the laboratory. Predators were reared in the laboratory on this aphid species in wide-mouth plastic jars. Fresh stock of leaves respective for aphids hosts were provided daily as oviposition substrate for female beetles. The eggs laid by female beetles on aphids-host leaves were transferred to plastic box for further rearing. The newly hatched larvae were reared to the fourth instar to be used as test insects.

Preparation of conidial suspension

Isolates of *Metarhizium* spp. were grown using autoclaved SDAY media. The fungal cultures were incubated at 25ºC for 15 days. Cultures with fully developed conidiospores were washed by 5 mL sterilized distilled water mixed with 0.05% Tween 80 to obtain the stock of conidial suspension. The conidial suspensions were passed through two layers of sterile muslin to remove any agar pieces and hyphae from the conidial suspensions. Conidia were counted in a compound microscope using a Neubauer hemocytometer.

Pathogenicity test

The conidial density was used 10^8 conidia mL^{-1} for all isolates. Two mL of the suspension was sprayed against the fourth instar larvae of *M. sexmaculatus* tested. For the control, larvae were sprayed with the same volume of distilled water mixed with 0.05% Tween 80. Then the fourth instar larvae of *M. sexmaculatus* were kept in the box provided. Each box contained only one of the fourth instar larvae of *M. sexmaculatus*. Each experimental unit consisted of 10 fourth instar larvae of *M. sexmaculatus*. The experiment was repeated four times. The mortality of larvae was recorded at 24 h intervals until adult emergence. Parameters observed were larval mortality, pupation, adult emergence and the ability of *M. sexmaculatus* to prey on aphids.

Data analysis

This experiment was arranged in the Completely Randomized Design (CRD) and data were analyzed by means of the analysis of variance (ANOVA) and Duncans Multiple Range Test (DNMRT) (P=0.05%).

RESULTS AND DISCUSSION

The results of pathogenicity test showed that all of the *Metarhizium* spp. isolates were pathogenic on the larvae of *Menochilus sexmaculatus*. Mortality of *M. sexmaculatus* larvae after application of Metarhizium spp., depended on the isolates. *Metarhizium* spp isolates tested induced cumulative mortalities between 27.50 to 67.50% in fourth instar larvae of *M. sexmaculatus*. ANOVA test revealed statistically significant differences amongst the treatments ($F=16.85$; $P<0.05$). All the *Metarhizium* spp isolates applied gave significant higher mortality than the control unit (Table 2). Three isolates (MetAKi, MetLKt and MetPKo) caused higher mortality (62.5-67.5%) on the fourth instar larvae, while MetKbCi isolates showed the lowest degree of pathogenicity (27.50%).

The fourth instar larvae of *M. sexmaculatus* infected by entomopathogenic fungi *Metarhizium* spp. was covered by fungus mycelia or conidia having green in color around the body surface *M. sexmaculatus* larvae (Figure 1).

The mortality of *M. sexmaculatus* larvae after *Metarhizium* spp application was due to the fact that *Metarhizium* spp was able to kill not only the pest insect but also predators. This is caused by the body structure of both being almost the same. Thungrabeab and Tongma (2007) reported that *Metarhizium anisopliae* could kill *Dicyphus tamanii* (Hymenoptera; Miridae) up to 10% and *Carnea chrysoperla* (Neuroptera; Chrysopidae) up to 4% at density of conidia 10^8 conidia mL^{-1}. The same result was also found by Ibrahim et al (2011) that isolates of *Metarhizium* spp. caused mortality for *Cryptolaemus montrouzieri* (Coleoptera; Coccinellidae) larvae about 7.7 %. Er et al (2008) also reported that *Metarhizium anisopliae* caused mortality of *Coccinella septempunctata* (Coleoptera; Coccinellidae) adults up to 47.61%. Sahayaraj et al (2008) reported that the survival rate and the particle carrying capacity of reduviid predator, *Acanthaspis pedestris* were reduced after application of *Metarhizium anisopliae* compared to the control. While Huang et al (2012) reported that there are no adverse effects of entomopathogenic fungus *Beauveria bassiana* application on the survival and reproduction of predator *Prynocaria congener* (Coleoptera; Coccinellidae).

Difference in pathogenicity among isolates is common for entomopathogenic fungi. Differences in pathogenicity among isolates were attributed to the differences in the ability to produce enzymes and mycotoxins during the infection process in insects such as the time of contact with the cuticle and hemocoel (Tanada and Kaya, 1993; Balachander et al. 2012). A toxin called destruxin which is produced by *Metarhizium* spp during infection was thought to be the cause of *M. sexmaculatus* death.

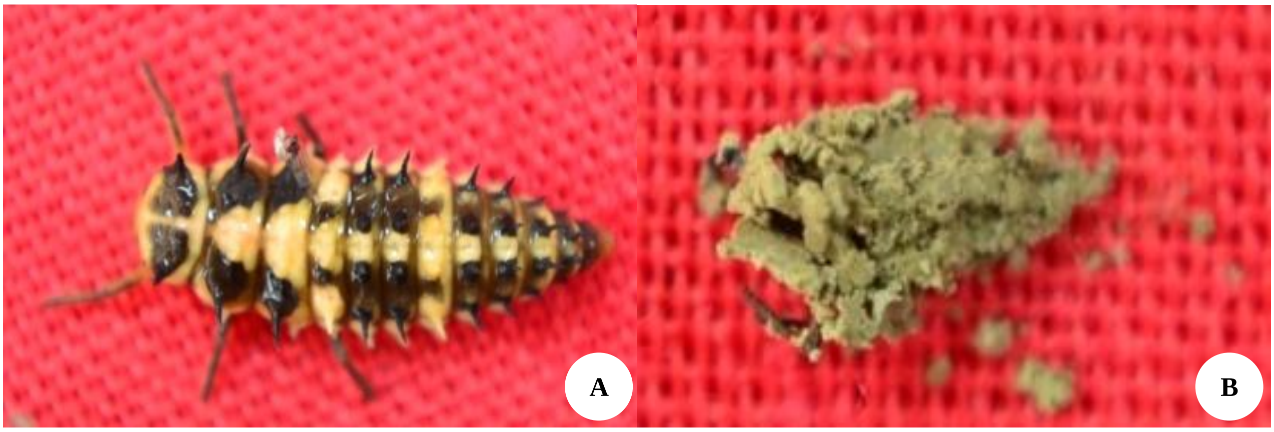

Figure 1. The fourth instar larvae of *M. sexmaculatus* in form of normal (A) and infected by entomopathogenic fungi *Metarhizium* spp. (B)

Table 1. The source of *Metarhizium* spp. isolates

Isolate	Host	Origin
MetPKo	Cacao	Pariaman
MetLKt	Rubber	Lintau (Tanah Datar)
MetKbCi	Chili	Koto Baru (Tanah Datar)
MetAKi	Coffee	Matur (Agam)

Table 2. Mortality of the fourth instar larvae of *M. sexmaculatus* after application of four isolates of *Metarhizium* spp with density conidia 10^8 conidia/mL.

Isolates	Mortality ± SD (%)
MetAKi	67.50 ± 9.57 a
MetLKt	65.00 ± 17.32 a
MetPKo	62.50 ± 9.57 a
MetKbCi	27.50 ± 18.93 b
Control	7.50 ± 5.00 c

Note: Different letters indicate a statistically significant difference according to DNMRT test, P<0.05

Besides killing larvae, fungi infections also affected the development of pupae. The amount of pupae formed (pupation) is inversely related to the mortality of larvae. Based on the analysis of variance, the percentage of pupae formed was significantly different among isolates (F=17.20; P<0.05), (Table 3). The highest percentage of pupa formed (70%) were found under application of isolate MetKbCi, while the lowest was under MetPKo, MetLKt and MetAKi.

As for larvae, pupae *M. sexmaculatus* infected by entomopathogenic fungi *Metarhizium* spp. were also covered by the fungal mycelia or conidia having green color at the body surface (Figure 2).

Percentage of adult emergence from larvae infected by *Metarhizium* spp. also reduced significantly. Application of *Metarhizium* spp. could only generate adults about 2.5% - 27.5%, while the control could reach 90% (Table 4).

Metarhizium spp. had also a significant effect in reducing pupation of *M. sexmaculatus* and adult emergence to below 30% and 3%, respectively. The low percentage of pupation and adult emergence was due to the fact that a high number of larvae and pupae were killed before becoming adult. This proves that the influence of *Metarhizium* spp. was not only active and destructive in certain stadia treated, but also had an impact on subsequent stadium. Malarvannan et al (2010) reported that entomopathogenic fungi, *Beauveria bassiana* can reduce pupation and adult emergence of *Spodoptera litura*, due to phagodepression and difficulty in molting.

The ability to prey on aphids of unapplied *M. sexmaculatus* larvae by *Metarhizium* spp. was also significantly different from those applied *M. sexmaculatus* (P <0.0001) (Table 5). In control, the predation ability of *M. sexmaculatus* on aphids reached 10.63 individuals per hour, while the predation ability of predators sprayed with entomopathogenic fungi, decreased significantly (from 6.93 to 1.08 individuals per hour).

The ability of the fourth instar larvae of *M. sexmaculatus* to prey on aphids was generally decreased following fungal infection. The reduction in preying insects was assumed due to production of toxic substances by fungi or mechanical disruption of the insect structural integrity by hyphal growth. Destruction affected target cell organelles (mitochondria, endoplasmic reticulum and nuclear membrane) and caused paralysis of cells. It also affected the mesenteron, Malpighian tubes, and tissue larval hemocyte dysfunction (Tanada and Kaya, 1993).

Based on the research conducted, it can be concluded that all isolates of *Metarhizium* spp tested were pathogenic to predators *M. sexmaculatus*. The lowest pathogenicity (27.5%) isolates were found at MetKbCi, while the isolates MetPKo, MetLKt and MetAKi had high pathogenicity (62.50%, 65%, and 67.50% respectively). Further research is required to determine how direct effects observed in laboratory play out in a field environment.

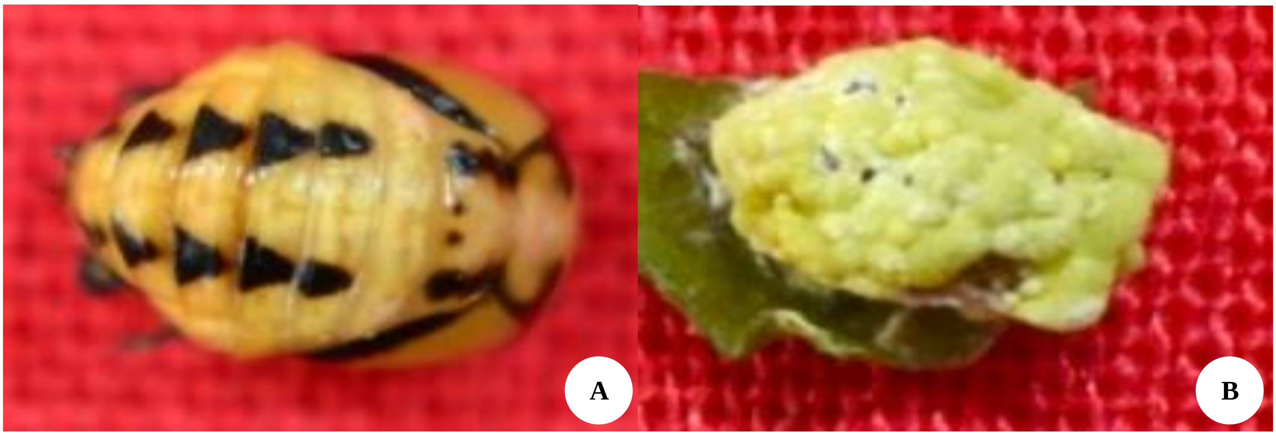

Figure 2. Pupae of *M.* s*exmaculatus* in form of normal (A) and infected by entomopathogenic fungi *Metarhizium* spp. (B)

Table 3. Formation of *M. sexmaculatus* pupae

Isolates	Pupae formed ± SD (%)
Control	92.50 ± 5.00 a
MetKbCi	70.00 ± 20.00 b
MetPKo	32.50 ± 8.16 c
MetLKt	30.00 ± 17.08 c
MetAKi	27.50 ± 9.58 c

Note: Different letters indicate a statistically significant difference according to DNMRT test, P<0.05

Table 4. Percentage of adult emergence of *M. sexmaculatus*

Isolates	Adult emergence ± SD (%)
Control	90.00 ± 0.00 a
MetAKi	27.50 ± 5.00 b
MetLKt	15.00 ± 12.91 b c
MetKbCi	15.00 ± 23.81 b c
MetPKo	2.50 ± 5.00 c

Note: Different letters indicate a statistically significant difference according to DNMRT test, P<0.05

Table 5. The predation ability on aphids of *M. sexmaculatus* larvae for 1 h after 24 h application of *Metarhizium* spp.

Isolates	Ability of predation (ind./hour) ± SD
Control	10.63 ± 1.56 a
MetPKo	6.93 ± 0.41 b
MetLKt	4.35 ± 0.79 c
MetAKi	1.48 ± 0.22 d
MetKbCi	1.08 ± 0.21 d

Note: Different letters indicate a statistically significant difference according to DNMRT test, P<0.05

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ASIAN JOURNAL OF AGRICULTURE
Volume 1, Number 1, June 2017
Pages: 6-8
E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g010102

Macrolepiota procera (Scop.) Singer (Agaricomycetes) – a new generic record of edible mushroom for Nagaland, Northeast India

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Manuscript received: 28 March 2017. Revision accepted: 9 June 2017.

Abstract. *Kumar R, Pandey S, Rishi RR, Giri K. 2017. Macrolepiota procera* (Scop.) Singer (Agaricomycetes) - a new generic record of edible mushroom for Nagaland, Northeast India. *Asian J Agric 1: 6-8.* In August 2013, an interesting mushroom was collected from the Puliebzie forest range in Kohima District of Nagaland state of India. The mushroom was identified as *Macrolepiota procera* (Scop.) Singer based on the macroscopic and microscopic characters.

Keywords: *Macrolepiota procera*, mushroom, Nagaland, new generic record

INTRODUCTION

The Northeast region of India abounds in forest wealth, including a wide range of flora and fauna. Nagaland, one of the Northeastern states of India, is a unique hotspot of rich biodiversity and has served as the habitat for a wide variety of mushroom species. The altitude varies from 194 m to 3,841 m and is characterized by the luxurious growth of conifers and mixed forests. Nagaland enjoys a humid tropical climate, where the plain areas and foothills both have a subtropical climate. The low to moderate ranges have varying degrees of slopes and have a submontane climate. Monsoon season lasts for five months, i.e. from May to September, with May, June, and July being the wettest months. Due to varied topography, annual rainfall ranges from 1000 mm to 3000 mm at different places with an average of 2000 mm. (http://www.moef.nic.in/sites/default/files/sapcc/Nagaland.pdf). These climatic conditions are conducive for the natural occurrence of a variety of mushroom species. Kumar et al. (2013) reported 15 wild edible mushroom species from 12 locations in different districts of Nagaland.

Mushroom genera having different forms and morphological characters comprise the white and green spored members of the Agaricaceae such as *Chamaemyces, Chlorophyllum, Coniolepiota, Cystolepiota, Eriocybe, Lepiota, Leucoagaricus, Leucocoprinus,* and *Macrolepiota* are called lepiotaceous fungi (Vellinga 2004 a, b). Historically mycologists characterized and defined these lepiotaceous taxa mostly from Netherlands in southern Limburg near Breukelen and Amsterdam (Kelderman 1994; Chrispijn 1999). They are also known in other countries (Bon 1993; Guinberteau et al. 1998; Henrici 2001; Sundberg 1967). *Macrolepiota* has about 40 species, and some of these have been used as foodstuffs and potentially cultivated. Using molecular and morphological data, the position and composition of *Macrolepiota* within the Agaricaceae and its phylogenetic relationship with other members of the family have been investigated. *Macrolepiota procera* clustered together with *M. mastoidea* and *M. clelandii* (Vellinga et al. 2003). Morphologically, *Macrolepiota* is readily recognized by its big and fleshy basidiocarp; universal veil that splits up into coarse to fine squamules on the pileus; thick walled spores with a germ pore, and an often complicated, double annulus. The majority of the characters of lepiotaceous fungi have been accepted from previous studies (Vellinga 2004; Vellinga et al 2011; Asef and Muradov 2012; Seyidova and Hüseyin 2012).

Macrolepiota procera, the parasol mushroom, is a well-known and highly esteemed edible mushroom in much of Europe, while *Macrolepiota albuminosa* is found in Chinese cuisine. They are harvested directly from natural habitats, grasslands, pastures, forest boundaries, hills, etc. (Boa 2004; Hu 2005; Kirk et al. 2008).

MATERIALS AND METHODS

Systematic and periodical surveys of Puliebzie forest range in Kohima District of Nagaland state of India were undertaken from June 2012 to December 2013. Field surveys and mushroom collection was done according to guidelines mentioned in "A Guide Collecting and Preserving Fungal Specimens for the Queensland Herbarium"(https://fungimap.org.au/Leonard2010_QLD_fungi_collect_manual_v3.2web.pdf). The specimens were immediately wrapped in aluminum foil or wax paper after taking photographs. Few specimens were preserved in 2 % formaldehyde at the time of collection. In the laboratory, the specimens were first dried in an oven at 40-50 oC. The specimens were then kept in plastic containers with some naphthalene balls in the Forest Protection Division, Rain Forest Research Institute, Jorhat, Assam. Identification numbers were assigned to the specimens for further studies. To obtain the spore prints, the face of the mushroom cap was placed over black and white paper (half white and half black) and covered with a bell jar overnight.

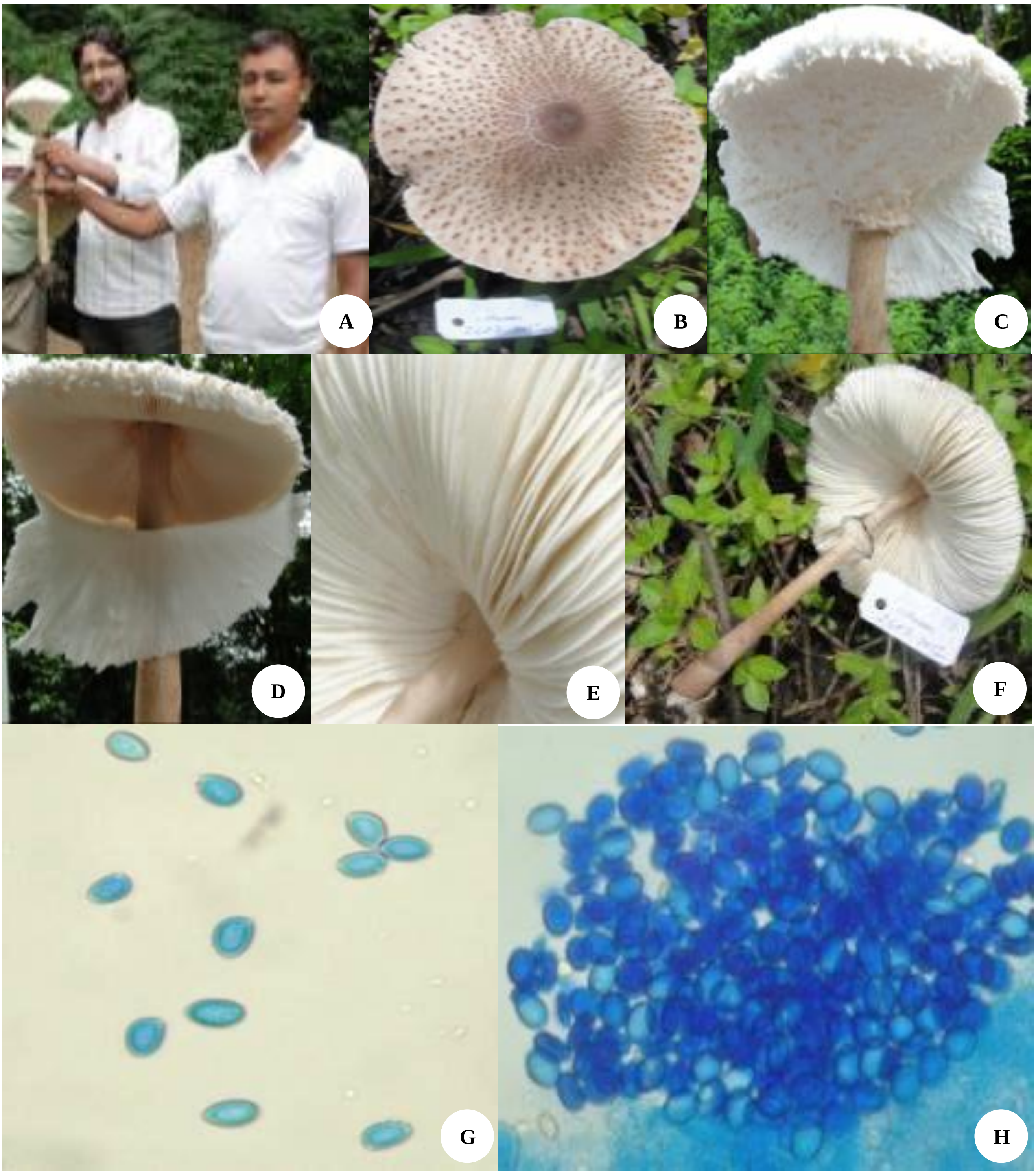

Figure 1.A. Team of scientist and research associates, B-F. Different views of *Macrolepiota procera,* G-H. Spores of *Macrolepiota procera*

RESULTS AND DISCUSSION

Mushroom collection and identification

In August 2013, an interesting mushroom was collected from the Puliebzie forest range in Kohima District of Nagaland state. Standard methods for collection, preservation, and description were followed (Atri et al. 2005;https://fungimap.org.au/Leonard2010_QLD_fungi_collect_manual_v3.2web.pdf). Color notations in the macroscopic descriptions are from Kornerup and Wanscher 1978. Microscopic characters were studied from freehand sections mounted in 5 % KOH, stained with 1 % Congo red. The spore shape quotient (Q = L/W) was calculated considering the mean value of length and width of 20 basidiospores. The specimens were kept in the Museum of Forest Protection Division, Rain Forest Research Institute, Jorhat, Assam with the identification number: ID No/RFRI/NL-000360. The morphological and microscopic characters revealed it to be *Macrolepiota procera*, a hitherto unrecorded species from Northeast India. The fungus, commonly called parasol mushroom is a basidiomycete fungus with a large, prominent fruiting body resembling a parasol. It belongs to the order Agaricales of the phylum Basidiomycotina. It is a soil-inhabiting saprophytic species growing either alone, scattered in woods, at the edges of woods, or in pastures on trails and other ground areas.

Nomenclature

Based on the presence or absence of clamp connection, Singer (1986) partitioned *Macrolepiota* into 2 groups namely *Macrolepiota* and *Macrosporae*. Bon (1996) divided the genus *Macrolepiota* into three sections. Molecular phylogenetic analysis recovered three major clades: macrolepiota clade, macrosporae clade and volvate clade.

Taxonomy

Cap 8-23 cm in diameter, oval, convex to broadly convex with age, white to cream, covered with brown, dark brown to grayish brown plate like squamules irregularly arranged towards the margin, below surface whitish, dark bump in the center. Lamellae are free, densely crowded, thin, white when young, white to cream colored when mature, lamellae in 2-3 lengths. Stem whitish, subcylindrical 17-33 x 1.2-2.4 cm, very fibrous and reaches full height before the cap has expanded, covered with brown squamules sometimes in irregular bands. Annulus, superior about 4 cm below the stem apex, underside brownish, upper side membranous whitish. Spore print white. Spores smooth; broadly elliptical with a small germ pore, thick-walled, hyaline, dextrinoid, congophilous, 10-16 x 9-13 µm. Basidia thin-walled, clavate, 4 spored, Pleurocystidia absent. Chelocystidia thin-walled, hyaline, in bunches forming a sterile edge, 19-37 x 9-23 µm. Clamp connection present.

Comments

Currently, there are about 117 species recognized worldwide (www.indexfungorum.org 2014). Several other genera are been found that are very similar to *Macrolepiota. Chlorophyllum* differs only in the distinctly green or ochre spores. The lamella trama is trabecular (Clemencon 1997), a character shared with *Leucoagaricus* Singer, *Leucoco prinus* Pat., and *Chlorophyllum* Mass. The spore print of genus *Chlorolepiota* is primrose yellow, provided with a germ pore (Vellinga et al. 2003). *Volvo lepiota* Singer also closely resembles *Macrolepiota*; a volva is present, the pileus covering is trichodermal, clamp connections present and the spores have a germ pore. (De Meijer 1996). *Macrolepiota procera* is an edible species and has been of interest to cultivate, but knowledge on this genus is poor and fragmentary in Nagaland. To facilitate easy identification, a photograph of the fungus and spores is provided (Figure 1-8). To the best of our knowledge, this is the first report on *Macrolepiota procera* from Northeast India.

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ASIAN JOURNAL OF AGRICULTURE
Volume 1, Number 1, June 2017
Pages: 9-16
E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g010103

Farmer adaptation strategy in paddy fields affected by climate variability in monsoon regions

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Manuscript received: 23 March 2017. Revision accepted: 15 June 2017.

Abstract. *Apriyana Y, Sarvina Y, Dewi ER, Pramudia A. 2017. Farmer adaptation strategy in paddy field affected by climate variability in monsoon regions. Asian J Agric 1: 9-16.* One of the strategies to minimize the impacts of climate variability and climate anomalies on paddy fields in monsoon regions is the adaptation of agriculture cultivation. It is important to minimize the impact of these two phenomena to reduce economic loss, particularly for food security in Indonesia. The objectives of this study are: (i) to identify onset and cropping pattern in irrigated land, rainfed and dry land in affected areas due to climate anomalies, (ii) to collect information on carrying capacity water resources and adaptation practices applied by farmers and, (iii) to identify strategies for farmers on irrigated land, rainfed and dry land in the region affected by climate variability. The desk work analysis and field survey were conducted in Serang district, Banten Province, Subang district, West Java Province and Pati district, Central Jawa Province, Indonesia. The study was undertaken in three cluster activities i.e. (i) correlation analysis of climate anomalies and rainfall, (ii) field survey and, (iii) analysis of onset planting season, cropping pattern, water availability, the best planting time and irrigation schedule. The results showed that the farmers in affected areas due to climate variability could adapt by shifting the onset of planting season. Farmers in irrigated lands changed their onset around 2-4 ten-days period to October II - December II. Furthermore, in rainfed areas, the onset around 4-6 ten-days period was shifted to November I - January III. For dryland their onset around 6-8 ten-days period was moved to November II - February I. The cropping pattern rice-rice-palawija/fallow was applied on irrigated land. Furthermore, the pattern of rice-rice/palawija/fallow-fallow was carried out in rainfed. Finally, the pattern of palawija-palawija/fallow-dormant was performed on the dry land. Adaptation programs dealing with climate variability in Serang and Pati districts varied more than in Subang district. In Serang and Pati, during the first planting season, farmers applied irrigation from approximately 20%-30% water pump from the river and during second planting season, farmers in Pati district used water from a well-pump, as well as in Serang that can reach 100% of the application.

Keywords: Farmer adaptation strategy, climate variability, paddy field, monsoon regions

INTRODUCTION

Climate variability and climate change are two phenomena of climate anomaly which has become a strategic issue and serious concern in Indonesia because it is believed to have a tremendous impact on life in various sectors and could have adverse impacts on global food production and food security (Apriyana 2011; Las et al. 2011). Climate variability in Indonesia driven by major inter-annual scales climate modes, such as the El Nino Southern Oscillation and Indian Ocean Dipole has been playing a vital role by often leading to droughts and decrease in crop yields (Ashok et al. 2001; D'Arrigo et al. 2006; Behera et al. 2008; Hansen et al. 2011)

The important strategy for Indonesian agriculture, especially for food crops, is a determination of planting season onset that is strongly related to climate anomalies (Naylor et al. 2001, 2007). Determination of onset is part of cropping calendar cultivation (Runtunuwu et al. 2007), and it has been traditionally developed by farmers for generations with a variety of different terms in each area (Koesmaryono et al. 2008). However, various indigenous knowledge cannot be fully used as a reference in determining the planting season onset due to climate change and finding indicators of season onset become more difficult. Rainfall fluctuation is very dynamic due to weather anomalies, and can cause a shift in the onset of rainy season and dry season. The impact of rainfall pattern alteration and a change of the season start caused alteration of planting date (Apriyana and Siburian 2014). Consequently, it is complicated for a farmer who has become accustomed to a particular cropping calendar.

In Indonesia, many farmers who are in a region with a monsoon climate, plant rice/paddy on a technical irrigation where water availability throughout the whole year, and there is an irrigation system flowing into the primary, secondary, and tertiary channel. Therefore, paddy cropping patterns in this area are more flexible than other rice fields. Other farmers plant the rice in fields with semi-technical irrigation, in which water availability is usually not enough for plant farming throughout the year. Another system of water supply for rice plantations is the rain-fed with water sources depending on rainfall. Paddy fields of this type are often found in areas of high altitude and dry land. The impacts of climate variability caused by El Niño and IOD were strongly felt in the region of irrigated land, rainfed, and dry land. These events could shift the planting start during the rainy season which was further delayed for 2-3 months in the next season (Las 2000). Planting time is also changing 10-20 days over the last decade (Linderholm. 2006).

The fact that climate variability significantly influences Indonesian food security, shows the necessity to create strategic efforts and programs to anticipate climate variability impacts is very urgent and important. Adaptation of agriculture cultivation is expected to minimize the impact of these anomalies (Apriyana 2011).

To support the development of information system of integrated cropping calendar, it needs an assessment of adaptation strategy notably related to planting time and crop pattern for irrigated land, rain-fed and dry land. The objectives of this study were (i) to identify the farmer's strategy in irrigated land, rain-fed and dry land affected by climate anomalies, (ii) to arrange and interpret capacity of water resources and the farmers' adaptation practices and, (iii) to identify planting time and crop pattern practices.

MATERIALS AND METHODS

Study area

This study was conducted in three districts, i.e. (i) Serang district, Banten Province, (ii) Subang district, West Java Province and (ii) Pati district, Central Jawa Province, Indonesia. The study was conducted using desk work and field surveys.

Analyses of climate anomaly and precipitation

The study was performed by conducting several activities including rainfall, ENSO, DMI, onset of planting season, and sensitivity analysis as well as dynamic cropping pattern determination. Information on ENSO and IOD was obtained through web browsing. ENSO was represented by an index of Nino 3.4 and IOD indicated by DMI index. Precipitation anomaly was analyzed monthly for each station using a formula:

$$AnoCH_{ij} = CH_{ij} - \overline{CH}_{ij}$$

$$\overline{CH}_{ij} = \frac{i}{n}\sum_{j-1}^{n} CH_j$$

Whereas:

$AnoCH_{ij}$ = rain fall anomaly for station -i, month- j

CH_{ij} = rain fall for station -i, month -j

$\overline{CH}_{ij}$ = rainfall average for station -i and month -j

n = the number of data

Correlation analysis was performed temporally to identify the relationship between rainfall anomaly and SST anomaly as an indicator of climate anomaly for each month. The correlation was investigated for several periods: December-February; March-May; June-August and September-November using statistic software *Minitab 14*. Correlation value (r) was calculated with the equation below:

$$r = \frac{n\sum_{i=1}^{n} x_i y_i - \left(\sum_{i=1}^{n} xi\right)\left(\sum_{i=1}^{n} y_i\right)}{\sqrt{\left[n\sum_{i=1}^{n} x_i^2 - \left(\sum_{i=1}^{n} x_i\right)^2\right]\left[n\sum_{i=1}^{n} y_i^2 - \left(\sum_{i=1}^{n} y_i\right)^2\right]}}$$

Whereas:
r = Correlation value
n = Number of pair data
x = SST Nino 3.4 anomaly or IOD anomaly
y = Rainfall anomaly

Correlation value ranged between -1 to 1 ($-1 \geq r \leq 1$). The positive correlation means that the increasing x value is followed by y value and negative correlation means that increasing x value affect to decrease y value. Correlation was determined by confidence interval; strong (99%), moderate (95%) and weak (90%). 18-years data length used in this study. The r value is generated and adopted from http://faculty.vassar.edu/lowry/ch4apx.html i.e.(i) Strong | ± 0.54 | ≤ r <| ± 1.00 |; (ii) Moderate | ± 0.39 | ≤ r <| ± 0.54 |; (iii) Weak | ± 0.33 | ≤ r <| ± 0.39 |; (iv) No correlation r <| ± 0.33 |.

The sea surface temperature (SST) Nino 3.4 (50N-50S, 1200-1700W) index was used to identify the influence ENSO on precipitation. The index was calculated by Kaplan Methods using season and monthly fluctuation. The value of SST Nino 3.4 was obtained from http://www.cpc.ncep.noaa.gov. Similar to ENSO, Dipole Mode (DM) or Indian Ocean Dipole (IOD) is represented by Dipole Mode Index (DMI). DMI represents differences between sea surface temperature in the West Indian Ocean (50°-70°BT, 10°LU-10°LS) and sea surface temperature in the Southeast Indian Ocean (90°-110°BT, 0°-10°LS). The DMI data was adopted from http://www.jamstec.go.jp/frsgc/research/d1/iod. From all analyses, the lag dominant was identified using validation correlation for each station.

Analysis of carrying capacity of water resources and farmers adaptation

Both primary and secondary data were required in this study. The primary data was obtained from direct observation in the field either collected from questioner or observed data from the specific location. Moreover, the secondary data was collected from a literature study. To collect data and information, the field survey was conducted by interview methods. Sampling technique was applied to represent the population to minimize the gap (bias).

Farmers or farmer leader groups were decided as respondents. Five respondents were chosen for each land type represented in the district. Respondents were asked to have minimum ten years' experience in paddy/rice cultivation. Furthermore, to obtain more accurate data, the in-depth interview was performed through a questioner technique.

The steps of data collection are (i) identification of the characteristics of the target group of farmers, (ii) interrogator arrangement, (iii) tabulation of the descriptive data, (iv) survey data analysis, (v) data report.

Carrying capacity of water and climate resources

The carrying capacity of water and climate resources was defined based on intrinsic values. This value was derived from the utilization of such resources. The carrying capacity was analyzed by using a sensitivity climate anomaly map (for the affected area or not) through inventory water and climate resources that affected cropping calendar dominantly and as unique characteristics for each zone. Carrying capacity of the environment and water resources were analyzed based on two approaches, namely the conditions of climate and water resources as well as its influence on the cropping calendar. The weather resources referred in this study are the intensity, pattern, and distribution of a ten-day period of rainfall. Whereas for water resources, some variables studied were the accessibility to water resources and the level of water availability. The accessibility to water resources was assessed based on: (i) the distance from the agriculture land to a water source, and (ii) the availability of tools to get the water. Furthermore, the level of water availability was accessed based on (i) the type of water source and (ii) Quantity water source.

Farmer adaptations to a cropping calendar

Indonesian farmers have applied various technologies to adapt to the season, climate and other natural phenomena both with simple or modern technology. All adaptation activity related to water management and climate conducted by the farmer was inventoried to add more information on cropping patterns for their region. This inventory will be carried out for each level of ENSO-IOD sensitivity in study area through interviews and field observation. To solve the issue of water shortage, farmers use well pumps, irrigation or pump the water from the river.

RESULTS AND DISCUSSION

Rainfall variability

In general, the rainfall in Serang, Subang and Pati districts, tended to decrease over the last 20 years (Figure 1). This downward trend may be influenced by the decline of precipitation which reached 1000 mm/year on dry years due to the climate anomalies in 2002/2003, 2004/2005 and 2006/2007.

The impact of climate anomaly in study area

Correlation analysis between sea surface temperature (SST) in Pacific Equator and Indian Ocean, and rainfall in study areas shows that not all rainfall stations have strong correlation with ENSO and IOD. The influences of IOD and ENSO can be seen from June to August and September to November. It coincides with the increasing SST anomaly in study regions commonly occurring around May and June. The ENSO (Nino 3.4) significantly influences the rainfall intensity in study region during June to August indicated by strong negative correlation. From September to November, ENSO and IOD increasingly influence all affected regions and it was considered as a period with strongest influence of ENSO and IOD. This was also referred to as a transition period from dry season to wet season. The influence of ENSO and IOD started to decrease between December to February and continued from March to May. This indicates that the influence of ENSO and IOD was weak.

Hendon (2003) concluded that the weak correlation between ENSO/IOD and rainfall in the wet (rainy) season addressed to the alteration of Indonesian sea surface (positive anomaly) during a transition period from dry season to wet season (from September to November to December to February). The sea surface temperature anomaly in Indonesia, is in contrast to the sea surface temperature anomaly in Pacific Ocean (center and east) and West India Ocean from June to August and September to November. However, it has the same condition from December to February and March to May.

Water sources used by farmers

The water sources used by farmers in the study area came from different sources for each planting season, during normal and dry years. The water availability in the dry year is different compared to the normal year. Farmers changed the water source in dry years as an adaptation strategy to irrigate their cultivation land as well as to avoid crop failure due to drought stress.

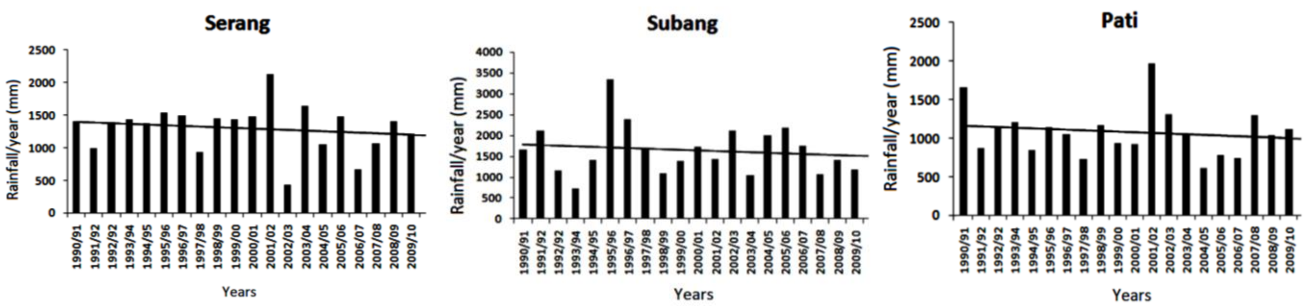

Figure 1. Downward trend of rainfall in Serang, Subang and Pati districts, Indonesia for period 1990/9991 to 2009/2010

Table 1. The responses of farmer on water shortages at normal and dry years in Serang, Subang, and Pati districts, Indonesia during Planting Season I and Planting Season II.

Irrigation type	Water resources	Planting season			
		Normal year		Dry year	
		I	II	I	II
Serang		%			
Technical irrigation	Irrigation	100	100	100	60
	River	0	0	0	30
	Well pump	0	0	0	10
Semi-technical irrigation	Irrigation	100	100	100	60
	River	0	0	0	30
	Well pump	0	0	0	10
Rainfed	Rainfall	100	100	80	50
	River	0	0	20	40
	Well pump	0	0	0	10
Dry land	Rainfall	100	25	60	0
	Well pump	0	75	40	100
Subang					
Technical irrigation	Irrigation	100	100	100	100
	River	0	0	0	0
Semi-technical irrigation	Irrigation	100	70	80	30
	River	0	30	20	70
Rainfed	Rainfall	100	70	60	0
	River	0	30	40	100
Dry land	Rainfall	100	70	60	0
	River	0	30	40	100
Pati					
Technical irrigation	Irrigation	100	100	100	100
	River	0	0	0	0
Semi-technical irrigation	Irrigation	100	70	80	30
	River	0	30	20	70
Rainfed	Rainfall	100	70	60	0
	River	0	30	40	100
Dryland	Rainfall	100	70	60	0
	River	0	30	30	70
	Well pump	0	0	10	30

Four area types in affected areas by climate anomaly in Serang district were: technical irrigation, semi-technical irrigation, rain fed and dry land (Table 1). The water availability in the normal year for these four area types was adequate. All respondents (farmers) that used semi technical irrigation in three land types could still access water from supplementary irrigation. But around 75% of farmers on dry land in the second planting season (PS II) anticipated the water shortages by using well-pump. A variety of water sources can be found during a dry year. In first planting season (PS I), the technical irrigation land can supply water adequately. However, the water shortage was still found starting from semi technical irrigation areas. Respondents used additional water from the river, 20% from semi technical irrigation land, 40% from the rainfall. On the other hand, 30% of respondents in the dry land area used water from a well-pump. During second plant season (PS II), 70% of farmers in semi-technical irrigation land and 100 % of farmers in the rain fed, used water from the river. Farmers in the dry land used 60% water from the river and used 40% water from well-pump.

For the second planting season in a normal year, rainfed farmers in Serang and Pati used the well-pump as another source to irrigate their land. While in Subang, farmers just used water from rainfall as a water source for irrigation. The difference is more clear in the dry year, in which the drought affected more significant water stress in Serang and Pati than in Subang (Table 1). To solve the drought situation, the alternative irrigation scheme in Serang and Pati was more varied than in Subang.

The previous data revealed that farmers used different water sources for planting season at different characteristics of the year (normal and dry year). The water availability in the normal year was different significantly compared to the dry year. A dry year is commonly caused by the El-Nino phenomenon and a positive IOD. Alteration in finding the source of water by farmers in a dry year is an adaptation strategy to supply water to their lands as well as to protect their crops.

The dynamical and sensitivity cropping season time in paddy field, rainfed and dry land

In general, the monsoon onset on Java Island is in September III. There were differences in both the cropping calendar and patterns for irrigated, rainfed and dry land in Serang district. Overall, the onset of planting season for irrigated lands was on November II/December II with cropping pattern rice-rice-fallow (Table 2). The onset of planting season in Serang, in general, started from November I/III to December II/III. Roughly 50% of respondents who used technical irrigation started to plant the crops on October I/II, and the remaining started to plant the crops on November I-December II. However, 50% of respondents from the rainfed area used the onset of planting season on October III/November I and even until December II. Furthermore, in dry land, most of the farmers (80%) cultivated peanuts on November II/III and the other 20% of respondents started to plant on October III/November I.

It was found that both the onset and crop rotation varied across the three land types. For technical irrigation, the onset of planting season is on October II/III and followed by technical irrigation. Nevertheless, in most irrigated lands, the water availability is limited, and there is even an alteration the irrigated lands to the rainfed lands with the onset November 1/II and the onset of dry land also in November I/II. Crop rotation rice-rice-palawija was only found in technical irrigation areas. In the irrigated and rainfed lands, the crop rotation was similar to rice-rice-palawija. On the other hand, the crop rotation for other land type was rice-rice-fallow.

Most of the farming lands in Pati district are rainfed, dry and a small part of irrigated lands. Like other districts, the onset of irrigated, rainfed, and drylands was also different. However, they have the same cropping pattern. In general, in irrigated land, the onset of planting season was on October III/February I with the crop rotation rice-rice-palawija (Table 2).

Farmers adaptation

In Serang district the onset of planting season started from November II/III to December I/II. Around 70% of respondents in technical irrigation lands planted the crop on November II/III. Furthermore, around 20% of respondents cultivated the plants on November III/December I. The rest of the respondents (10%) had planting season on December I/II. Moreover, in the rainfed lands, many of the respondents (80%) planted the crops on January I/II and the rest was on January II/III. In drylands, 70% of respondents started to plant palawija, especially peanut and maize on November I/II and around 30 % on November II/III (Figure 2).

For the irrigated areas in Subang District, most respondents (80%) determined the onset of planting season on October II/III and the remaining 20% on October III/November I. Moreover, the onset of planting season of 70% of respondents in semi-technical irrigation areas was on October II/III and for 30% on November II/III. While for dry land, the onset of 50% of respondents was executed on November I/II and the rest was on November II/III (Figure 3).

The onset of planting season in Pati district was on October II/III applied by around 80% of farmers in irrigated land. The remaining respondents were on October III/November I and November I/November II. In the rainfed areas, the onset of planting season for 70% of respondents in Pati Districts was on November/November, 20% on November I/II and 10% on December I/II. Whereas in dry land, around 80% of farmers cultivated palawija (secondary crop) mainly maize on November I/II and the others on November III/December I (Figure 4).

For all cases of study areas in Pati, Subang, and Serang, farmers in the irrigated lands planted earlier 2-4 decades than in farmers in another land type. In general, farmers in semi-technical irrigation in Subang district, don’t have to access the irrigation water. Therefore, they use rain fall and well pump to irrigate their land.

The differences in water availability in Serang and Subang districts can be represented by the variation of crop rotation. There were significant differences in crop rotation patterns, especially in the rainfed areas. Crop rotation in Serang district was dominated by rice cultivation twice a year while in Subang district was dominated by three-time cultivation Rice-Rice-Palawija (Table 3).

Table 2. Onset and crop rotation in different site/land types

Land type	Onset	Planting rotation
Serang		
Irrigation	Nov II – Dec II	Rice-rice-fallow
Rainfed	Jan I – Jan III	Rice-palawija crops-palawija crops
Dryland	Jan I – Feb I	Palawija crops-palawija crops-fallow
Subang		
Technical irrigation	Oct II – Nov I	Rice-rice-palawija crops
Semi technical irrigation	Nov I – Nov II/III	Rice-rice-fallow
Rainfed	Nov I – Nov III	Rice-rice/palawija crops/fallow-fallow
Dryland	Nov I – Nov III	Palawija crops-palawija crops/fallow-fallow
Pati		
Technical irrigation	Oct II – Nov II	Rice-rice-fallow
Semi technical irrigation	Oct II – Nov II	Rice-rice-fallow
Rainfed	Nov I – Dec II	Rice-rice/palawija crops/fallow-fallow
Dryland	Nov I – Dec I	Palawija crops-palawija crops/fallow-fallow

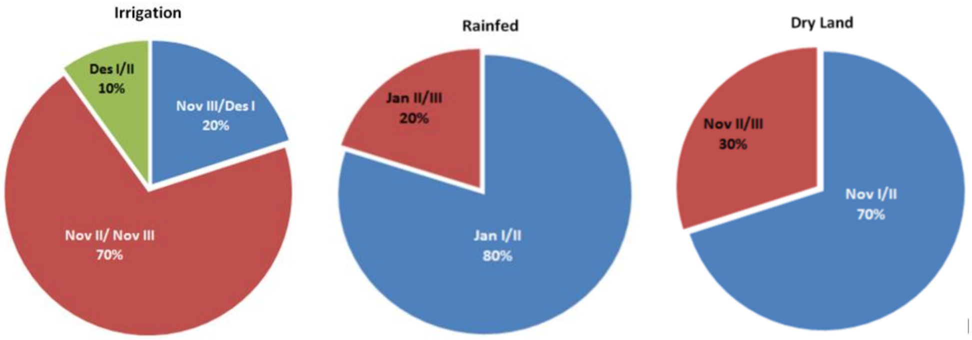

Figure 2. The existing onset of planting season in Serang district, Banten Province, Indonesia

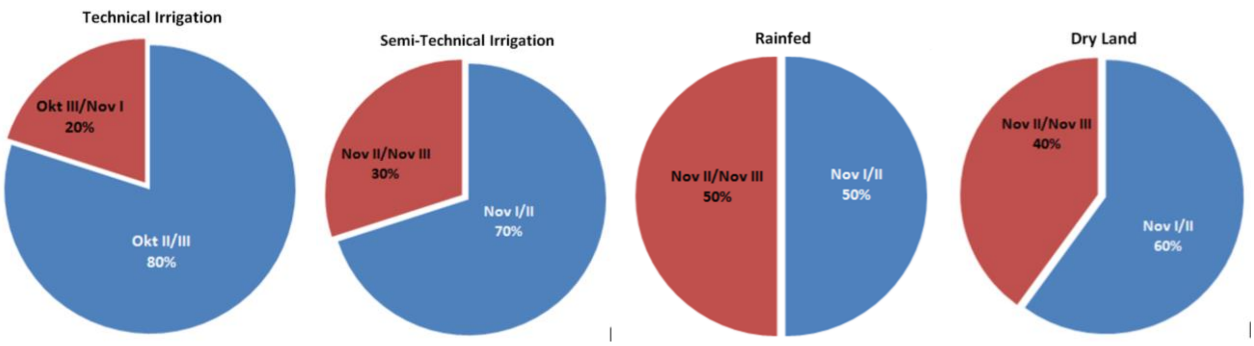

Figure 3. The existing onset of planting season in Subang district, West Java Province, Indonesia

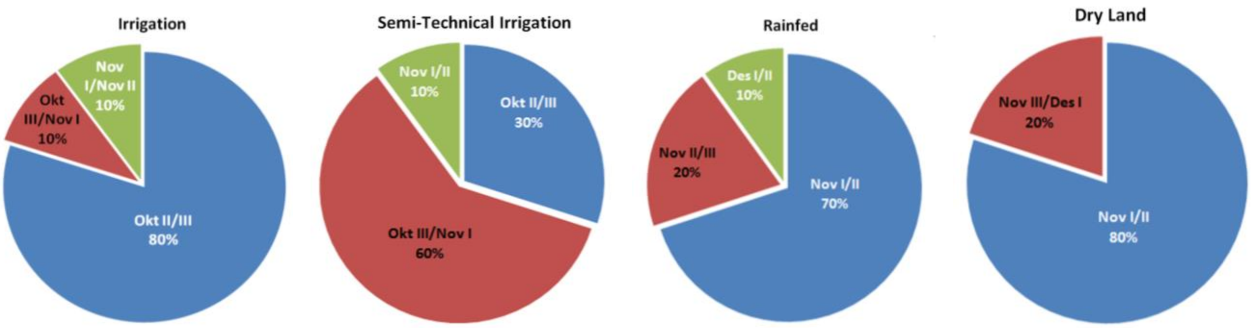

Figure 4. The existing onset of planting season in Pati district, Central Jawa Province, Indonesia

The irrigation management in Serang and Subang districts is better than other study areas since the location of this district is near the Jatiluhur reservoir, West Java, Indonesia. This reservoir is one of irrigation sources for the many surrounding farming areas. Therefore, water supply for technical irrigation was not influenced by the climate anomaly. The same case was also like the irrigated areas in Pati district. The alteration of crop rotation was conducted by farmers in rainfed lands and dry lands in Serang districts. In Subang, the crop modification was found on the technical irrigation, rainfed and dryland areas, while in Pati the plant rotation was found in semi technical irrigation, rainfed and dryland areas.

In the third planting season (PS III) the cropping index is decreased and no crop farming in almost all agriculture lands. For PS I in a dry land, the farmer generally didn't grow rice but palawija (secondary crop). In Serang for the same planting season, farmers cultivated peanut and maize, whereas, in Subang and Pati, farmers planted corn.

In a dry year, farmers in the study area dealt with the dry condition in several ways (Table 4). Most of the farmers try to irrigate their land from other water sources or delay the onset of planting season. Roughly 75 % of farmers in Serang in irrigated land anticipated drought conditions by delaying the onset for around two weeks and 25 % of farmers don’t postpone the onset since water is still available. In the rain fed areas, 65% of farmers decided to find another water source and 35% agree to postpone the onset for even more than four weeks. Moreover, in dryland during dry conditions, around 30% of farmers pump water from the river to irrigate their land but 70% of farmers adapt to that situation by delaying their onset.

In Subang, around 80% of respondents in irrigation areas decided to plant, and 20% of respondents delayed their onset approximately two weeks. Farmers in irrigated lands adapt to dry conditions by delaying the onset for about two weeks instead of finding the other water source. However, in technical irrigation 4-5, 85% of farmers delay the onset two weeks to deal with drought, and 20% try to find other water sources from the river. In the rain fed, 65% of farmers decide to find other water origin and 35 % delayed the onset for more than four weeks. In dryland, 30% of farmers pump water from a river, and 70% delayed the onset.

In Pati, 75% of farmers in irrigated land anticipate drought by delaying the onset of planting season approximately two weeks and 25% decide to plant as water is still available. In technical irrigation, the farmer decides to delay the onset two weeks while waiting for water supply. But in semi technical irrigation, most of the farmers adapt to dry conditions by delaying the onset two weeks, leaving 20 % of farmers to pump water from a river. In the rain fed, 65% of farmers try to find other water sources and 35% delay their onset four weeks. In dryland, 40% of farmers pump water from the river and 30% decide to delay the onset (Table 4).

Table 3. Farmer's response to adjust the crop rotation

Irrigation type	Normal year (%)				Dry year (%)				
	Rice-rice-palawija	Rice-rice	Rice-palawija-palawija	Palawija-palawija	Rice-rice-palawija (%)	Rice-rice	Rice-palawija-palawija	Palawija-palawija	Rice/palawija
Serang									
Technical irrigation	0	100	0	0	0	60	40	0	0
Semi technical irrigation	0	100	0	0	0	60	40	0	0
Rainfed	0	1	100	0	0	10	40	30	20
Dryland	0	1	0	100	0	0	0	20	80
Subang	0	0	0	0	0	0	0	0	0
Technical irrigation	100	0	0	0	20	80	0	0	0
Semi technical irrigation	0	100	20	20	0	20	80	0	0
Rainfed	0	60	0	100	0	0	60	40	0
Dryland	0	0	0	0	0	0	0	40	60
Pati									
Technical irrigation	100	0	0	0	20	80	0	0	0
Semi technical irrigation	0	100	0	0	20	80	0	0	0
Rainfed	0	60	20	20	0	0	50	50	0
Dryland	0	0	0	100	0	0	0	30	70

Table 4. The farmers responses to dry conditions in different land type

Land type	Farmer respondent (%)			
	Planting	Finding other water sources	Delayed 2 weeks	Delayed more 4 weeks
Serang				
Technical irrigation	25	0	75	0
Semi technical irrigation	0	25	75	0
Rainfed	0	65	0	35
Dryland	0	30	0	70
Subang				
Technical irrigation	80	0	20	0
Semi technical irrigation	0	20	85	0
Rainfed	0	65	0	35
Dryland	0	70	0	30
Pati				
Technical irrigation	25		75	0
Semi technical irrigation		20	80	0
Rainfed		65	0	35
Dryland		40	30	30

Since dry conditions cannot be avoided, farmers adapt to this situation by finding other water source or by delaying the onset of planting season and other activity to reduce the damage and loss caused by drought. It found that the response of farmers in different locations was varied. One reason for this is that the water availability for each site is different.

In general, in Subang farmers decide to find other water sources both in irrigation land or in the rain fed land. But several respondents delay the onset for about two weeks because the water source was far from their land. Unlike farmers in Subang, farmers in Pati in both technical or semi-technical irrigation delay their onset approximately two weeks instead of finding other water sources. This activity can be seen clearly in PS II in a dry year. In the rain fed land, the response of farmers is different significantly than the response in irrigated land. In the rain fed, the farmer decides to find other water sources instead of delaying the onset, and most of the farmers plant twice a year. The other water source that they used is pump water from the river. To do so, they must pay an extra cost. Those that delay their onset roughly one month are those farmers who plant only once a year.

In conclusion, the farmers in affected areas due to climate variability could adapt to shift the onset of planting season. Farmers in irrigated lands change their onset around 2-4 ten-days period becomes October II - December II, in rainfed area around 4-6 ten-days period becomes November I - January III and in dryland around 6-8 ten-days period from November II - February I. The cropping pattern rice-rice-palawija/fallow was applied in irrigated land, rice-rice/palawija/fallow-fallow in rainfed and palawija-palawija/fallow-fallow in dryland. In irrigated land, 75-80% of farmers decide to delay the onset two weeks while waiting for water supply. In rainfed areas, 65% of farmers try to find other water sources and 35% of them delay their onset four weeks. In dryland, 30-70% of farmers pump water from the river and 30-70% decide to delay the onset more than four weeks. Adaptation programs to deal with climate extremes in Serang and Pati vary more than in Subang. In Serang and Pati, during first planting season, farmers applied irrigation roughly 20%-30% pump water from river and during second planting season, in Pati farmers use water from well pump and even in Serang reach 100%.

ACKNOWLEDGEMENTS

We would like to acknowledge the valuable funding support given by Indonesian Agroclimate and Hydrology Research Institute, Bogor, Indonesia. We would also like to express our gratitude for the many constructive comments provided by Dr. Haris Syahbuddin.

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ASIAN JOURNAL OF AGRICULTURE
Volume 1, Number 1, June 2017
Pages: 17-21

E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g010104

Fecundity performance of nilem *(Osteochilus vittatus)* from Cianjur, Tasikmalaya and Kuningan Districts, West Java, Indonesia

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Manuscript received: 15 May 2017. Revision accepted: 15 June 2017.

Abstract. *Rostika R, Andriani Y, Junianto. 2017. Fecundity performance of nilem (*Osteochilus vittatus*) from Cianjur, Tasikmalaya and Kuningan Districts, West Java, Indonesia. Asian J Agric 1: 17-21.* The aim of this study was to know the reproduction aspects of nilem (*Osteochilus vittatus* Valenciennes, 1842; syn. *Osteochilus hasseltii* Valenciennes, 1842). The study was conducted from October 2015 to March 2016. This research included elements of body length, weight, gonad maturity level (GML) and fecundity. There were 27 samples collected from farming activities in Cianjur, Tasikmalaya and Kuningan West Java, Indonesia. The parameters observed were the correlation between weight and fecundity, correlation between body length and fecundity and GML. The method used was explorative approach and the data were analyzed by descriptive approach. The results showed that the correlation between weight and fecundity in Cianjur District was $y= e^{0,024x}$, while the body length and fecundity was $y=e^{0,413x}$. In Tasikmalaya District was $y= e^{0,0728x}$, while the body length and fecundity was $y=e^{0,4903x}$. In Kuningan District was $y= e^{0,2902x}$, while the correlation between the length of the fish and fecundity was $y=e^{0,6859x.}$ The dominant gonad maturity level of *O. vittatus* in Cianjur was GML IV (50%), Tasikmalaya was GMLVI (100%), and Kuningan was GML V (55,56%) respectively. The relationship between length and body weight of *Osteochillus vittatus* from Cianjur, Tasikmalaya and Kuningan District were $W=0,03L^{2,95}$, $W = 0.04L^{2.89}$ and $W = 0.04L^{2.79}$ respectively. The implication of this research was to understand the proper size of *O. vittatus* that can be harvested that have an optimum egg volume.

Keywords: Fecundity, GML, LW relationship, *Osteochilus vittatus,* West Java

INTRODUCTION

Nilem or *Osteochilus vittatus* (Valenciennes, 1842), syn. *Osteochilus hasseltii* (Valenciennes, 1842) is a freshwater fish that has been known as one of the prominent fish farming commodities in Java Island, particularly in the areas of Priangan, West Java. However, in recent years, the activities related to the industry have been gradually changed by the other similar emerging industries (Subagja et al. 2006) whereas the fish possesses propitious characteristics suitable for business, as the egg performance has shown good aspects of fecundity. *O. vittatus* has potential for both superior reproduction and fecundity. A pair of fish with weights between 100-150 g may produce 15,000-30,000 eggs (Yudhistira 2013). Their fecundity also may increase in logarithmic patterns as the fish grows both in length and weight. The eggs have been consumed widely for the taste and exported to several countries as an alternative for caviar.

Measurement of length-weight fish correlation shows standardized size of the fish at various locations related to the length and weight. This measurement is used to perform the character of the species. This correlation is important information to identify the growth rate (Isa et al. 2010). It is also one of the factors considered in determining fishery management strategies (Mansor et al. 2015).

Farming and industrial activities of *O. vittatus* in West Java are mainly centralized in the District of Ciamis and Tasikmalaya (Department of Fisheries and Marines, West Java 2010). The objective of this research was to recognize the reproductional growth pattern of *O. vittatus* that includes length-weight correlation, gonad maturity level (GML), fecundity, as well as the management strategies for preparing maximum egg for the “egg crispy cookies”.

Fecundity is related to the total length and body weight of fish. The environment, especially the availability of food in the habitat, is an important factor influencing the quality of eggs and the timing of reproduction. Lack of food can cause a delay in the maturation of the gonad resulting in low fecundity. Fish nutrition and diet can be determinant factors controlling population density, growth and fish condition. The feeding behavior of a species usually depends on the age, place/habitat, time and digestive tract of the fish itself (Syandri et al. 2015).

MATERIALS AND METHODS

This research was conducted from October 2015 to March 2016. Fish samples were taken from 3 different farming locations (Muchlisin et al. 2015) i.e., Cianjur, Kuningan, and Tasikmalaya in the West Java Province (Figure 1). Samples were observed and identified in the Laboratory of Aquaculture, Faculty of Fisheries and Marines, Universitas Padjadjaran, Sumedang, West Java, Indonesia.

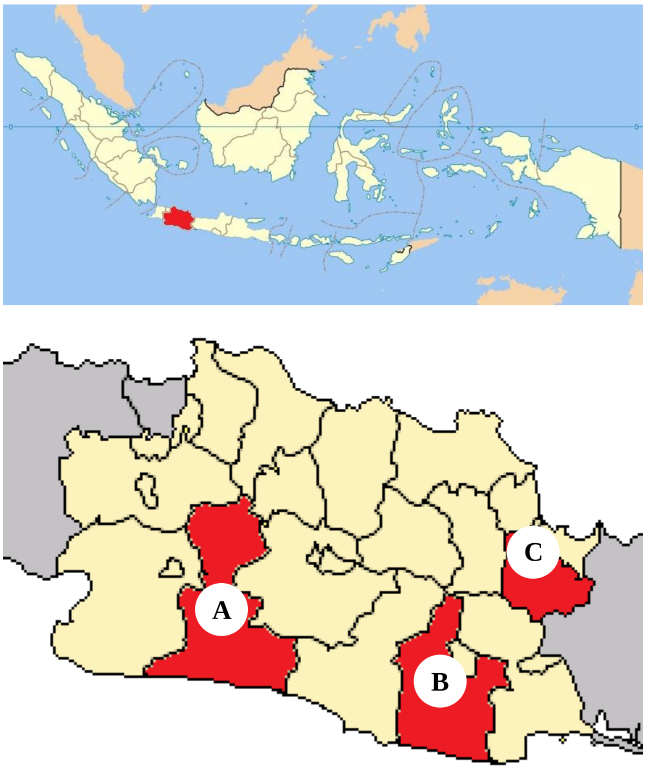

Figure 1. Research area in West Java, Indonesia (red color). A. Cianjur, B. Tasikmalaya, and C. Kuningan

The female breeder consists of 27 fish taken and froze for further observation in weight (in gram), length (in centimeter) and gonad maturity level (GML). Identification of Gonad Maturity Level refers to Kesteven (Bagenal and Braum 1968). Number of eggs was counted by gravimetric method. Fecundity was measured with the following formula (Effendie 1979):

$$F = \frac{G.x}{g}$$

Note:
F = fecundity;
x = number of sampling eggs;
G = total gonad weight (g);
g = sample gonad weight (g)

The length-weight correlation was analyzed using the following formula (Effendie 1979; Brodziak 2012):

$$W = a\, L^{b}$$

The linearization could be done through logarithmic transformation using the following equation:

$$\text{Log } W = \text{Log } a + b \text{ Log } L$$

Note
W = weight (g)
L = length (mm)
a = Intercept (curved intersection of length-weight correlation with the y-axis)
b = Length-weight coefficient assumption

RESULTS AND DISCUSSION

Reproductions aspect

The following are the results from the observation and treatment of the sampling in Cianjur, Kuningan, and Tasikmalaya that include total length (TL), body weight, gonad weight, gonad maturity level (GML), and fecundity (Table 1).

Fecundity

Fecundity is the number of eggs per unit of weight or length (Effendi 1979). The ovary weight may be resorted to make a presumption of the fecundity to get the expected results (Murtejo 2008). The observed fish weighed approximately 108-418 g, and the fecundity is 26,200-123,880 eggs and the gonad weight range is 25-95 g (Table 1). The fecundity level in this research fluctuated possibly due to the age and size differences of the fish that results in the variation of the number of fecundity. It means that the bigger the fish, the higher the fecundity. Longer fish also tended to have higher fecundity (Figure 2-4). Fish with the highest fecundity (total length of 28.5 cm) is in the GML VI (the spawning period).

It is also noted that the fish weight measurement is more reliable to presume the fecundity than the total body length. Absolute fecundity is often shown by the relation to the body weight, as it could approach the fish condition better than their body length (Effendie 1979). The relationship between fecundity and weight (Figure 5-7) appears as in general; heavy fish also have high fecundity, even the highest fecundity (weight 373 g) in this research is achieved by fish in the spawning GML. Fluctuation occurs due to the age difference and the intensity of the spawning.

Table 1. Average reproductions aspects of *O. vittatus* in Cianjur, Tasikmalaya and Kuningan, West Java, Indonesia

Sample origin	TL (cm)	SL (cm)	Weight (g)	Gonad (g)	GML	Fecundity (eggs)
Cianjur	21.1	17.30	139.8	29.8	GML IV-V	30418
Tasikmalaya	27.4	22.16	356.0	72.3	GML VI	88954
Kuningan	10.6	7.84	20.9	8.62	GML IV-VI	109530

Note: TL = total length, SL = Standard length, GML = gonad maturity level

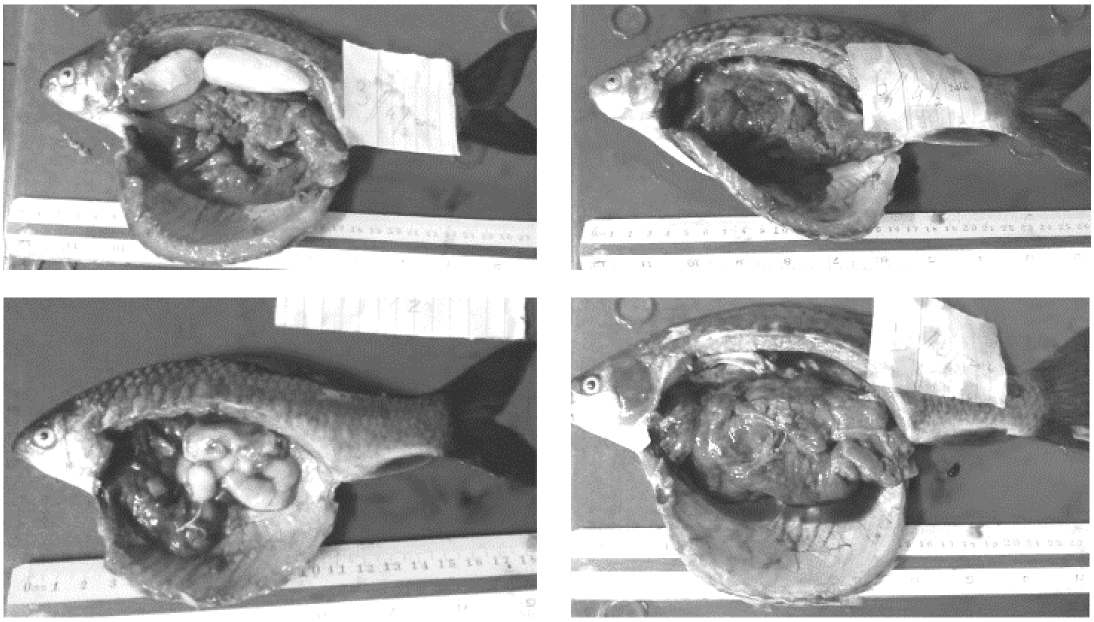

Figure 2. Egg profile of *Ostechilus vittatus* from Cianjur District, West Java, Indonesia

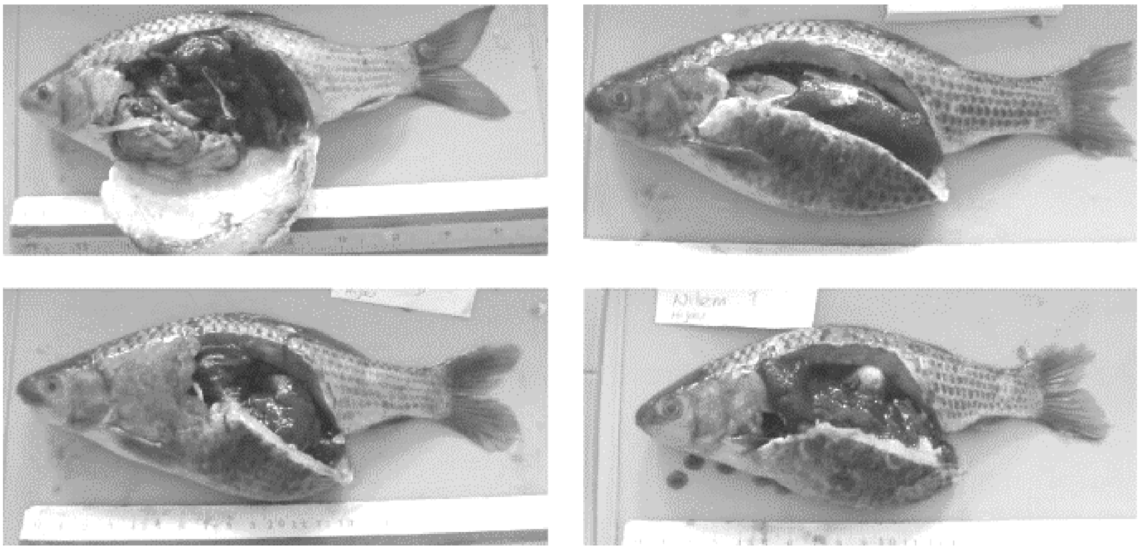

Figure 3. Egg Profile of *Ostechilus vittatus* from Tasikmalaya District, West Java, Indonesia

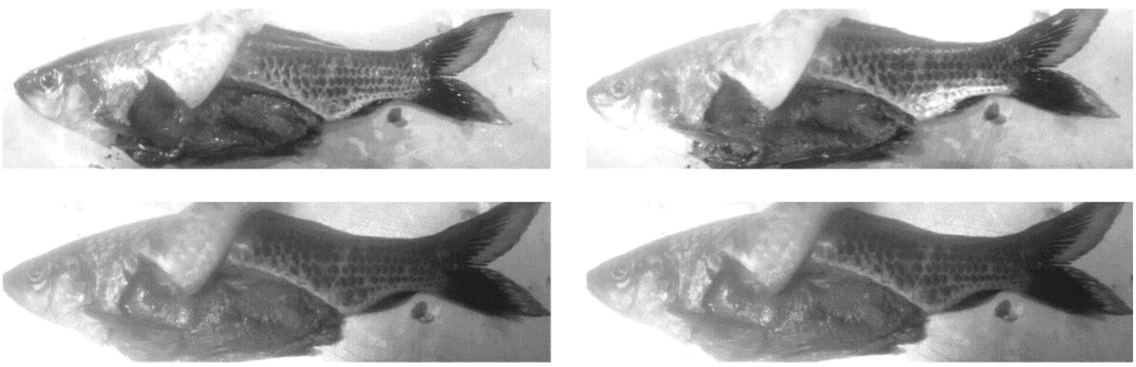

Figure 4. Egg profile of *Ostechilus vittatus* from Kuningan District, West Java, Indonesia

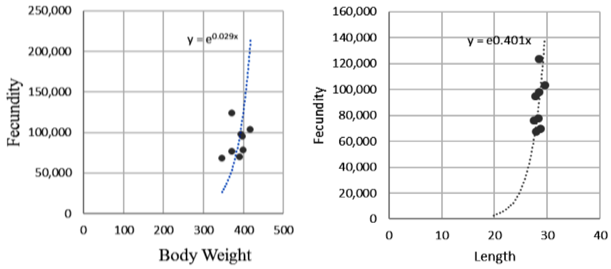

Figure 5. The relationship between body weight and fecundity (*left*) and the relationship between length and fecundity (*right*) in Cianjur District, West Java, Indonesia

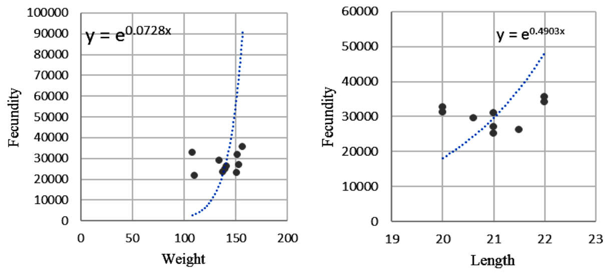

Figure 6. The relationship between body weight and fecundity (*left*) and the relationship between length and fecundity (*right*) in Tasikmalaya District, West Java, Indonesia

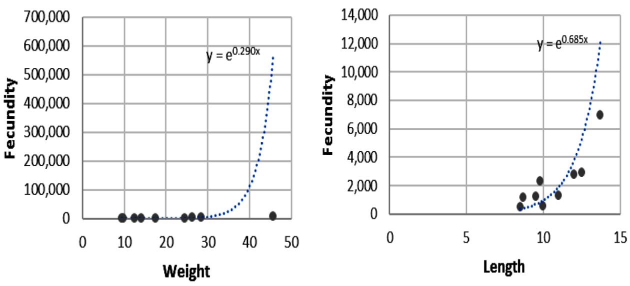

Figure 7. The relationship between body weight and fecundity (*left*) and the relationship between length and fecundity (*right*) in Kuningan District, West Java, Indonesia

Table 2. Average gonad maturity level of *Osteochilus vittatus* in Cianjur, Tasikmalaya and Kuningan districts, West Java, Indonesia

Districts	Gonad Maturity Level (GML)
Cianjur	VI (100%)
Tasikmalaya	IV (50%)
Kuningan	V (55,56%)

Table 3. *Osteochilus hasseltii* length and weight relationship in Cianjur, Tasikmalaya and Kuningan districts, West Java, Indonesia

District	WL relationship	a value	b value	R^2
Cianjur	$W=0.03L^{2.95}$	0.03	2.95	0.94
Tasikmalaya	$W = 0.04L^{2.89}$	0.04	2.89	0.93
Kuningan	$W = 0.04L^{2.79}$	0.04	2.79	0.95

Distribution of Gonad Maturity Level (GML) in Cianjur is in the range of 22-25 cm length at the level of GML VI of 100%. The length of the fish in Tasikmalaya had a range from 16 to 18 cm at GML level IV, V and VI, but dominated on GML IV at 50%. The length of the fish in Kuningan ranged from 6.5 to 7.4 cm both at the level GML IV, with the size 8.3 to 10.2 at GML V and 11-13 at GML VI but dominated at GML V that is equal to 50% (Table 2). This is in line with research by Rochmatin et al. (2015) which examines the aspects of the growth and reproduction of fish in Rawa Pening, Semarang showing the average GML V.

Length-weight correlation

Weight can be considered as a function of length. By analyzing the length-weight correlation of the fish, the growth pattern can be identified. Furthermore, it can be seen whether the fish's body is fat or thin (Effendie 1997). Effendie (1979) quotes if the length and weight are plotted in a picture, it will get an equation W = aLb. b is a constant, the square value showing the growth pattern. Length-weight correlation can be used to look at the fish water condition factor. The greater the b value, the better the condition of the water environment.

Length-weight correlation can be identified from the constant b value, i.e., if b = 3, the formed correlation is isometric (the length is balanced with weight gain). If $b \neq 3$, the correlation is allometric and if $b > 3$, the correlation is positive allometric meaning that weight gain is faster than the length, showing the plump body type. If $b < 3$, the correlation is allometric negative that the length is faster than weight gain, showing the bony body type (Effendie 2002).

From the analysis of the length-weight correlation, it could be identified that the equation of the correlation in Cianjur is W = $W=0.03L^{2,95}$; Tasikmalaya is $W = 0.04L^{2.89}$ and Kuningan is $W = 0.04L^{2.79}$. From the b value, the fish from the sampling area have a negative allometric growth pattern (Table 3). This is consistent with the growth pattern of blue mackerel (*Scomber australasicus*) in the Natuna Sea, which means that the length is faster than weight gain (Nugroho et al. 2013). Similarly, according to Nehemia et al. (2012) who conducted research on tilapia farmed in freshwater, with b value = 2.94. In addition, flathead gray mullet (*Mugil cephalus*) and freckled hawkfish (*Ambassis kopsii*) also have a negative allometric growth pattern (Mulfizar et al. 2012).

According to Bagenal and Braum (1968), factors that cause the b value differences are environmental conditions, fish stock differences within the same species, stage of development, sex, gonad maturity, even the time differences due to changes in the entail.

The R^2 values of length-weight correlation in various districts show the value from 0.93 to 0.95, which means the data is very closely related. This is in line with research report by Koffi et al. (2014) that the R^2 was scored between 0.46 to 0.94 and by Satrawaha and Pilasamorn (2009) with R^2 approaching 0.95.

In conclusion, the relationship between body weight and fecundity of *Osteochillus vittatus* from Cianjur, Tasikmalaya and Kuningan District are $y= e^{0,024x}$, $y= e^{0,0728x}$, $y= e^{0,2902x}$ b < 3 so the growth was allometric negative. The relationship between body length and fecundity of *Osteochillus vittatus* from Cianjur, Tasikmalaya, and Kuningan district are $y=e^{0,413x}$, $y=e^{0,4903x} y=e^{0,6859x}$. The GML of *Osteochillus vittatus* in Cianjur is GML IV (50%), Tasikmalaya is GML VI (100%), and Kuningan is GML V (55,56%). The relationship between length and body weight of *Osteochillus vittatus* from Cianjur, Tasikmalaya and Kuningan District are $W=0,03L^{2,95}$, $W = 0.04L^{2.89}$ and $W = 0.04L^{2.79}$. The *O. vittatus* from Kuningan proved to be of the size that was best used as a sample fish with eggs of optimum volume.

ACKNOWLEDGEMENTS

We would like to express my sincere gratitude to the research assistant, Lantun Paradhita Dewanti and Akhmad Hafiz Kurniawan of Faculty of Fisheries and Marine Science, Universitas Padjadjaran Jatinangor, Sumedang, Indonesia. We also would like to acknowledge the generosity of Rector of Universitas Padjadjaran, Sumedang, Indonesia for the Academic Leadership Grant.

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ASIAN JOURNAL OF AGRICULTURE
Volume 1, Number 1, June 2017
Pages: 22-28

E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g010105

The influence of fertilizer type and time of application on growth and forage productivity of mung bean

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Manuscript received: 30 September 2016. Revision accepted: 18 June 2017.

Abstract. *Mohammedaltom AAA, Dagash YMI. 2017. The effect of fertilizer type and time of application on growth and forage productivity of mung bean. Asian J Agric 1: 22-28.* A field experiment was conducted on April 3, 2016, at the Demonstration Farm of College of Agricultural Studies, Sudan University of Sciences and Technology, Shambat, Khartoum, Sudan to study the effect of some fertilizers and their time of application on growth and forage productivity of mung bean. The treatments were arranged factorially in split-plot trial with four replications. Application time was assigned to the main plot as three times of application: before sowing, with sowing and after sowing. Types of fertilizers as the subplots, included four types of fertilizers: without fertilizer (control), 50 kg ha^{-1} (organic manure), 100 kg ha^{-1} (diammonium phosphate), and 10 L ha-1 (humic acid). Different characteristics measured included: plant height (cm), stem thickness (cm), number of branches per plant, number of leaves per plant, fresh weight/plant (g) as well as dry weight/plant (g). The results revealed that there was a highly significant difference for types and application time of fertilizers and their interaction on plant height, number of leaves, and fresh forage and dry forage. Highly significant differences ($p \leq 0.01$) were recorded in types of fertilizers and interaction between different application times and types of fertilizers for number of branches and significant difference ($p \leq 0.05$) of application time for number of branches and stem diameter. There was no significant difference in types of fertilizers for stem diameter. The highest height of the plant (28.78 cm), the highest number of branches/plant (9.37), the maximum stem diameter (6.43 cm), the largest number of leaves/plant (31.69), the best fresh weight (815 kg ha^{-1}) and dry weight (161 kg ha^{-1}) were recorded for the treatment of 50 kg ha^{-1} organic manure applied after sowing.

Keywords: Fertilizer, plant growth, forage, mung bean

INTRODUCTION

Fodders are a very vital resource for the development of the agricultural economy within poor countries for livestock raising (Zahid et al. 2013). Fodder crops are crops that are cultivated primarily for animal feed. All fodder crops whether grasses, legumes and root crops are fed to animals, either as green, hay or silage products (Wanas et al. 2007). The traditional system for forage production in Sudan favors high yields at the expense of nutritive value. This is because fodders were mainly produced as cash crops. Such system requires fast growing, highly productive cultivars to minimize costs of production. These requirements are largely met by fodder sorghum, Abu Sab'in (Maarouf and Zeinab 2013). The total area of forage production in Sudan is estimated to be about 126,000 ha, or almost half in Khartoum state (Zaroug et al. 1997). The recent statistics of the Sudan Ministry of Agriculture (2015) showed that the area of forage crops represented about 80% of the area cultivated in Khartoum state. This area was almost doubled from 114513 in (2006) to 239535 feddans in (2015). 81% of the area under forage production was occupied by fodder sorghum, Abu Sab'in and alfalfa (Sudan Ministry of Agriculture 2015). The system of forage crop production adopted in Sudan, the green chopping system, does not allow a continuous supply of animal feed. Referring to the recent statistics, about 90% of the animal wealth in Sudan relies on natural pastures and crop residues (NCS 1999). This expansion was due to growing importance of forage crops, due to the increased attention to dairy production, particularly around urban centers and to satisfy the needs of increasing animals for meat. The demand is continuously increasing due to population growth and mass immigration of rural communities. In addition to this, a remarkable activity of cattle and sheep export has resulted in increasing the area of fodder crops grown primarily under irrigation (Idris et al. 2013).

Legume fodder is important for livestock production because it is rich in protein, minerals, phosphorus, calcium, and vitamins (Bogdan 1977, Unkovich et al. 1997). Dairy animals require a green legume crop to cover up the balance of their protein requirement. Mung bean (*Vigna radiata* L.) Wilczek *syn.* also called green gram and golden bean is an important summer annual pulse crop, belongs to genus *Vigna* and family Leguminosae. India is considered its native country, and it is cultivated in Pakistan, USA, Europe (Zaid et al. 2012), Bangladesh, Cambodia, Indonesia, the Philippines, Thailand, Vietnam (Somashekaraiah et al. 1992), Uganda (Apioibedo 2014), Australia, China (Imrie and Lawn 1991) and Egypt (Ashour et al. 1994) and Iran (Paroda et al. 1987). From Asia, it spread into the Middle East, the Pacific Islands, East Africa, Australia, and the Americas, but Asia continues to be the region of major production (Nassar 2013). India is the largest producer of mung bean in the world (54%), the average productivity is 550 kg ha^{-1} (ICAR. 2008) and produced higher forage 2.2-ton ha^{-1} (Twidwel et al. 1992). In South Asia, improved varieties of mung bean are planted on an area of 3 million hectares, and with a total annual production of 3.1 million tons both under rain-fed and irrigated conditions (Shanmugasundaram et al. 2009). In Pakistan, it was planted on an area of 2.5 million hectares with a total annual production of 1.8 million tons, and with an average yield of 723 kg ha-1. Out of the total area in Pakistan, Khyber Pakhtunkhwa covered an area of 10.1 thousand hectares with the production of 6.4 thousand tons producing an average yield of 634 kg ha^{-1} (Minfal 2008) and, the average yield in Pakistan during the year 2009-2010 was 709 kg per hectare (Ali et al. 2000).

In Sudan, mung bean is a new crop, and it may be a commercially promising pulse crop and can be grown as a forage crop. Local production of pulses is not sufficient to meet the increasing demand for human utilization. Therefore, to meet the situation, it is necessary to boost up the production. Inadequate supply of feed-in quantity and quality is responsible for the low productivity of animals.

Animals depend entirely on natural pastures for their feed. This source is only adequate for their survival during the wet season but inadequate during the dry season. This has resulted in the characterized limitation posed by non-availability of all-year-round feed resources due to prolonged dry season (Oladotun et al. 2003, Odeyinka and Okunade 2005). There is a need to improve pasture production through properly planned management and a need for better forage cultivars that maintain a continuous supply of forages. Such management practices include cutting management and introducing high-yielding new crops with short-growing seasons. With proper management practices, it can be considered an effective tool for narrowing the food gap in Sudan as well as cultivation of mung bean and the use of fertilizers. Genetic potential of legumes is not obtained at field due to poor soil nutrient status, mineral deficiency (Maskey et al. 2004) and nodulation are poor on Shambat soil. They are worldwide agricultural problems causing yield and quality loss (Liu 2001). In this context, a low-cost technique is needed to incorporate nutrient (micronutrient and macronutrient) into the plant system, thus it enhances growth, and boost up crop yield and nutrient status in plants, thus nutrient deficiency can be remove and higher yield and vigor seedling can be achieved in mung bean, by using the best fertilizers and optimum application time.

The objectives of this study were: (i) to examine the influence of different types of fertilizers on growth and forage productivity of Mung bean, (ii) to determine optimum application time of fertilizer to enhance Mung bean forage productivity.

MATERIALS AND METHODS

Site of experiment

This experiment was conducted at the experimental farm in the College of Agricultural Studies-Sudan University of Science and Technology (Shambat), from April to June 2016. Shambat is located between latitudes (15.40ºNorth and 32.32 º East) and altitudes of 380 meters above sea level. The climate is characterized by semi-desert tropics with a low percentage of humidity, average rainfall of 158 mm per annum, temperature of 20.3-36.1 ºC clay soil (Khairy 2010), and soil pH 7.5-8.7 (Hamdon 2001).

Experimental design

The treatments were arranged factorially in split-plot design with four replications. The main plot consisted of three application times, i.e., T_1 = before sowing, T_2 = with sowing and T_3 = after sowing. The subplot consisted of four fertilizers i.e., F_0=control (no fertilizer), F_1 =diammonium phosphate, F_2 =organic manure, F_3= humic acid.

Plant material

Mung bean seeds used in the study were obtained from the College of Agricultural Studies, Sudan University of Science and Technology, Shambat.

Cultural practices

Preparation of soil samples. The soil mixture consisted of clay and sand percentage (2: 1) in plastic pots. Pot area was 1.4 m2 and each pot contained 10 kg soil sample. Sub samples were taken before sowing and analyzed in the Laboratory Soil and Water Science, College of Agricultural StudiesCAS), Sudan University of Science and Technology, Shambat, Khartoum, Sudan. EC, pH, and soluble salts were determined on paste saturation extract (Ritchard 1954) by the use of pH meter (model 3510), EC meter (model M35). Na and K were estimated using direct flame photometry in soil extract (flame photometer (model 410)). CaCO3 was estimated using a calcimeter, Model (Eijkelkamp). Total nitrogen was performed using the Kjeldahl method (Ryan et al. 2001). For available phosphorous, O'lsen (1954) method was using a spectrophotometer model (6305). Organic carbon was determined by the Walkley and Black method (1934). The amount of exchangeable potassium was estimated using direct flame photometry in soil extract (Ryan et al. 1996). Soil texture was determined using Particle Size Determination (Pipette Method), and textural classes were defined USDA textural triangle, appendix (1). As activation dose, recommended N (40 kg ha^{-1}) by using urea 6 g per pot was applied in each pot before sowing.

Sowing. 30 g of seeds were mixed thoroughly with 0.15g Thiram, and immediately sown on the 3rd of April 2016 in pots at the rate of 3 seeds per hole.

Irrigation. The first irrigation was done immediately after sowing and then when necessary.

Treatment. Pots were fertilized as per treatments described below. Diammonium phosphate 100 kg ha^{-1} (15 g per pot), organic manure 50 kg ha^{-1} (7.5 g per pot) and humic acid 10 L/ha (1.5 ml per pot). All fertilizers were incorporated into the soil before sowing (15 days), at sowing and after sowing of seeds (15 days).

The treatments were arranged as follows:

Application time: (i) 15 days before sowing (B.S), (ii) With sowing (W.S), (iii) 15 days after sowing (A.S)

Fertilizers: (i) Diammonium Phosphate (46% P2O5, 18% N) at 100 kg ha^{-1} or 15 g per pot. (ii) Humic Acid (12% N, 15% Humic Acid, 3% K2o) at 10 L/ha or 1.5 mL per pot. (iii) Organic manure (0.062% O.M, 0.107% O.C, 1.232% N, 61% P, 4.6mL/L K, 40.7 mL/L Ca, 119.3mL/L Mg, 32.6mL/L Na, 6.45mL/L Fe, 1.797mL/L Mn, 0.028mL/L Su, 0.108mL/L Co,0.15mL/L Pb, 0.114mL/L Zn, 36.1% m, 58.16% Ash, 1: 2 C: N, 6.5 pH, 23.5/D.S.ME.C, at 50 kg ha^{-1} or 7.5g per pot.

Harvesting. After 75 days from sowing the crop was ready for harvesting. Removal of the vegetable parts at soil surface was done manually using clipper for stover production.

Data collection

Plant height growth. After four weeks from sowing (30 days), four plants of mung bean were randomly selected from each pot to determine growth stages and the period between readings was 15 days.

Plant height (cm). Plant height was measured from the ground level to tip of the stem, from four plants of mung bean randomly selected from each pot, using a measuring tape then the mean plant height was recorded.

Stem diameter (cm). Four plants of mung bean were randomly selected from each pot and stem diameter was measured for each plant separately at the middle internodes using meter and the average per plant was recorded

Number of branches per plant. Four plants of mung bean were randomly selected from each pot and number of branches was counted and then the mean per plant was recorded.

Number of leaves per plant. The number of leaves was counted from four plants of mung bean randomly selected from each pot and then the mean per plant was recorded.

Fresh yield per plant (g). At harvest (75 DAS) four plants were weighted and the mean fresh yield weight per plant was recorded.

Dry yield per plant (g). The fresh yield of four plants were oven dried at 80 C for 48 hours to constant weight and the mean dry yield weight per plant was recorded. The fresh yield and dry yield (kg ha^{-1}) were calculated as follows:

$$\text{Yield} = \frac{\text{Area in hectare } (10000 \text{ m}^2) \times \text{forage weight per m}^2 \text{ (g)}}{\text{Weight unit } (1000)}$$

Statistical analysis

Data were statistically analyzed according to split-plot design using MSTAT-C package. The mean values were separated by Least Significant Difference (L.S.D) (Gomez and Gomez 1984).

RESULTS AND DISCUSSION

Plant height (cm)

The analysis of variance showed that a higher significant effect of time of fertilizer application and types of fertilizers as well as their interaction on plant height (Table 1). The tallest plants were recorded at application time after sowing while shorter plants were recorded at application time at sowing (Table 2). The types of fertilizers promoted significantly different plant heights. The highest plants were observed in organic manure and the shortest plants were recorded in humic acid (Table 3). Plant height ranged from 15.41cm to 28.78 cm. Maximum plant height (28.78 cm) was found with 50 kg organic manure per ha application after sowing which was statistically higher than other treatments. Minimum plant height was observed for organic manure added before sowing (15.41 cm). It was clear that with the fertilizer added after sowing, the plant height was increased with the organic manure (Table 4). These results agreed with those of Malihe et al. (2014) who reported a significant effect of organic fertilizers on plant height and while Bhuiyan et al. (2008), Shukla and Dixit (1996) and Sharma and Singh (1997) reported that application of phosphorus enhanced the plant height significantly.

Stem thickness (cm)

The results showed that the time of fertilizer application and types of fertilizers had higher significant effect on stem thickness per plant, while their interaction had no significant effect on stem thickness per plant (Table 1). Stem thickness was thick at application time before and at sowing and the thin stem, thickness was observed at application time after sowing (Table2). Organic manure produced thick stem thickness per plant (5.68 cm), while thin stem thickness per plant (5 cm) was produced by humic acid (Table 3). Interaction between time of fertilizer application and types of fertilizers had also significant effect on stem thickness per plant pots treated with 50 kg ha^{-1} organic manure added after sowing produced thick stem thickness (6.43 cm), while thin stem thickness (4.18 cm) was recorded in pots with 100 kg ha^{-1} diammonium phosphate added at sowing (Table 4). These results were not in agreement with those of Iqbal et al. (2012) who showed that significant results were obtained in stem with an appropriate supply of phosphorus.

Stem increase with organic manure 50 kg ha^{-1} added after sowing was recorded (6.43 cm) (table 4). These results were not in accordance with those of Nemat et al. (2000) who reported that increasing levels of phosphorus lead to the increment in stems.

Number of branches

Number of branches per plant was significantly higher at the time of fertilizer application and types of fertilizers and significantly different with their interaction (Table 1). The lowest number of branches per plant was noticed in application at sowing while higher number of branches per plant was recorded in application after sowing (Table 2).

Number of branches was increased with types of fertilizers. The highest number of branches (8.10) was observed with the organic manure application at 50 kg ha^{-1} which was statistically higher than other treatments. The lowest values (7.37 and 7.35) were recorded with the diammonium phosphate and control respectively (Table 3). These results were confirmed earlier by Ali (1993) who showed that the number of branches per plant was not affected by level of phosphorus. In contrast, Muhammad et al. (2014) found that the number of branches per plant was significantly influenced by phosphorus application.

With 50 kg organic manure per ha added after sowing produced maximum number of branches per plant (9.37), while minimum number of branches per plant (5.12) was recorded with 100 kg ha^{-1} diammonium phosphate added at sowing (Table 4). Malihe et al. (2014) stated that the number of branches was not affected when using organic fertilizers which did not agree with results of this study.

Number of leaves

Time of fertilizer application and types of fertilizers, as well as their interaction, had higher significant effects on number of leaves per plant (Table 1). Number of leaves per plant was influenced significantly by the time of fertilizer application. The highest values of the number of leaves were found after sowing application. While the lowest values of the number of leaves were observed both before sowing and at sowing application (Table 2). The number of leaves was significantly affected by types of fertilizers. The highest number of leaves was recorded with the organic manure application. Number of leaves in pots treatment with humic acid fertilizer was lowest (Table 3). This contradicted with Eldm, (2004) who reported that number of leaves per and yield was gradually and significantly increased with the application of humic substances. The interaction between time of fertilizer application and types of fertilizers had higher significant effect on number of leaves per plant, the pots treated with 50 kg organic manure per ha added after sowing produced maximum number of leaves per plant (31.69), while minimum number of leaves per plant (17.88) was recorded in at sowing application by 100 kg ha^{-1} diammonium phosphate (Table 4). These results were supported earlier by El-Banna et al. (2006) who found that the increase in leaves number due to the application of organic components had stimulatory effects on cell division and enlargement, protein and nucleic acid synthesis. But this is not in line with Bhuiyan et al. (2008) who found that plants produced a significantly higher number of leaves with phosphorus and highest number of leaves (22.84) was found with phosphorus added at the rate of 40 kg ha^{1}, which was statistically significant.

Fresh forage yield (kg ha^{-1})

The fresh forage yield was affected by time of fertilizer application and types of fertilizers as well as their interaction (Table1). Time of fertilizer application had high significantly affected the fresh forage yield. The highest fresh forage yield (kg ha^{-1}) was recorded from the pots application time after sowing. While the least fresh forage yield (kg ha^{-1}) was counted in pots application time at sowing (Table2). 50 kg ha^{-1} organic manure performed better than other fertilizers doses. The lowest fresh forage yield was recorded in humic acid fertilizer. Diammonium phosphate increased fresh forage yield significantly over control (Table 3). These results confirm the findings of Bhuiyan et al. (2008) and Manpreet *et al*. (2004) who reported that the effect of phosphorus on Stover yield of mung bean was influenced significantly at harvest. The highest fresh forage yield (815.8 kg ha^{-1}) was recorded in 50 kg ha^{-1} organic manure after sowing application, which was significantly higher than other treatments. After sowing application humic acid fertilizer and control were statistically similar. The lowest fresh forage yield was 146 kg ha^{-1} recorded with 100 kg ha^{-1} added at sowing. Their results agreed with those of Bhuiyan et al. (2008) who reported with increasing Phosphorus rate, Stover yield decreased significantly.

Dry forage yields (kg ha^{-1})

Dry forage yields of mung beans were influenced significantly by the time of fertilizer application, types of fertilizers and their interaction (Table 1). The highest forage yield 106.1 kg ha^{-1} was found with application after sowing, which was significantly higher than other treatments. The lowest forage yield was 85.3 kg ha^{-1} recorded with application at sowing (Table 2). Moreover, the single effect of types of fertilizers on mung bean forage yields was also significantly influenced. The forage yield with organic manure was significantly higher than the forage yield recorded with humic acid fertilizer (Table 3). Sarwar et al. (2014) showed that the maximum straw yield on mung beans was recorded in the treatment where humic acid was applied at 50 kg ha^{-1}. Dry forage yield of mung bean was significantly influenced by interaction of time of fertilizer application, types of fertilizers. The maximum forage yield 161 kg ha^{-1} was obtained from pots fertilized by 50 kg organic manure per ha after sowing application. The minimum forage yield 40 kg ha^{-1} was obtained from diammonium phosphate application at sowing (Table 4). This result is not in agreement with that of Bhuiyan et al. (2008) who showed the significant effect of phosphorus, on dry weight of mung bean. Singh and Agrawal, (2007) stated that higher availability of nutrients in organic fertilizer was the main factor contributing to higher biomass of plants, which in line with the results of this study.

Plant height growth

A significant effect of time of fertilizer application and types of fertilizers and their interaction in early growth stage such as up to 30 days after sowing (days) was revealed (Figure 1), but at later stage, there were higher significant difference among the of time of fertilizer application and types of fertilizers and their interaction.

Table 1. F-value of plant height (cm), number of branches per plant, number of leaves per plant, fresh weight (kg ha^{-1}) and dry weight (kg ha^{-1}) of mung bean under time of fertilizer application and types of fertilizers.

Source of Variation	Degree of freedom	F-values					
		Plant height (cm)	Stem thickness (cm)	Number of branches per plant	Number of leaves per plant	Fresh weight (kg.ha^{-1})	Dry weight (kg.ha^{-1})
Replication	3	1.62	4.27	0.31	3.35	15.52	7.36
Application Time (A)	2	34.03**	4.69*	9.16*	26.58*	2223.13**	68.22**
Error 1	6	-	-	-	-	-	-
Fertilizers (F)	3	40.39**	1.36^{NS}	14.17**	17.38**	383.98**	34.31**
(A × F)	6	130.99**	3.38*	33.29**	37.64**	954.38**	81.70**
Error	27	-	-	-	-	-	-
Total	47	-	-	-	-	-	-
Error Mean Square (EMS)	-	0.78	0.74	0.24	2.22	173.90	77.66
Coefficient of Variance (C.V. %)	-	4.10	15.90	6.68	6.03	3.14	9.80
L.S.D at 5%	-	0.369	0.360	0.20	0.62	5.52	3.69
Se ± (A)	-	0.19	0.14	0.15	0.25	1.96	1.74
Se ± (F)	-	0.25	0.24	0.14	0.43	3.80	2.54
Se ± (A×F)	-	0.44	0.43	0.24	0.74	6.59	4.40

Note: NS= not significant, *= statistically significant difference at p = 0.05, **= statistically significant difference at p = 0.01

Table 2. Mean comparison of parameters studied of mung bean under time of fertilizer application

Treatments	Plant height (cm)	Stem diameter (cm)	Number of branches per plant	Number of leaves per plant	Fresh weight (kg.ha^{-1})	Dry weight (kg.ha^{-1})
Before sowing application	20.31C	5.55 A	7.28 B	24.03 B	398 B	85.31 B
With sowing application	21.71 B	5.04 B	7.01 C	23.89 B	339.8 C	78.38 C
After sowing application	22.62 A	5.62 A	7.92 A	26.20 A	521.3 A	106.1 A

Note: The same letter in each column shows non-significant differences using LSD 5%.

Table 3. Mean comparison of parameters studied of mung bean under types of fertilizers

Treatments	Plant height (cm)	Stem thickness (cm)	Number of branches per plant	Number of leaves per plant	Fresh weight (kg ha^{-1})	Dry weight (kg ha^{-1})
Control	21.48 C	5.45 A	7.35 B	24.94 B	388.8 C	84.33 C
Organic Manure	23.05A	5.68 A	8.10 A	27.06 A	523.8 A	109.7 A
Diammonium phosphate	22.35B	5.50A	7.37 B	24.02 C	416.4 B	91.42 B
Humic acid	19.32D	5B	6.79 C	22.81 D	349.7 D	74.33 D

Note: The same letter in each column shows non-significant differences using LSD 5%.

Table 4. Interaction effects of time of fertilizer application time and types of fertilizers of parameters studied of mung bean

Treatments	Plant height (cm)	Stem thickness (cm)	Number of branches per plant	Number of leaves per plant	Fresh weight (kg ha^{-1})	Dry weight (kg ha^{-1})
B.S ×C	23.08 D	6 BC	7.85 D	27.31 C	471.8 E	101.8 D
B.S × O.M	15.41 I	4.8 F	6.25 H	20.50 I	218 J	51.50 H
B.S × D.P	24.85 B	6.18 AB	8.37 C	26.63 D	482 D	103.8 D
B.S×H.A	17.92 G	5.25 DE	6.62 G	21.69 H	420.3 F	84.25 E
W.S ×C	23.90 C	5.37 D	7.56 E	25 E	372.8 G	85.25 E
W.S × O.M	24.95 B	5.8 C	8.68 B	29 B	537.5 C	116.5 C
W.S ×D.P	17.17 H	4.18G	5.12 I	17.88 J	146 K	40 I
W.S ×H.A	20.83 E	4.81 F	6.68 G	23.69 F	302.8 I	71.75 F
A.S ×C	17.46 H	5 EF	6.62 G	22.50 G	322 H	66 G
A.S × O.M	28.78 A	6.43 A	9.37 A	31.69 A	815.8 A	161 A
A.S ×D.P	25.04 B	6.12 ABC	8.62 B	27.56 C	621.3 B	130.5 B
A.S ×H.A	19.21 F	4.93 EF	7.06 F	23.06 G	326 H	67 G

Note: BS: Application fertilizer before sowing, WS: Application fertilizer with sowing, AS: Application fertilizer after sowing, C: control, MO: Manure Organic, DP: Diammonium Phosphate, HA: Humic Acid. The same letters in each column show non-significant differences using LSD 5%.

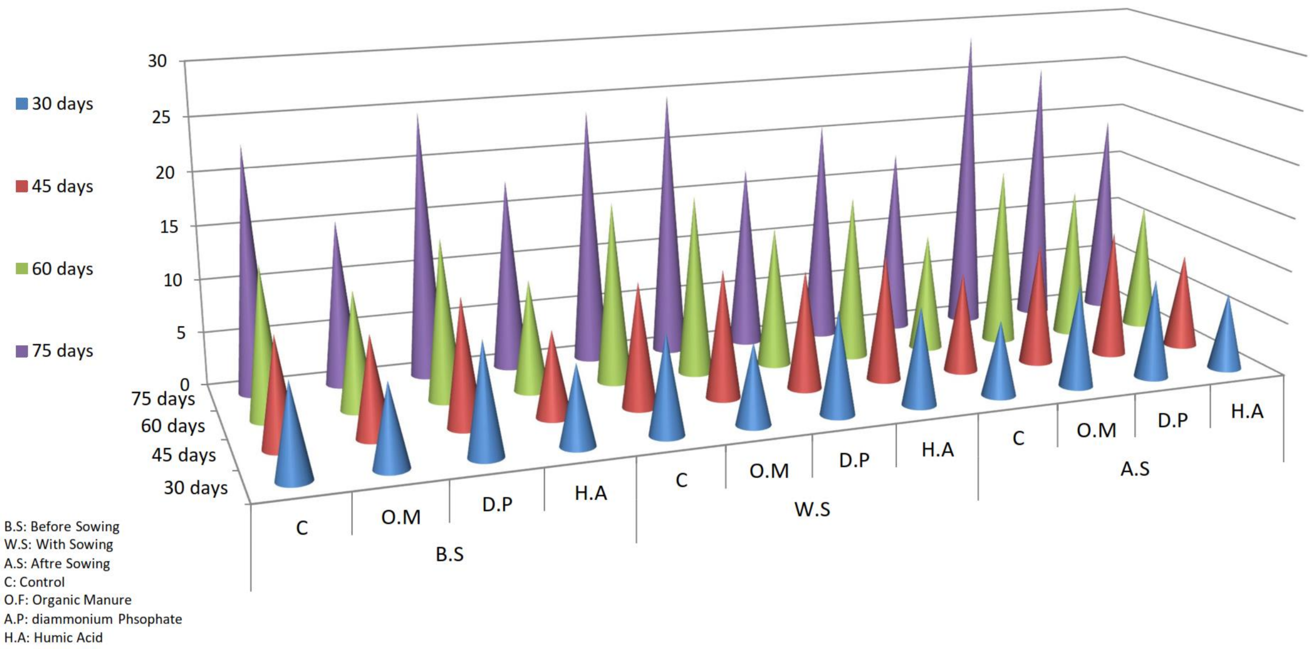

Figure 1. Mean values comparison of effect application time and fertilizers and their interaction of plant height growth on mung bean

The highest plant height was observed from the diammonium phosphate before sowing application (10.3 cm) at 30 days, diammonium phosphate after sowing application, organic manure, and humic acid both application at sowing (11.9 cm) at 45 days, organic manure application at sowing (16.8 cm) at 60 days and organic manure application after sowing (28.7 cm) at 75 days. The lowest was observed after sowing control (7 cm) at 30 days, humic acid application before sowing (8 cm) at 45 days, humic acid application before sowing (10.4 cm) at 60 days and organic manure application before sowing (15.4 cm) at 75days (Figure 1).

A similar trend was also found by Thakuni and Saharia (1990) and Salah et al. (2009) showed that in early growth stages such as up to 20 days after sowing (DAS) there was no significant difference among the treatments (Nitrogen, Phosphorus, Potassium and organic fertilizer (Bio fertilizer) but at later stage the highest plant height was observed from the organic fertilizer (Bio fertilizer) plots (45.93 cm, 60.63 cm, and 69.73 cm) at 35 DAS, 50 DAS and 65 DAS respectively (Table 1) while lowest (52.27 cm) was observed with control at 65 DAS.

To summarize, producing leguminous crops such as mung bean for forage is considered an alternative method to provide supplemental protein. The humic acid, organic manure and diammonium phosphate as fertilizers can increase the quality and improve the output paving the way for sustainable agriculture. The major targets of this study were to examine the impact of humic acid, organic manure and diammonium phosphate fertilizers on growth and stover productivity of mung bean and determine optimum application time for enhancing mung bean productivity. To accomplish these objectives, three different applications time and four types of fertilizers were studied using an experimental factorial split plot with random complete blocks design with four replications. The results obtained from the present research work indicated that highly significant difference of time of fertilizer application and types of fertilizers and their interaction for growth characters of mung bean on plant height, number of leaves per plant, and fresh forage yield and dry forage yield were significantly differenced at ($p \leq 0.01$). There were highly significant differences of types of fertilizers and interaction between time of fertilizer application and types of fertilizers at ($p \leq 0.01$) for number of branch per plant, significant difference of time of fertilizer application for number of branch per plant and stem thickness at ($p \leq 0.05$), significant difference of interaction between time of fertilizer application for stem thickness at $p \leq 0.05$ and no significant difference of types of fertilizers at ($p = 0.05$) for stem diameter. The treatment of 50 kg ha^{-1} of organic manure after sowing applications, gave highest plant height (28.78 cm), thick stem thickness (6.43 cm), optimized number of branches per plant (9.37), large number of leaves per plant (31.69), best fresh forage yield (815 kg ha^{-1}) and dry forage yield (161 kg ha^{-1}) were recorded.

Based on findings of this study the attributes of mung bean combined application of 50 kg ha^{-1} organic manure with application after sowing was considered the most balanced and suitable combination of fertilizer nutrients for achieving the maximum output of mung bean.

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ASIAN JOURNAL OF AGRICULTURE
Volume 1, Number 1, June 2017
Pages: 29-34

E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g010106

Sustainable aquaculture development in floating nets at Cirata reservoir (West Java, Indonesia) through single sex nilem fish introduction

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Manuscript received: 25 Mei 2017. Revision accepted: 20 June 2016

Abstract. *Yustiati A, Andriani Y, Herawati T. 2017. Sustainable aquaculture development in floating nets at Cirata reservoir (West Java, Indonesia) through single sex nilem fish introduction. Asian J Agric 1: 29-34.* This research aimed to develop a sustainable aquaculture system using single-sex nilem fish (*Osteochilus hasseltii* Valenciennes, 1842; syn. *Osteochilus vittatus* Valenciennes, 1842) in trophic level-based farming and to analyze the feeding habits, types of meal and preference level of the feed in *O. vittatus* farmed in Cirata Reservoir. In addition, it also aimed to determine the fish's ability as a biocontrol agent for water cleaning by measuring the ability to utilize periphyton attached to the floating nets. Research was conducted at the Laboratory of Ciparanje, Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran and floating nets of Cirata reservoir. Observations of the performance of biological testing and food feeding were applied with the experimental design method of Completely Randomized Design (CRD) consisting of 5 treatments and 3 replications. The five treatments include non-artificial feeding (treatment A), 1% feeding of body weight per day (treatment B), 2% feeding of body weight per day (treatment C), 3% of feeding of body weight per day (treatment D), and 4% feeding of body weight per day (treatment E). The results showed there was no difference in both the survival and growth levels of the female single-sex fish that were given both commercial and non-commercial feed. The highest feed efficiency was observed in the 3% of commercial feeding treatment, amounting to 55.30%. The fish are herbivorous and generalist with trophic levels between 1.03% and 1.45% eating plankton for living. Fish fed with artificial food to 4% of the biomass still grazed on phytoplankton as the main source of food but adapted by changing the natural feeding and tend to be ineffective in utilizing the available food resources in the waters. During the study, both the types and amount of periphyton attached to the nets decreased. Higher commercial feeding level results in a higher density of periphyton in the floating nets.

Keywords: Female single sex nilem fish, food and feeding habits, grazing level, sustainable aquaculture

INTRODUCTION

Cirata reservoir (West Java, Indonesia) is a site for fish farming activities; one of which is the growing fish farming system of floating nets (Indonesian: *keramba jaring apung* or KJA). The number of floating net units in Cirata Reservoir has increased expeditiously, even reaching three times the number regulated by the government. The extensive and intensive activities in floating net system have carried great consequences in feeding. Generally, in lakes or reservoirs, feeding is supported with ad libitum system, i.e., the feeding is available at any time. Some researchers have indicated that about 20%-50% of unconsumed or wasted feed from the fish in the bottom of the water could be released into the body of the lake as pollutants. The food remains and solid discharge from the fish are decomposed to form organic and inorganic compounds; some of which are nitrogen compounds (NH_3, NO_2, NO_3) and phosphorus (PO_4) (Juaningsih 1997). In an anaerobic condition, decomposition may function, but this process also produces a variety of toxic gases that can pollute the water in the lake or reservoir.

Several approaches are suggested to address the deteriorating conditions and at the same time to initiate environment-oriented floating net improvements. First and foremost, it is important to note that the application of feeding should be in accordance with the needs of the fish. Secondly, it is also recommended to the increase utilization of feed to reduce the possible feed wasting in the waters by applying layered nets system in the trophic-level-based aquaculture. This can be achieved by farming fish with different feeding characteristics in each layer, such as combination of key commodities (carps and tilapias) respectively in the first and second layers of netting, while the herbivorous ones are kept in the third layer. One of the herbivorous species that can be used as a commodity is the nilem fish (*Osteochilus hasseltii* Valenciennes, 1842; syn. *Osteochilus vittatus* Valenciennes, 1842).

This research aimed to find out optimum commercial feed rate to make operational cost economic. Other than that, finding the optimum rate can reduce organic material waste entering the reservoir.

MATERIALS AND METHODS

Study area

Research was conducted from July to October 2014 in floating nets system of Cirata Reservoir, West Java, for the field experiment component of fish stocking. The Laboratory of Aquatic Resources Management was used for water quality analysis, as well as the Laboratory of

Aquaculture was used for food production and analysis. All laboratories and fish stocking belong to the Faculty of Fisheries and Marine Sciences, Universitas Padjajdaran, Sumedang, Indonesia.

Fish materials

The tools used in this study were: a 7 x 7 m^2 floating cages, 20 pieces of 1m x 1m x 1m nets, digital scales, ruler, plastic cup, and pH meter. Additionally, a Dissolved Oxygen (DO) meter, thermometers, the field equipment such as a dipper, bucket and others, camera, microscope, object glass and cover glass, hand counter, and dissecting kit were all used.

Materials used in this study were juvenile *O. vittatus* female fish, 3-4 cm in size and between 0.2-0.4 g of weight. The number of fish used for the study was 1,500 fish juveniles and 500 juveniles for each treatment. *O. vittatus* juveniles were obtained from the experiment ponds of Ciparanje, Faculty of Fisheries and Marine Science, Universitas Padjajdaran. The artificial fish feed used in the study was Shinta brands with protein content of 28%.

Meanwhile, in control treatment, the fish were not given any commercial feed and they utilize natural feed in Cirata Reservoir water, namely plankton and periphyton.

Procedures

Experimental design

The experiment was designed as a completely randomized design (CRD) consisting of 5 treatments and 3 replications. The treatments are as follows: (i) Treatment A = natural feed, (ii) Treatment B = 1% feeding of body weight per day, (iii) Treatment C = 2% feeding of body weight per day, (iv) Treatment D = 3% feeding of body weight per day, (v) Treatment E = 4% feeding of body weight per day.

Linear model used in this design is as follows (Gasperz 1991).

$$Xij = \mu + \tau i + \varepsilon ij$$

Where:

Xij : Yield of the experiment unit for the treatment of-i and the replicates of-j

μ : General mean value

τ : Effect of treatment of all i

εij : Effect of random factors from treatment of –i and replicates of- j (General error)

Data collection and observation variables

Survival rate. The survival rate was calculated by the equation:

$$SR = \frac{N_t}{N_o} \times 100\%$$

Where:

SR : survival rate (%)

N_0 : number of fish juveniles at the beginning of culture

N_t : number of fish juveniles at the end of culture

Daily growth rate. Daily growth rate is calculated using the equation:

$$G = \frac{\ln Wt - \ln Wo}{t} \times 100\%$$

Where:

G : daily growth rate (%)

Wt : average of individual weights at the end of the study (g)

Wo: average of weight of individuals before treatment (g)

T : Length of maintenance time (days)

Feed efficiency

$$EP\ (\%) = \frac{(Wt + D) - Wo}{JKP} \times 100\%$$

Where:

EP : Feed efficiency (%)

Wt : Biomass of fish at the end of maintenance/ culture (g)

Wo: Biomass of fish before treatment (g)

D : The weight of the fish that died during the study (g)

JKP: Amount of feed consumed during the study (g)

Data analysis

Data were analyzed using the analysis of variance (ANOVA). If the treatments resulted in a significant effect, the mean values among treatments were then analyzed using the Duncan's Multiple Range Test (DMRT) with a confidence level of 95% to determine the differences between treatments. The water quality was analyzed descriptively as additional information.

RESULTS AND DISCUSSION

Survival rate

Survival rate value is derived from the comparison between the number of organisms living at the end of maintenance and that of organisms living at the beginning of the stocking in percent where greater percentage indicates more organisms living during the maintenance period in a cultivation container (Effendie 1997). The results showed that for 42 days of treatment, female single sex *O. vittatus* have a high survival rate at above 95%. The survival rate of the control treatment (non-artificial feeding) and of artificial feeding at various levels ranged from 96% to 99.33% (Figure 1).

The female single sex *O. vittatus* reached the highest average of survival rate in treatment of 4% feeding of body weight per day about 99.33%, while the lowest was reached in the treatment of non-artificial feeding about 96%. Based on the analysis of variance, it can be concluded that the non-artificial feeding treatment did not give significant effect on the survival rate.

Compared with others, the result of this research is better than Rejeki (2013) culturing Nile tilapia 'larasati' in floating cage with the highest survival rate of 79.6%. According to Aquarista et al. (2012), survival rate can be used to measure tolerance and its ability to live in certain environments. By obtaining survival rates of 96-99.3%, rearing *O. vittatus* in floating cage in Cirata Reservoir is more suitable.

Daily growth rate

Growth is a change in the size of fish due to the increase in the length and weight in each period (Effendie 1997). Observation of the fish growth for 42 days of the treatment resulted in varied individual weight gain as a response to treatments (Figure 2). The daily growth rate of *O. vittatus* range from 4.13% to 5.17%, with the lowest value is in the non-artificial feeding treatment (4.13%), which continues to increase until the 3% feeding (5.17%). On the latest treatment, the growth rate tends to decline only to 4.99%.

The effects of the treatments on the growth rate can be determined from the analysis of variance. Results indicate significant effect of the artificial feeding treatment on the growth rate of the fish. The further mean value test using Duncan's multiple Range resulted in the fact that the growth rate of the non-artificial feeding treatment was different from those with artificial feeding at various levels.

The result is also like Andriani et al. (2016) and proves that the use of only natural feed is not sufficient to meet the fish requirement, because it does not contain complete nutrition. The use of two or more protein sources in the feed is better than one source only (Madinawati et al. 2011).

The best growth rate from this research is higher than that found by Yudhistira (2013) where fermented herb given to *O. vittatus* gives the higher rate of 1.46%. The higher rate of *O. vittatus* is associated with a warm water temperature of Cirata reservoir. This is like the statement of Wicaksono (2013) that external factors such as ambient environment, quantity and quality of food in meeting fish nutrition requirement, energy content in the food, and availability of materials in the food influence fish growth rate.

Feed efficiency

Feed efficiency is the utilization of feed to increase fish growth (Nugraha 2011). Based on the research results, the highest feed efficiency occurs in the 3% artificial feeding treatment of 55.30% and the lowest feed efficiency is in the 1% artificial feeding treatment of 40.04% (Figure 3).

Analysis of variance of feed efficiency showed that the feeding material and levels did not give significant effect. Non-artificial feeding differs from treatments with artificial feeding. *O. vittatus*es responded remarkably to the feeding treatment during the research shown by no trace/remains of feed at each feeding period. This indicates that the fish have a wide range of adaptability to feeding variations. Furthermore, utilization of feed by fish is reflected by the relatively high growth and feeding efficiency value (55.30%.).

The bigger food efficiency value the more food portion that can be taken and accumulated as biomass or the increment of its flesh. A linear correlation was found between amylase activity and digestibility in response to dietary. Furthermore, rates of food efficiency and growth are correlated linearly with the apparent digestibility of dry materials (Chen 2013).

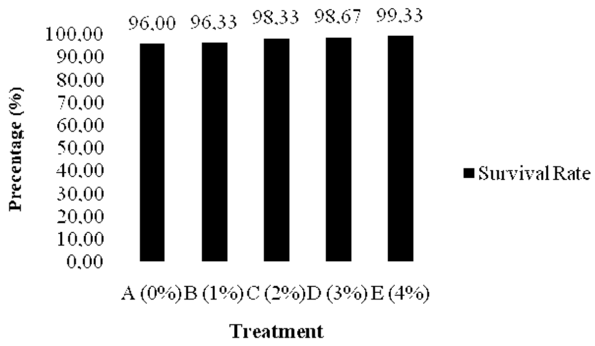

Figure 1. Survival rate of female single-sex *Osteochilus vittatus* during the experiment.

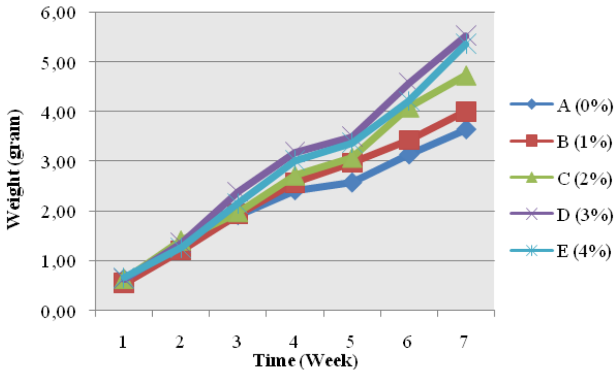

Figure 2. Weight gain of *Osteochilus vittatus* during the research

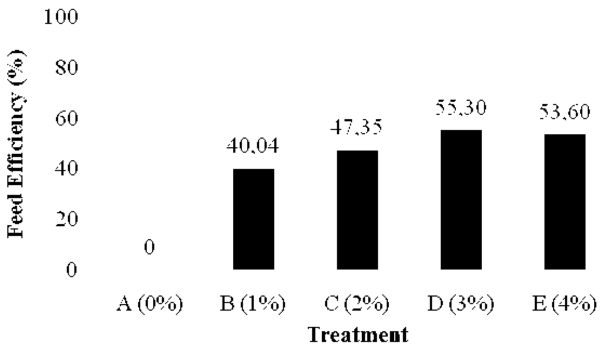

Figure 3. Feed efficiency of female single-sex *Osteochilus vittatus* during the research

Treatment with 4% of commercial feeding showed a decrease in feed efficiency compared to that of in 3% feeding. Feeding 4% affects insignificantly to the growth due to the nature of herbivorous behavior of *O. vittatus* on the lacking the enzymes and digestive fluids to absorb the artificial food (German, 2004). The research of German (2004) shows that there is a difference between enzyme activity in carnivorous and herbivorous fish. Fish will modulate the activity of its digestive enzyme as a response to the change of the food.

This illustrates that if the fish in floating nets of Cirata are cultivated with commercial/artificial feeding, it should be no less than 3%. The feed efficiency value in this study is 55.30%, higher compared to the research results from Yudistira (2013), in which the feed efficiency of fermented lettuce is only 24.85%.

Feeding habits

The composition of plankton as natural feed in the waters of Cirata consists of three classes and 23 genera of phytoplankton and three classes and 4 genera of zooplankton. Phytoplankton of Bacillariophyceae class consists of 11 genera with an average abundance of 605 individuals per liter, Chlorophyceae of 13 genera with an average abundance of 389 individuals per liter, and Cianophyceae of 4 genera with an average abundance of 152 individuals per liter, while the zooplankton consists of 2 genera of Crustaceans, 1 gene for each Euglenoidea and Rhizopoda with an average abundance of 118 individuals per liter (Figure 4).

The individual abundance of phytoplankton was larger than zooplankton population. Such condition is considered normal in an ecosystem as primary producers remain at the bottom of food pyramid and occupy the largest space in the system (Taofiqurohman et al. 2007).

Analysis of the *O. vittatus* digestive organs of female single sex found three types of food including phytoplankton, zooplankton, and artificial feed. Fish graze on 23 genera of phytoplankton comprising three classes of Bacillariophyceae, Chlorophyceae and Cyanophyceae, and zooplankton from Crustaceans class of *Cyclops*, and Rhizopoda class of *Arcella*. Nikolsky (1963) classifies the feeding habits based on three categories i.e., the natural food when the preponderant index is more than 25%, supplementary feed when the preponderant index is between 2% to 25%, and complete feed when the preponderant index is less than 5%. The research suggests that *O. vittatus* cultured in floating nets of Cirata Reservoir possess 0% to 57% of preponderant index indicating the full utilization of all types of feed in the water.

Trophic level

Trophic level is the order in the use of foods or materials and energy as defined by the food chain. Calculation on the trophic level is based on the correlation between the organism's trophic level and feeding habits of the fish to determine the position of the fish in an ecosystem (Tjahyo 2000). *O. vittatus* in this research occupied the trophic level of 1.03 to 1.45 that indicating the herbivorous character of the species (Figure 5). The lowest trophic level was found in the group that was given by non-artificial feed in the first week, and the highest number was given by the group fed up with artificial food of 4% in the third week. The varied numbers do not change the trophic level status as fish still graze on the phytoplankton in the water as food resource as found in the digestive tract.

The abundance of phytoplankton and periphyton species

The types and abundance levels of phytoplankton and periphyton in the floating nets of Cirata varied in both the number and kind, during observations. The largest average number of phytoplankton comprised the groups of *Oscillatoria* (1078.00 species), *Navicula* (585.47 species), and *Nitzschia* (315.53 species), while the zooplankton showed the biggest number of *Arcella* with the average number of individuals of 387.27 species (Figure 6).

The periphyton found in the floating nets consists of phytoplankton groups of Bacillariophyceae (8 species), Chlorophyceae (5 species), and Cyanophiceae (3 species). Furthermore, the periphyton consists of Euglenoidea (1 species), Rhizopoda (1 species) and Rotatoria (2 species). The nine species of phytoplankton were from Bacillariophyceae (6 species), Chlorophyceae (2 species), and Cyanophiceae (1 species) decline at the sixth week; seven of them are *Symbella, Spyrogyra, Crucigenia, Spirulina, Merismudesmus,* and *Pinularia* decreased to 100% or were fully consumed. Similarly, the number of species of zooplankton has decreased from the first week and it is identified that from four species at the first week have declined remaining two species at the sixth week. The species *Diurella* and *Branchionus* were fully consumed at the sixth week (Figure 7).

Osteochilus vittatus have high preference towards periphyton, as seen from the declining types, from four species to 2 species with a significant amount of consumption from 55.59% to 100%. The preference is derived from, not only from the size that fits into the larva's mouth opening, but also from highly active character of zooplankton that stimulates the fish juveniles to consume (Hardjamulya 1979).

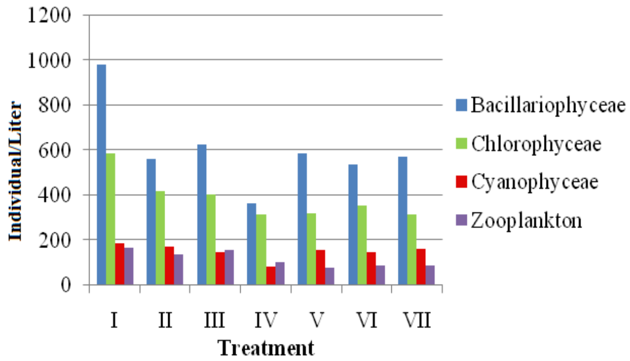

Figure 4. Abundance of plankton as food resources in water of Cirata reservoir

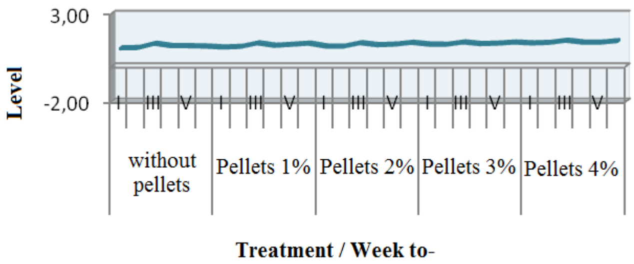

Figure 5. Trophic level of *Osteochilus vittatus* for 6 weeks of search

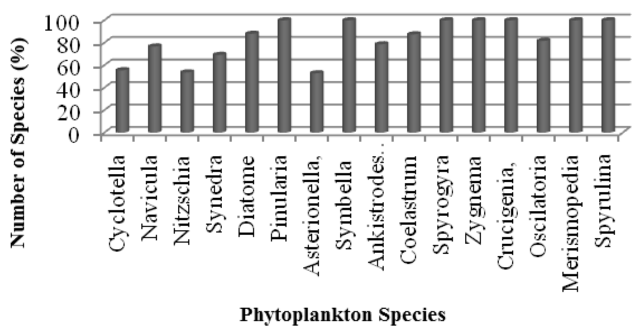

Figure 6. Percentage of periphyton (from phytoplankton) consumed by *Osteochilus vittatus* juveniles

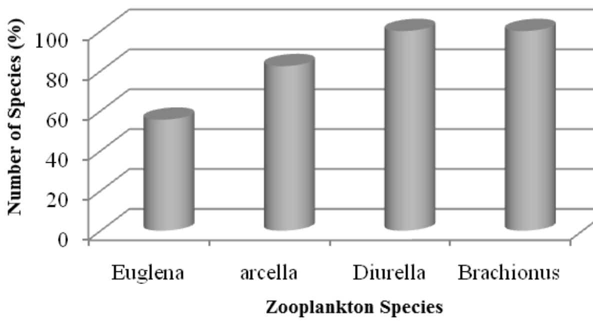

Figure 7. Percentage of periphyton (from zooplankton) consumed by fish juveniles

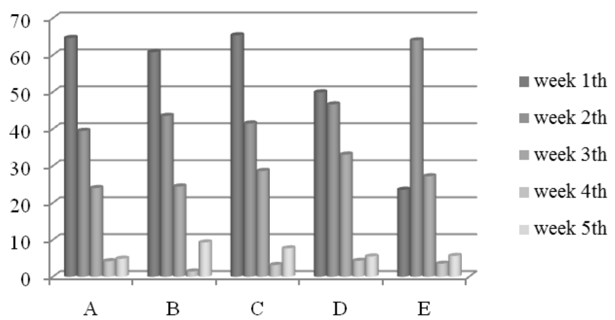

Figure 8. Grazing level of *Osteochilus vittatus* juveniles due to the treatment

The grazing levels

A 5-gram *O. vittatus* requires 6.373 g of periphyton attached to 19 m^2 net substrate to grow to 100 g because periphyton contains 0.46% of protein and 97.06% of water (Harris (2006). This indicates that the *O. vittatus* is suitable species as an alternative to overcome the problem of blooming phytoplankton in the waters.

Treatments A, B, C, and D showed similar pattern of the grazing level trend from the first week of sixth week. The Grazing level of *O. vittatus* was decreased during the time for all treatments. The condition could be due to the declining availability of periphyton in the nets. In treatment E, where the fish were fed with 4% of artificial feed, the grazing level declined from the third week, and it is assumed that on first and second week, the *O. vittatus* are still not attracted to consume the artificial food. In natural water, living food is in motion so that the fish are attracted to go after it. Attractive food will stimulate fish appetite and hence they can survive (Widodo 2010).

On those weeks, the grazing level is still high i.e. 134 $ind.cm^{-2}$ and 111 $ind.cm^{-2}$ respectively, while the grazing level slowly declined on the third week to the fifth week, from 47 $ind.cm^{-2}$, 20 $ind.cm^{2,}$ 16 $ind.cm^{-2}$. Deterioration in grazing occurs because of the option between artificial food and periphyton as food resources.

In conclusion, analysis of the survival rate and growth proves that there is no difference between female single sex of *O. vittatus* in floating nets Cirata Reservoir fed with non- and artificial food. The highest feeding efficiency is obtained by artificial feeding of 3% treatment amounting up to 55.30%. Female single sex *O. vittatus* are herbivores with trophic level between 1.03 to 1.45. *O. vittatus* is generalist and grazes on plankton as a food source. The *O. vittatus* fed with artificial food of 4% from biomass still consume phytoplankton as a main source of food, but the experience changes in the feeding habits and tends to be ineffective in utilizing the available food resources in the waters. During the study, the types and amount of periphyton attached to the nets tend to decrease. Higher level of artificial feeding causes higher density of periphyton. The grazing level of each treatment is relatively similar, but higher commercial feeding level tends to decrease the grazing level on the periphyton.

It is suggested that female single-sex *O. vittatus* could be used as a controlling organism for the growth of phytoplankton and as a bio-cleaning agent of periphyton in Cirata Reservoir.

ACKNOWLEDGEMENTS

This material used in this work is supported by the Department of Fisheries, Faculty of Fisheries and Marine Sciences, Padjajaran University, Indonesia and we are highly indebted. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors.

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ASIAN JOURNAL OF AGRICULTURE
Volume 1, Number 1, June 2017
Pages: 35-39
E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g010107

Microclimate modification through shading and watering frequency treatments as efforts for ex-situ conservation of pule pandak (*Rauvolfia serpentina*)

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Manuscript received: 21 June 2016. Revision accepted: 22 June 2017.

Abstract. *Samanhudi, Purwanto E, Sulandjari, Setiyaningsih A. 2017. Microclimate modification through shading and watering frequency treatments as efforts for ex-situ conservation of pule pandak (*Rauvolfia serpentina*). Asian J Agric 1: 35-39.* Pule pandak (*Rauvolfia serpentina* Benth) is a rare medicinal plant. To meet the needs of the drug, people continue to explore nature. Thus, proper cultivation technique for *R. serpentina* is required, by giving appropriate shading level and watering frequency to the growth and yield of *R. serpentina*. This research aimed to examine the effects of shading and watering frequency on the growth and yield of *R. serpentina*. The study was conducted from February to July 2009 in BBP Mondromino, Pokoh Village, Wonogiri at 141 meters above sea level. The experimental design used in this research was a Split Plot Design, which consisted of two factors and three repetitions. The first factor (main plot) was a shading level that consisted of three levels: 55%, 65%, and 75%. The second factor (subplot) was watering frequency that consisted of three levels: once every three days, once every five days and once every seven days. Data were subjected to analysis of variance, followed by post hoc test of Duncan Multiple Range Test at 5% level. The reserpine content was analyzed descriptively. The results showed that no interaction between shading level and watering frequency was seen on all variables. The shading level caused no significant effect on growth and yield of *R. serpentina*, except the leaf area. Watering frequency caused no significant effect on growth and yield of *R. serpentina,* apart from the chlorophyll content. The results showed that plants treated with watering frequency of five-day intervals produced the highest chlorophyll content. Plants treated with shading level of 65% and watering frequency of once every five days produced the highest reserpine content.

Keywords: Pule pandak, *Rauvolfia serpentin*a, reserpine shading, watering frequency

INTRODUCTION

Indonesia is a country that is rich in biodiversity, including medicinal plants. There are thousands of medicinal plant species that exist in various areas in Indonesia, especially in forest ecosystems. As the need for health care increases, there is a global issue of "back to nature" and strong sociocultural support in the use of traditional medicine, which makes medicinal plants more sought after. Lifestyle changes such as these, will provide greater market opportunities that can provide a positive impact on the development of traditional medicine industry and phytopharmaca.

One of the important medicinal plants is *Rauvolfia serpentina* Benth, also known by the local name of *pule pandak*. Roots of *R. serpentina* are a major part of the plant that is exploited for medical purposes, although the leaves and stems are also useful. *R. serpentina* contains about 50 kinds of alkaloids that have been isolated. Among the alkaloids is reserpine, the most important element in the roots of *R. serpentina* which acts as an anti-hypertension (Sulandjari et al. 2005).

Now, *R. serpentina* is still considered a rare medicinal plant. Exploration of nature continues to happen to meet the needs of drugs. This will probably make the plant even more scarce, thus there is a need for efforts to cultivate *R. serpentina* to produce large quantities of the crops' root extract with a high alkaloid content. Cultivation technologies that can be applied to this plant include setting the most appropriate levels of shading and watering frequency. Application of appropriate levels of shading and watering frequency will enable the plant to grow well and produce high reserpine.

The intensity of light received by the plant at a level lower than 40%, inhibits plant growth since light intensity is closely related to the opening of stomata. Water availability and sunlight are also crucial for plants and be important limiting factors for plant growth. Water stress may hamper plant growth, particularly the enlargement of cells and decrease the turgor potential (Sitompul 1995 *cit* Herlina 1996). Drought stress causes a decrease in photosynthesis, as seen from the reduction in net assimilation rate, that could result in reduced yield components of both quality and quantity (Herlina 1996).

A secondary metabolite is a chemical rather than a nutrient, with diverse chemical structures which limit the spread of its biosynthesis process influenced by the number and activity of enzymes. Secondary metabolite biosynthesis is genetically controlled and highly influenced by the environment. Reserpine is the primary alkaloid in *R. serpentina* and is a secondary metabolite that belongs to the indole alkaloids complex. In general, water shortage and low light intensity could induce the formation of secondary metabolites as a defense reaction.

This study aimed to examine the influence of shading level and watering frequency on growth and yield of *R. serpentina* and to assess the interaction between the level of shading and watering frequency on growth and yield of *R. serpentina*.

MATERIALS AND METHODS

This research was conducted from February until August 2009 at the BBP Mondromino, Pokoh Village, Wonogiri with an elevation of 141 m above sea level. This study employed a Split Plot Design consisting of two factors and three replications. The first factor (the main plot) was the level of shading which consisted of three levels, namely: 55% shading (N1), 65% shading (N2) and 75% shading (N3). The second factor (the sub-plot) was the frequency of the water supply consisted of three levels, i.e., watering in three-day intervals (A1), five-day intervals (A2) and seven-day intervals (A3). Materials used in this study included *pule pandak* (*Rauvolfia serpentina* Benth.), paranet, soil, water, polybags, and manure. The equipment used was a hoe, trowel, digital balance, ovens, ruler, cutter, lux meters, thermohygrometer, and a shovel.

Implementation of this research included nurseries, land preparation, planting preparation, planting, maintenance, and harvesting. The variables measured were plant height, leaf number, leaf area plant^{-1}, chlorophyll content, root length, root diameter, specific leaf area, roots-crown ratio, the weight of simplicia, and the reserpine content.

Obtained data were subjected to analysis of variance according to the split-plot design approach. A post hoc test of Duncan's multiple range test (DMRT) at 5% level was used to compare the treatment means. Reserpine content was analyzed descriptively.

RESULTS AND DISCUSSION

Rauvolfia serpentina is considered a medicinal plant. The main part that is used for medicinal purposes is the roots. *R. serpentina* contains no less than 50 kinds of alkaloids that have been isolated. Among the alkaloids is reserpine; the most important element in the root of *R. serpentina* which acts as an antihypertensive (Sulandjari et al. 2005). The availability of water and shade at the appropriate level are both important aspects that can affect growth and yield of *R. serpentina*. The analysis of variance showed no interaction between shading level and watering frequency on all variables. The significant effect of shading treatment only occurred on the variable leaf area, whereas the significant effect of water frequency was observed only on the chlorophyll content.

Plant height

Plant height is the most frequently observed variable both as an indicator of growth as well as the parameter used to measure the environmental effect of the treatment applied (Sitompul and Guritno 1995). Analysis of variance showed no significant interaction effect of shading level and watering frequency on plant height. This means that the plant experienced no etiolation during the experiment. Presumably, the plants were tolerant to low-light intensity and that the metabolism of the plants under stress conditions was similar to that at field capacity level.

Number of leaves

The analysis of variance showed that the treatments of shading level and watering frequency caused no significant interaction effect on the number of leaves. This is probably because the number of leaves tends to be more influenced by genetic factors. We found in the present research that several plants suffered leaf loss. In addition, the number of leaves will increase with increasing plant height; however, we found no significant increase in the number of leaves in this study because the plant height of the tested plants did not increase significantly.

Leaf area

The leaf is the organ where the process of photosynthesis occurs. If leaf area increases, the photosynthesis rate will increase, and the plant growth and plant dry weight will also increase. Based on our analysis of variance, shading treatment significantly affected leaf area, whereas the watering frequency treatment caused no significant effect on leaf area.

DMRT 5% (Table 3) test shows that 75% shading level produced a lower leaf area as compared to other shading levels. This is, presumably, due to the low light intensity, which in turn caused inhibition of photosynthesis and resulted in a decrease of number of leaves and ultimately, a decrease of leaf area of the plant.

Chlorophyll content

Chlorophyll is the photosynthetic pigment found in plant that functions to absorb red, blue, and purple lights and reflects green light, which causes plants to get their color characteristics. Chlorophyll is found in the chloroplasts and uses the absorbed sunlight energy as the light reactions in photosynthesis (Streitweiser and Heathcock 1981). Based on the analysis of variance, we found that shading treatment had no significant effect on chlorophyll content, which may be because not the entire chlorophyll content is influenced by light. Lakitan (1993) stated that the shaded leaves contained more chlorophyll (especially chlorophyll b) per unit of leaf weight because the shaded leaves formed more grana as an adaptation strategy to absorb light more efficiently.

The present study results revealed that the frequency of watering in five-day intervals could raise the levels of chlorophyll content. Presumably, in this water shortage condition, the plants tended to produce higher amino acid. One of amino acids is nitrogen, which is the main component of chlorophyll. Differences in the water supply for three-day and five-day intervals showed significantly elevated levels of chlorophyll. However, more severe water shortage conditions may show a decrease in chlorophyll content. The accumulation of photosynthetic pigments because of exogenous application of salicylic acid may be due to increased photosynthesis efficiency as reflected by increasing contents of both chlorophylls a and chlorophyll b on *R. serpentina*.

The occurrence of water loss due to water stress that is not followed by the entry of water into the plant at the same speed will cause decrease in cell turgor. This caused leaves to wither and light interception into the plant decreased (Islami and Utomo 1995). It is suspected that provision of water once every seven days, causing very low light interception into the plants, which in turn caused the inhibition of photosynthesis process and hence damage to the plant tissues, including the chlorophyll.

Root length

The root is the lower part of the plant that usually grows beneath the soil surface and serves to absorb water, minerals, and materials essential for plant growth and development (Hidayat 1995; Gardner et al. 1991). Based on analysis of variance we found that shading and watering frequency treatments caused no significant effect on root length. Gardner et al. (1991) stated that water shortage that was relatively short in time but is severe, may not significantly affect crop yields; on the other hand, an extended period of water shortages that are not so severe may have a greater influence on the yield. In this research, the shortage of water, was relatively short so that root growth was not significantly affected.

Root diameter

One indicator of root growth is the root diameter increment. The analysis of variance of root diameter showed that both the treatments of shading and watering frequency had no significant effect on root diameter. This is probably because root growth is more influenced by the availability of adequate nutrients, so that the levels of shading and watering frequency had no significant effect on root diameter.

Specific leaf area

Not all light received by plants is absorbed by the leaves. The lights are partially reflected and partly transmitted by the plant leaves. Transmission of light through the leaves is determined by several factors, including leaf thickness that is expressed in specific leaf areas. Specific leaf area is the weight of leaf per unit leaf area (Guritno and Purnomo 2006). Based on analysis of variance of the specific leaf area, the treatments of shading and watering frequency had no significant effect on specific leaf area, presumably because the plants are resistant to the drought.

Based on Figure 1, the highest specific leaf area was found at 55% shading level, i.e., 52.44 cm^2 g^{-1} and the lowest was at 75% shading level. According to Fosked (1994), a plant that grew in an environment of low radiation intensity will etiolate with a more dominant longitudinal growth. At dark condition, proplastids do not turn into a chloroplast but become an etioplast, so that the lower the specific leaf area is in line with the increasing shading level. Presumably, this is what makes leaves slimmer at the high shading level condition. However, according to analysis of variance, the differences in specific leaf areas due to increasing shading levels are not significant, which means the leaves had not yet shown symptoms of etiolation.

Table 1. Means of plant height at various levels of shading and watering frequency

Level of shading (%)	Watering frequency (days)			Means (cm)
	3	5	7	
55	29.70	30.03	32.46	30.73 a
65	25.10	23.67	26.50	25.09 a
75	17.63	23.80	19.73	20.39 a
Means (cm)	24.14 c	25.83 c	26.23 c	(-)

Note: Means on the same column or same line followed by the same letter are not significantly different at 5% DMRT.(-): no interaction.

Table 2. Means of leaf number at various levels of shading and watering frequency

Level of shading (%)	Watering frequency (days)			Means
	3	5	7	
55	30.42	26.75	30.83	29.33 a
65	24.08	22.75	25.08	23.97 a
75	19.25	22.92	19.75	20.64 a
Means	24.58 c	24.14 c	25.22 c	(-)

Note: Means on the same column or same line followed by the same letter are not significantly different at 5% DMRT. (-) : no interaction

Table 3. The effect of shading level on leaf area of *Rauvolfia serpentina*

Shading level (%)	Leaf area (cm^2)
55	2014.98 a
65	1503.16 a
75	842.64 b

Note: Means followed by the same letter are not significantly different at 5% DMRT.

Table 4. The effect of watering frequency on chlorophyll content of *Rauvolfia serpentina*

Watering frequency (day)	Chlorophyll content (%)
3	33.30 a
5	40.90 b
7	38.98 b

Note: Means followed by the same letter are not significantly different at 5% DMRT

Table 5. Means of root length at various levels of shade and watering frequency

Level of shading (%)	Watering frequency (days) 3	5	7	Means (cm)
55	26.33	21.33	18.83	22.16 a
65	21.67	21.50	19.50	20.89 a
75	20.67	19.00	21.50	20.39 a
Means (cm)	22.89 c	20.61 c	19.94 c	(-)

Note: Means on the same column or same line followed by the same letter are not significantly different at 5% DMRT. (-) : no interaction

The ratio of the root/crown

Comparison of root growth and tip growth (canopy) is usually expressed as the ratio of roots/crown that has physiological importance as it illustrates one type of drought tolerance mechanism. Analysis of variance showed that shading and watering frequency caused no significant effect on the ratio of the root/crown. This is, presumably, because the influence of genetic factors on root/crown ratio was greater than that of environmental factors (Gardner et al. 1991).

Weight of simplicia

Simplicia is the parts of plants that have been dried and used as ingredients in pharmaceutical manufacturing. Parts of *R. serpentina* plants commonly used for medicine are the roots. Our analysis of variance revealed no significant effect of shading and watering frequency treatments on the weight of simplicia. The weight of simplicia is affected by root length and root diameter. In the present study, we found that the plant root was not significantly affected by shading and watering frequency treatments so that the weight of the simplicia was also not significantly affected by the treatments.

Reserpine content

The main purpose of cultivation of medicinal plants is to obtain maximum biomass containing the highest secondary metabolites. A secondary metabolite is a compound produced by plants to sustain their life and is mostly a defense mechanism for plants to cope with attacks from outside, such as viruses and bacteria (Rogerio et al. 2010).

Sulandjari (2008) stated that both the climate and the sun affected the photosynthesis process in relation to primary and secondary metabolite formation. Figure 2 shows that the highest levels of reserpine were found at 65% shading level treatment with the watering frequency of five-day intervals, while the lowest was at 75% shading treatment with the watering frequency of three and five-day intervals. Figure 2 also shows that at 75% shading level, the reserpine levels started to decline. This is apparently because, at 75% shading, the plant metabolism was obstructed so that the reserpine content generated was the lowest. *R. serpentina* is a C_3 plant, so the saturation level of light intensity is low. However, the very low-light intensity can decrease photosynthetic activity, which then reduces the secondary metabolites production. Presumably, the light intensity of less than 25% has inhibited the *R. serpentina* growth, so that its reserpine level was the lowest.

Table 6. Means of root diameter at various levels of shade and watering frequency.

Level of shading (%)	Watering frequency (days) 3	5	7	Means (cm)
55	0.72	0.84	0.58	0.71 a
65	0.61	0.76	0.50	0.62 a
75	0.60	0.61	0.56	0.59 a
Means (cm)	0.64 c	0.73 c	0.55 c	(-)

Note: Means on the same column or same line followed by the same letter are not significantly different at 5% DMRT. (-) : no interaction

Table 7. The means of the ratio of the root/crown at various levels of shade and watering frequency

Level of shading (%)	Watering Frequency (days) 3	5	7	Means
55	0.57	0.31	0.23	0.37 a
65	0.54	0.50	0.31	0.42 a
75	0.89	0.89	0.72	0.83 a
Means	0.67 c	0.57 c	0.42 c	(-)

Note: Means on the same column or same line followed by the same letter are not significantly different at 5% DMRT. (-) : no interaction

Table 8. The means of the weight of simplicia at various levels of shade and watering frequency

Level of shading (%)	Watering frequency (days) 3	5	7	Means (g)
55	5.703	2.403	5.714	4.61 a
65	2.877	4.765	2.024	3.22 a
75	1.852	1.978	2.714	2.18 a
Means (g)	3.48 c	3.05 c	3.48 c	(-)

Note: Means on the same column or same line followed by the same letter are not significantly different at 5% DMRT. (-) : no interaction

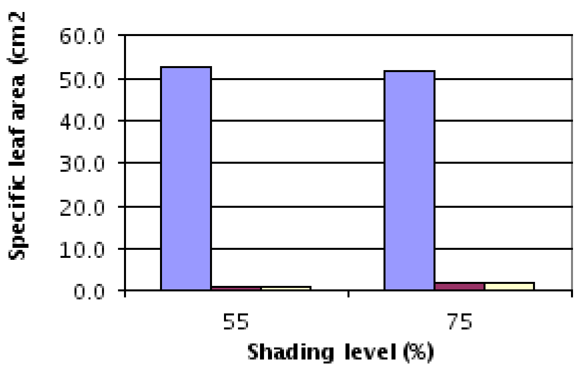

Figure 1. Specific leaf area of *Rauvolfia serpentina* at various levels of shading

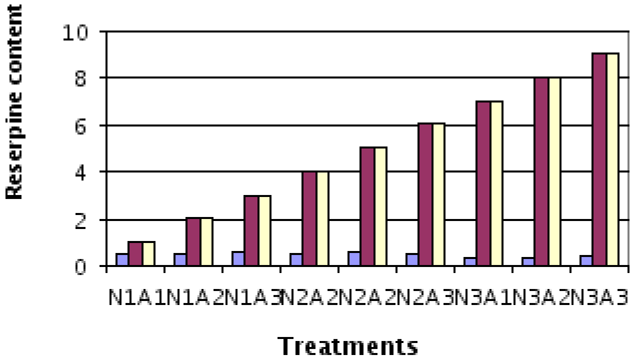

Figure 2. Reserpine content of *Rauvolfia serpentina* at various treatments of shading level and watering frequency

In conclusion, there was no significant interaction effect of shading level and watering frequency on all the observed variables. The shading level treatment did not affect the growth and yield of *R. serpentina*, except on leaf area that was the narrowest at the 75% shading level. Different watering frequencies caused significant influence on growth and yield of *R. serpentina*, except for the chlorophyll content that increased in the watering frequency every five days. The highest reserpine content was found in 65% shading level treatment with the watering frequency of five-day intervals.

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ASIAN JOURNAL OF AGRICULTURE
Volume 1, Number 1, June 2017
Pages: 40-45
E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g010108

The role of plant-parasitic nematodes on productivity reduction of banana and tomato in East Kalimantan, Indonesia

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Manuscript received: 27 September 2016. Revision accepted: 27 June 2017.

Abstract. *Suyadi, Rosfiansyah. 2017. The role of plant-parasitic nematodes on productivity reduction of banana and tomato in East Kalimantan, Indonesia. Asian J Agric 1: 40-45.* Plant-parasitic nematodes are one of the most limiting factors of agricultural ecosystem productivity in East Kalimantan. But their occurrence in agricultural fields as a crop's pest is generally undistinguished, due to their microscopic size and their existence being wrapped in the roots or soil particles. However, plant-parasitic nematodes might cause yield loss of up to 75%, without showing any disease symptoms on crop morphologically. Both perennial crops and annual crops under intensive cultivation usually experience high yield loss due to plant-parasitic nematodes if crop protection management was not implemented properly. This research aimed to determine the role of plant-parasitic nematodes in reducing crop productivity in East Kalimantan, in relation to agricultural practices implemented by farmers. Descriptive research and comparative analyses were implemented to determine the role of plant-parasitic nematodes on yield reduction of banana and tomato as the indicator of productivity. Based on the field observation related to pest management in East Kalimantan, it was determined that plant-parasitic nematode existence was neglected by farmers and no significant effort was implemented to control plant-parasitic nematodes population. Therefore, low productivity of agricultural ecosystem in East Kalimantan was reported by Statistical Office. This was not only caused by soil fertility problems, but in some crops, it was also the impact of population outbreak of plant-parasitic nematodes. The first ranking three genera of plant parasite nematodes observed in East Kalimantan were *Meloidogyne*, *Radhopholus*, and *Rotylenchulus*, respectively. *Meloidogyne* and *Rotylenchulus* were major pests on vegetables and caused a yield loss of >50% on tomato. While genus of *Radhopholus* was the major pest on banana and caused a yield loss of >75% on banana.

Keywords: Banana, East Kalimantan, nematode, productivity reduction, tomato, yield loss

INTRODUCTION

Plant-parasitic nematodes (PPN) were important pests in East Kalimantan Province of Indonesia, compared to other pests, PPN caused highest yield loss on some crops. However, their existence in the field is still neglected by farmers because they could not distinguish the existence of PPN directly in the field, with plants being symptomless during the early stage of infection. Additionally, no serious efforts were implemented to control their populations. Worldwide crops yield loss due to PPN has been estimated at $US 80 billion per year, and this is likely to be a significant underestimate of the true figure, as many growers, particularly in developing nations, are unaware on PPN existence (Jones et al. 2013).

Yield loss due to PPN varies as it can be affected by environmental factors, cropping systems and pest management practices. Reported by Jones et al. (2013) that *Meloidogyne graminicola* caused yield losses of up to 87% on rice. Whereas cyst nematodes (*Heterodera* and *Globodera* spp.) under specific environmental conditions caused yield losses of up to 90%. *Pratylenchus* spp. caused yield losses of up to 30% on wheat in Australia. Finally, reniform nematode (*Rotylenchulus reniformis*) caused yield losses of 40%-60%. Furthermore, reported by Singh and Kumar (2015) that yield loss on vegetable crops in Western Uttar Pradesh varied from 4% on bitter gourd (*Momordica charantia*) to up to 43% on eggplant (*Solanum melongena*), and *Meloidogyne incognita* was determined as the most important PPN in Uttar Pradesh, this nematode genus involved in the first rank of causal yield losses, on eggplant (43%), tomato (40%), and okra (38%).

Banana yield loss due to burrowing nematodes can reach more than 50% (Bartholomew et al. 2014) or range from 30 to 60% (Brooks 2014). Moreover, reported by Hölscher et al. (2014) that burrowing nematodes might cause yield loss of up to 75% on banana. Higher banana yield loss commonly occurred in developing countries, because control efforts for this parasite is still limited (Chitamba et al. 2013; Kamira et al. 2013; Srinivasan et al. 2011), and there were some important genera of PPN attacking banana altogether with burrowing nematodes, i.e., *Meloidogyne* (root-knot), *Pratylenchus* (lesion), *Helicotylenchus* (spiral), *Rotylenchulus* (reniform) (Brooks 2014; Kamira et al. 2013; Srinivasan et al. 2011). Whereas, banana yield loss was relatively reduced under commercial banana cultivation, where integrated pest management (IPM) technology was implemented (Ricède et al. 2010).

Tomato yield loss due to PPN ranged from 25% up to 100% and the main nematode genus attacking tomato was *Meloidogyne* (Seid et al. 2015). As well as in the banana case, yield loss due to PPN on tomato mostly occurred in developing countries, because of poor control efforts implemented to the parasite. Whereas PPN in developed countries was relatively well managed and more control technique alternatives were available. The availability of resistant cultivars apparently was the most prospective control technique for PPN on tomatoes (Rani et al. 2009; Gharabadiyan et al. 2013). However, the most practical control measure applicable in East Kalimantan was soil amendment of dung manure (Aalders et al. 2009) which prevented tomato yield loss direct and indirectly.

Research activities related to PPN and their management in East Kalimantan are still limited. The most intensive research on PPN which was conducted in East Kalimantan, was focused on bananas and tomatoes. Because the symptoms of PPN attacking both crops are relatively easy to observe, i.e., root galls on tomato, root rot or blackhead on banana which caused toppling disease. The purpose of this research was to determine the role of PPN on productivity reduction of banana and tomato, as the indicator of crop yield loss due to PPN in East Kalimantan. Furthermore, this research aims to expand farmers understanding of the existence of PPN as an important pest in the province, with the hopes that farmers could control them properly.

MATERIALS AND METHODS

Study area

Field observations and surveys to determine the existence of PPN on tomato and banana were conducted at production centers of banana and tomato in East Kalimantan Province, Indonesia. Banana production areas were distributed in almost all the districts in East Kalimantan, whereas tomato production areas were only found in Balikpapan municipal, Kutai Kartanegara and Penajam Paser Utara districts. Production centers of bananas are commonly located along province main road and along rivers in the province (Figure 1).

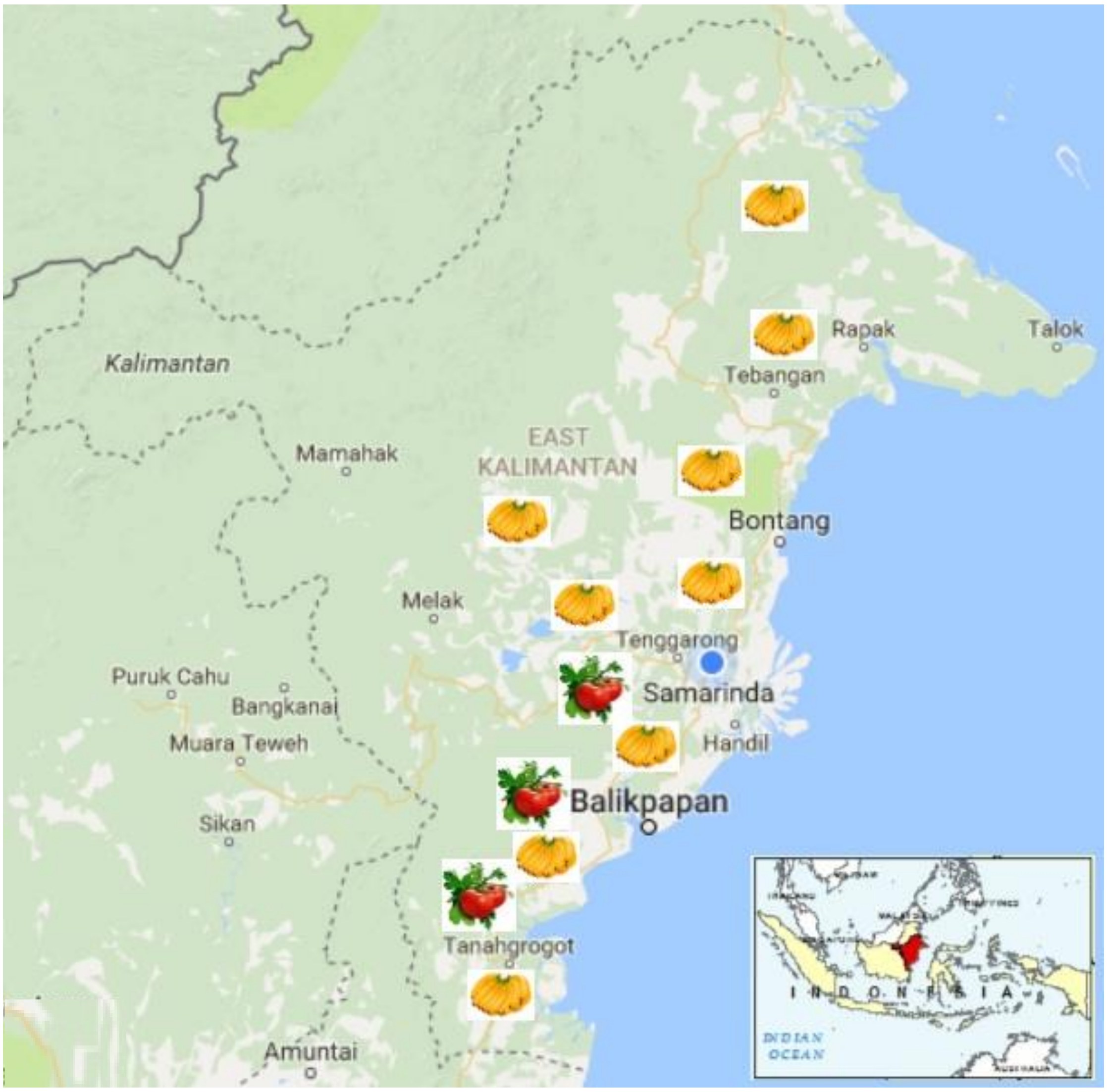

Figure 1. The production center sites of banana and tomato in East Kalimantan Province, Indonesia. The banana and tomato centers are indicated by the banana and tomato symbols

Procedures

This research was a combination of field and desk research. Field research was focused on the determination of the existence of PPN attacking the crops, and their impact on crop productivity reduction. The primary data was gathered from field research, i.e.: PPN genera attacking tomato and banana, population densities of each PPN genus, and the actual yield of banana and tomato under traditional cultivation system implemented by farmers. Secondary data about productivity, production, and harvested or cultivated area of banana and tomato, was gathered from the formal data reported by the Statistic Center Bureau of East Kalimantan Province.

Field research

Field surveys and observations were carried out to gather primary data, to determine the existence of PPN attacking tomato and banana at the sites of production center, as well as actual or attainable yield of both crops at each farmer's field. Crops yield and productivity were directly observed and measured or counted in the field. While PPN genera and their population densities were gathered from soil and roots samples. Then, nematodes extractions were conducted at the laboratory, soil samples were processed by Baermann Funnel Method and root samples were processed through the Direct Maceration Method.

Desk research

The main purpose of desk research was to gather a time series of secondary data, which was available and reported formally by the Statistic Center Bureau of East Kalimantan Province. Secondary data required included: crop production (yield), productivity, and harvested area of banana and tomato in East Kalimantan. The secondary data used in this research covered at least a five year period, to elaborate the dynamic of banana and tomato production in the province.

Data analysis

Comparative data analysis was implemented in this research to describe the role of PPN on banana and tomato productivity reduction. Data analysis was conducted based on the secondary data and justified by the primary field data, comparatively. Therefore, the description of time series secondary data of banana and tomato production in the province was elaborated to explain the relationship occurrences between the existence of PPN and productivity reduction on bananas and tomato.

RESULTS AND DISCUSSION

The productivity of tomato

Tomato farmers in East Kalimantan always cultivated their crops under intensive management, especially in terms of fertilizer application and aboveground pest control, but plant-parasitic nematodes control efforts were still neglected. Consequently, they harvested only limited yields of tomato, and the average annual productivity was still very far from the potential and attainable yield of the crop.

Based on the field observation and experiment in Samarinda, it was determined that the average attainable yield of tomato *Ratna* variety was 20 tons. ha^{-1}, and the occurrence of PPN (*Meloidogyne* spp.) at population density of 2-3 juveniles. g^{-1} soil at harvest time reduced tomato productivity by about 57-77% (Table 1). The experiment was conducted at the field where tomato was previously planted, and with an unknown initial population density of *Meloidogyne* spp. but evenly distributed through soil tillage management. The population density development of plant-parasitic nematodes was prevented by applying chicken dung as a soil amendment treatment.

The potential productivity of tomato commercial varieties which are available in East Kalimantan was always above 40 tons. ha^{-1} (Purwati 2009). However, the annual average productivity of tomatoes reached in the East Kalimantan province was considered very low compared to the potential productivity, only ranging from 14.88% (2012) and up to 30.83% (2014) (Figure 1).

The highest attainable productivity of tomatoes occurred in Penajam Paser Utara district, being roughly 24.44 tons. ha^{-1} (Table 2). But the productivity of tomatoes in other districts and cities was lower, therefore the annual average productivity of tomatoes in the province was still low. Based on the standard of the highest attainable productivity (24.44 ton. ha^{-1}), the annual average productivity of tomatoes in the province ranged from 24.39% (2012) up to 50.45% (2014).

The productivity of banana

Banana is the number one fruit produced in East Kalimantan. Its production reached 72,114 tons in 2015 (BPS, 2015) or about 30% of total fruit production in the province which was composed of about 20 varieties of fruits. Banana farmers commonly cultivated their crop traditionally, and production inputs were not implemented to the crop. The risk of PPN infection at new banana plantations could come from the planting materials. Because banana farmers commonly used suckers as planting material, where small amounts of soil and root may have carried PPN in it. Afterward, the population of PPN increased and could cause significant yield loss and productivity reduction.

Based on the field survey and observation of PPN population density and banana yield in Kutai Kartanegara district, it was determined the relationships between PPN population density and productivity reduction on banana. The dominance of PPN genera at the observation area were *Radopholus*, *Meloidogyne*, and *Pratylenchus*. They all caused the productivity reduction on bananas, of Saba variety, in the upland area by about 41.67% and about 33.33% at the watershed area (Table 3). The productivity reduction on bananas was from the impact of PPN, at a population density of about 5891 and 4919 nematodes per 100 g of root, which generated the average root lesion at level of 55.9% and 39.8%, respectively (Table 3). In the older banana plantations, without PPN control activities, the root lesion incident generally reached up to 90% - it was the precondition for toppling disease and productivity reduction by more than 75%.

Table 1. The relationships between plant-parasitic nematode (*Meloidogyne* spp.) population densities and productivity reduction of tomato in East Kalimantan Province, Indonesia

Chicken dung application (ton.ha^{-1})	Nematode population (juvenile per 50g soil)	Tomato productivity (ton.ha^{-1})	Productivity reduction (%) based on attainable tomato yield (20 ton.ha^{-1})
0 (Po)	170	4.55	77.25
5 (P_1)	140	5.34	73.30
10 (P_2)	134	6.76	66.20
15 (P_3)	122	7.91	60.45
20 (P_4)	120	7.98	60.10
25 (P_5)	110	8.59	57.05

Table 2. Harvested area, productivity, and production of tomato at districts and cities of East Kalimantan province, Indonesia.

Districts/cities	Harvested area (ha)	Productivity (ton.ha^{-1})	Production (ton)
Berau	98	1.77	174
Kutai Barat	23	2.34	54
Kutai Kartanegara	467	12.47	5,823
Kutai Timur	68	1.88	128
Mahakam Ulu	-	-	-
Paser	22	5.30	117
Penajam Paser Utara	103	24.44	2,517
Balikpapan	191	17.66	3,374
Bontang	15	3.89	58
Samarinda	14	7.34	103

Source: BPS (2015)

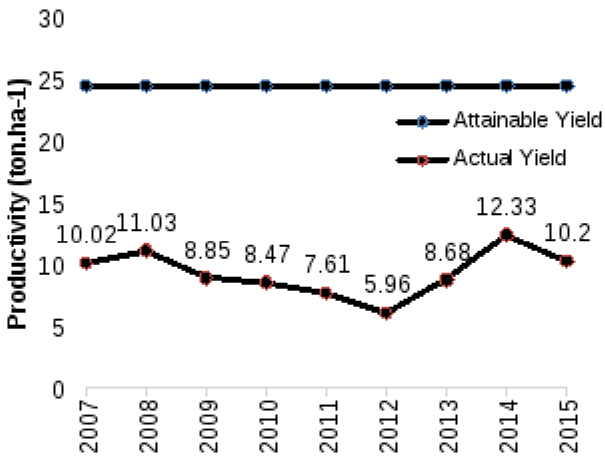

Figure 1. Tomato productivity trend in East Kalimantan (Sources: BPS, 2012 and 2015)

Table 3. The Relationships between plant-parasitic nematodes population density and productivity reduction on banana of Saba variety in Kutai Kartanegara district of East Kalimantan province, Indonesia

PPN genera	PPN genera population density (ind per 100 g root)	Total of PPN population density (ind 100 g root)	Average root lesion (%)	Productivity reduction (%)
Watershed (periodically flooded area)				
Radopholus	9106			
Meloidogyne	4070	14756	39.8	33.33
Pratylenchus	1580			
Upland area				
Radopholus	9967			
Meloidogyne	4610	17672	55.9	41.67
Pratylenchus	3095			

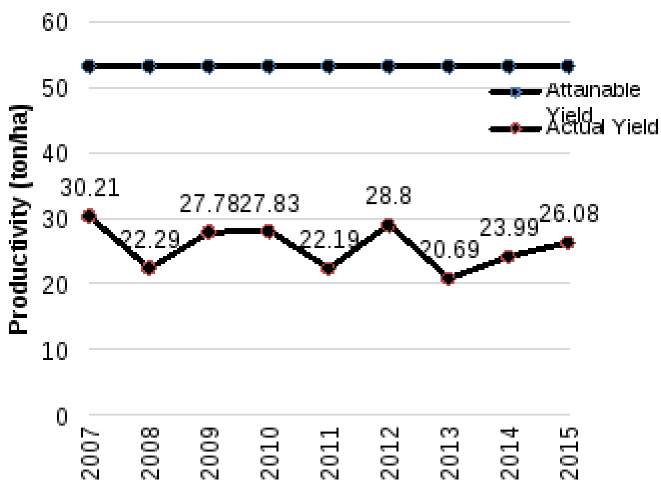

Figure 2. The banana productivity trend in East Kalimantan (Sources: BPS 2012 and 2015)

Table 4. Harvested area, productivity, and production of banana at districts and cities of East Kalimantan Province, Indonesia

District/City	Harvested area (ha)	Productivity (ton/ha)	Production (ton)
Paser	500.77	18.48	9252
Kutai Barat	14.85	16.90	251
Kutai Kartanegara	717.27	22.12	15868
Kutai Timur	694.12	30.37	21081
Berau	200.45	12.89	2584
Penajam Paser Utara	264.94	11.34	3004
Mahakam Ulu	-	-	-
Balikpapan	306.77	53.11	16292
Samarinda	188.28	20.10	3784
Bontang	5.28	12.12	64

Source: BPS (2015)

The average annual productivity of banana in the province (Figure 2) ranged from 20.69 ton.ha^{-1} (2013) up to 30.21 ton.ha^{-1} (2007) (BPS 2015), based on the highest attainable productivity (53.11 ton.ha^{-1}) as the standard of determination (Table 4). It meant that the average annual productivity of banana in the province only reached 38.96% and up to 56.88%. The banana productivity would be lower the well manage banana plantation, including integrated management of PPN, was considered.

Discussion

Plant-parasitic nematodes are a serious pest of agricultural ecosystems in East Kalimantan, but their existence is still neglected by farmers as well as Agricultural Government Officers. A special effort of extension and technologies dissemination for controlling this pest in provincial and districts level was still limited. However, research and field observations conducted by lecturers and students of Mulawarman University since 1990 proved that PPN was one of the important (silent) pests in East Kalimantan (Hambarwati 2004; Nur 2004; Nurmina 2006; Zulaichan 2007; Ngaini 2008; Sanjaya 2008; Sartono 2008; Septian 2011; Hidayat 2015, *unpublished data*). Population densities of plant-parasitic nematodes in agricultural ecosystems of East Kalimantan under intensive cultivation were always above the economic injury level. It was justified that plant-parasitic nematodes were causal pests that contributed to agricultural ecosystem productivity reduction.

East Kalimantan is situated in a tropical rain forest climatic zone and is considered a zone of flora and fauna diversity center, including plant-parasitic nematodes. Therefore, various genera of plant-parasitic and free leaving nematodes were observed in East Kalimantan, major plant-parasitic nematodes genera which frequently determined in agricultural ecosystem were: *Meloidogyne*, *Radhopholus*, *Rotylenchulus*, *Helicotylenchus*, *Pratylenchus*, and *Tylenchorhynchus*.

Meloidogyne was the most distributed nematode genus in East Kalimantan, this genus was the major pest on vegetable crops, and attacked both food crops and fruit crops. Genus of *Radhopholus* was the major pest of banana causing toppling disease in all banana development areas in the province. Genus of *Rotylenchulus* mostly attacked vegetables and food crops, especially those of the family *Solanaceae* and *Leguminosae*. Genera of *Helicotylenchus* and *Pratylenchus* have mostly attacked food crops of grasses family, and together with *Radhopholus* attacking banana. While *Tylenchorhynchus* seem to be adapted to perennial crops and cause serious damage to oil palm seedlings in Paser District of East Kalimantan (Suyadi 2010, *unpublished data*).

The role of plant-parasitic nematodes on the tomato productivity reduction

In relation to the role of plant-parasitic nematodes as the causal agent of tomato productivity reduction, it could be elaborated by the fact that the higher tomato yield was reached at the area of tomato production centers, such as Balikpapan city, Penajam Paser Utara and Kutai Kartanegara districts (Table 2). It was due to the impact of the organic soil amendment which is commonly applied by farmers at the area of tomato production centers. Organic soil amendments, especially animal dung, are beneficial for soil fertility improvement. It also played a role as nutrient source for natural enemies of plant-parasitic nematodes. Therefore, those natural enemies suppressed the population of plant-parasitic nematodes and can be more effective than synthetic nematicides (Aalders et al. 2009).

Tomato is one of the important vegetable crops in East Kalimantan, and tomato farmers always manage their crops intensively, including pest control activities. Essentially, the major pest of tomato was successfully solved, except for PPN - because the existence of PPN is undistinguished due to their microscopic size and wrapped in the soil particles or plant roots. However, most farmers in the province were still applying inorganic fertilizers and pesticides as the component of production inputs for their tomato crop. Consequently, the population of PPN was always above the economic injury level and caused a significant yield loss.

The impact of inorganic fertilizers and pesticides application will reduce the diversity of soil micro-flora and fauna, including those natural enemies of PPN. Whereas the surviving PPN of tomatoes will be free from their enemies, and their population will increase rapidly to reach the economic injury level. Therefore, the productivity reduction caused by the PPN cannot be rejected, and the average annual productivity of tomatoes in the province is considered low, only ranging from 24.39% (2012) up to 50.45% (2014) compared to the attainable yield (Figure 1 and Table 2). This means that the productivity reduction of tomatoes caused by PPN in East Kalimantan occurred at about 49.55% up to 75.61%. Furthermore, the productivity reduction will be higher, if the potential yield (40 ton.ha.$^{-1}$) is considered as the comparison standard, so therefore productivity reduction of tomato caused by PPN in East Kalimantan will be about 69.17% up to 85.12%.

The role of plant-parasitic nematodes on the banana productivity reduction

The opposite crop management of tomato was implemented on bananas, as most banana farmers managed their crop traditionally and they never provide any production input to their crop. They just plant the banana suckers and sometimes implement weed control, and then leave them up to harvest time. Fortunately, bananas in East Kalimantan was relatively not faced with a serious pest attack, except for the outbreak of bacterial wilt disease in early 2000 (Suyadi 2007). However, the impact of bacterial wilt disease could be easily differentiated from the PPN in the banana productivity reduction. Because eradication treatment was implemented to bananas infected by bacterial wilt.

Plant-parasitic nematodes distributed simultaneously with the distribution of planting material, because together with the suckers were carried a small amount of soil and/or banana roots that contained PPN in it. Then, those PPN will reproduce rapidly on suitable hosts. As we know that the life cycle of plant-parasitic nematodes is just about one month under suitable environmental conditions. It means, the population density of plant-parasitic nematodes in a year will almost reach the economic injury level, if natural enemies are not functioning effectively to reduce the population of PPN. Consequently, banana yield will drastically reduce at third or fourth harvest and afterward, but farmers often don't pay any attention to control the PPN. They just expect that yield reduction was due to nutrient shortage and soil fertility reduction.

In relation to the role of PPN in the reduction of banana productivity, field observations determined that PPN was the causal agent of drastic yield reduction on saba cultivar banana (the most common banana cultivar planted by farmer in East Kalimantan). At first harvest of the newly planted banana, farmers will harvest bunches of bananas with >10 combs which are composed of 18 fingers each comb on average, but from the fourth harvest farmers will only get a banana bunch with five or fewer combs and smaller fingers, if the proper management of plant-parasitic nematodes was not implemented (Swandono et al. 2009; Suyadi 2013). Based on the explanations stated above, and determination of the average annual productivity based on the attainable yield (Figure 2 and Table 4) experiencing yield loss on banana of about 61% was common in the province, because quantitatively the number of combs was reduced and qualitatively the size of fingers become smaller. The highest productivity reduction of bananas in the province could reach 78.65% (Table 4).

Just like in the tomato management case, farmers controlling PPN on banana was only indirect, as it was the impact of organic manure application as soil amendment. Then, the organic manure will supply a nutrient to the crop as well as provide a "food" for natural enemies of PPN. That could easily be distinguishable on the banana planted around the farmers housing, where those banana plants always received organic material from the household waste.

ACKNOWLEDGEMENTS

We are very grateful to the students who were involved in the field research, gathering primary data. Also, we are thankful to the Statistic Center Bureau of East Kalimantan Province for providing the secondary data.

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ASIAN JOURNAL OF AGRICULTURE
Volume 1, Number 1, June 2017
Pages: 46-51

E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g010109

Review: Genetic diversity studies using microsatellite markers and their contribution in supporting sustainable sheep breeding programs

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Manuscript received: 1 May 2017. Revision accepted: 28 June 2017.

Abstract. *Sheriff O, Alemayehu K. 2017. Review: Genetic diversity studies using microsatellite markers and their contribution in supporting sustainable sheep breeding programs. Asian J Agric 1: 46-51.* Microsatellites have been widely accepted and employed as useful molecular markers for measuring genetic diversity and divergence within and among populations. The various parameters developed so far to measure genetic diversity within and among populations are observed and expected heterozygosity (Ho and He), the mean number of alleles per locus (MNA), polymorphic information content (PIC), genetic distance and phylogenetic or tree building approach. The objective of this review was therefore to quantify the genetic diversity studies of domestic sheep populations using microsatellite markers and their contribution in supporting sustainable sheep breeding programs. From the review, it is possible to see that there were high population genetic variations in all the studied sheep populations, poor levels of population differentiation and high levels of inbreeding. On the other hand, low estimates of heterozygosity and mean number of alleles and employing only few and weak markers were observed in some of the studies. The gaps observed in the previous genetic diversity studies of the sheep populations may demand further works to reveal more information on the population structures and to start appropriate and sustainable breeding programs.

Keywords: Genetic diversity, microsatellites, sheep, sustainable breeding

Abbreviations: DA: Cavalli-Sforza genetic distance, DS: Nei's standard genetic distance, FAO: Food and agricultural organization of the united nations, F*IS*: Level of inbreeding, F*ST:* Genetic differentiation between subpopulations, He: Expected heterozygosity, Ho: Observed heterozygosity, HWE: Hardy-Weinberg equilibrium, MNA: Mean number of alleles per locus, PIC: Polymorphic information content

INTRODUCTION

Domestic farm animals are crucial for food and agriculture, providing 30 to 40 percent of the agricultural sector's global economic value (FAO 2000). Despite their invaluable contribution to the global economy, there is a rapid loss of genetic resources in farm animals and the world loses two breeds of its valuable domestic diversity every week (FAO 2000). Hence, there should be an urgent mechanism to maintain and document the diversity of livestock genetic resources and design appropriate strategies for conservation and sustainable use, particularly in developing countries (Hanotte and Jialin 2005).

Maintenance of livestock genetic diversity is a key to the long-term survival of most species and should be done based on comprehensive information regarding the structure of the populations, including sources of genetic variability within and among populations. It also requires adequate implementation of conservation priorities and sustainable management programs (Mahmoudi et al. 2011) to be widely used to categorize livestock species in the world (Cardellino and Boyazoglu 2009).

Genetic diversity (the variation of alleles and genotypes present in a population) provides a basis for adaptive and evolutionary processes (Frankham et al. 2002). The current pool of diversity in livestock has been created by the forces of both natural and artificial selection (Groeneveld et al. 2010). These forces encompass processes such as mutations, adaptations, segregation, selective breeding, and genetic drift (Groeneveld et al. 2010). Future generations of domesticated species are wholly dependent on genetic variation which will be observed from genetic differences between breeds, between populations within a breed and between individuals within a population (Groeneveld et al. 2010).

Globally, sheep have the highest number of recorded breeds, contributing 25% to the total mammalian breeds adapted to a broad range of environments (Gizaw et al. 2008). The adaptation of different breeds to a broad range of agroecology provides the necessary variability that offers opportunities to meet the increased future demands for food and provide flexibility to respond to changing markets and needs (Wollny 2003). To date, more than 1078.2 million sheep populations are kept in different parts of the world with the following share in million: Asia (452.3), Africa (287.6), Northern America (6.9), Central

America (8.1), Caribbean (3.1), South America (73.1), Europe (133.9) and Oceania (113.1) (Mahmoud, 2010).

Microsatellites have been widely accepted as useful tools for measuring genetic diversity and divergence within and among populations (FAO 2011). So far, several genetic diversity studies on sheep have been conducted using microsatellite markers (Adamov et al. 2011). Their abundance, high level of repeat-number polymorphism, manifested as the occurrence of many alleles per locus, and co-dominant inheritance has facilitated their extensive use in genome mapping, phylogenetic inference, and population genetics in farm animals (FAO 2011). However, most of the genetic diversity studies of sheep using microsatellite markers, conducted so far, may not be as supportive as expected in revealing the required information for designing appropriate and sustainable sheep breeding programs and conservation strategies. Therefore, the objective of this review was to quantify the genetic diversity studies using microsatellite markers and their contribution in supporting sustainable sheep breeding programs.

GENETIC DIVERSITY AND WITHIN POPULATION VARIATION

Some of the parameters which can help to study genetic diversity within a population are the mean number of alleles per locus (MNA), the average expected and observed heterozygosity values (Halima et al. 2012b). Additionally, testing for deviations from the Hardy-Weinberg equilibrium (HWE) per population gives insight about those primary forces viz., natural selection, mutation, genetic drift, nonrandom mating, and genetic migration that derive evolutionary change (Ojango et al. 2011). On the other hand, the precision of estimated genetic diversity is a function of the number of loci analyzed, the heterozygosity of these loci and the number of animals sampled in each population (Barker 1994).

ESTIMATION OF MEAN NUMBER OF ALLELES (MNA)

The mean number of alleles is a good indicator of the genetic polymorphism within the population (Halima et al. 2012b) and it depends on sample size of the population because of the potential presence of unique alleles in a population that may occur at low frequencies (Sithembile 2011). The number of detected alleles may increase with an increase in population size. A high number of alleles imply more genetic variation (Nei 1987). Mean number of alleles that indicate the genetic polymorphism within the studied microsatellites were reported for several sheep populations (Table 1).

The mean number of alleles (MNAs) (Table 1) showed relatively lower estimates for some Ethiopian, Chinese, and South African sheep populations. For the other sheep populations, relatively encouraging estimates of MNA were reported. A high number of alleles imply more genetic variation (Nei 1987), and it is the key relevance in conservation programs. However, though those reports explain the existence of high polymorphism, the average number of alleles depends on sample size; number of observed alleles tends to increase with increasing population size (Aljumaah et al. 2012). Therefore, it is important to compare sample population sizes that are close to equal (Sithembile 2011). However, some of the studies used not only very small number of animals which is quite far from FAO recommendation for microsatellite marker analysis (FAO 2011), e.g., Hirbo et al. 2006 used only 9 animals to represent a population, but also, they used unequal sample size. This may lead to biasedness in estimating genetic parameters such as HWE and MNA, additionally, there was not any technique indicated in the papers which were employed to handle such a limitation.

It was observed that most of the sheep genetic diversity studies (Table 1) were undertaken by using few numbers of microsatellite markers. All 30 microsatellites, the maximum coverage recommended by FAO (2011), were covered only for Merino derived and Albanian sheep breeds (Ceccobelli et al. 2009; Hoda and Marsan 2012). Genetic diversity studies with a greater number of microsatellite markers, not only reveal more information on the population structures but also offer more opportunities to compare with results from previous studies undertaken with various subsets of the markers (FAO 2011).

ESTIMATION OF OBSERVED (*H*O) AND EXPECTED (*H*E) HETEROZYGOSITIES

Observed heterozygosity, the proportion of heterozygotes observed in a population, and expected heterozygosity, the percentage of loci heterozygous per individual or the number of individuals heterozygous per locus (Ojango et al. 2011) are the most widely used parameters to measure genetic diversity in a population (Toro et al. 2009). Literature suggests that heterozygosity estimates having greater than 0.5 heterozygosity values are believed to be appropriate for genetic diversity studies (Davila et al. 2009; Dorji et al. 2012). However, the heterozygosity estimates observed in some Indian, South African, Ethiopian, Chinese, Chilean, Kenyan and Nigerian sheep populations (Table 1) were below 0.5 or closer to the margin. These low heterozygosity estimates might be due to maintaining microsatellite loci that had registered values below 0.5 in the respective breeds during the analysis. On the other hand, very low heterozygosity estimates maybe because of the effect of small population size, high selection pressure in closed population, inbreeding and minimal or null immigration of new genetic materials into the population (Canon et al. 2006). The heterozygosity (both observed and expected) estimates in the remaining sheep populations are relatively high, concluding that the studied sheep populations have high amount of within-population genetic diversity.

Table 1. Estimated heterozygosity, mean number of alleles, polymorphic information content and level of inbreeding

Breed	**Country of origin**	***He***	***Ho***	**MNA**	**PIC per locus**	***F*IS**	**MS (No.)**	**Author**
Vembur sheep	India	0.73	0.52	5.88	0.69	0.29	25	Pramod et al. (2011)
Kail sheep	India	0.72	0.77	5.27	0.60	0.053	11	Ahmed et al. (2014)
sheep breeds (7)	South Africa	0.63	0.45	5-16	0.95	NA	24	Buduram (2004)
Turkish breeds (4)	Turkey	0.87	0.66	7.04	NA	0.07	17	Yilmaz et al. (2015)
Turkish native and cross sheep (11)	Turkey	0.75	0.72	5.8-11.8	NA	0.09-0.16	15	Evren (2004)
traditional sheep populations (14)	Ethiopia	0.66-0.75	NA	6.79	NA	*F*ST (0.046)	17	Gizaw (2008)
sheep breeds (3)	Ethiopia	0.50	0.33	3-23	0.69	0.236	22	Nigussie (2015)
Italian merino derived sheep (3)	Italy	0.64-0.75	0.61-0.70	5.17-8.43	NA	0.048-0.118	30	Ceccobelli et al. (2009)
Pelt sheep (3)	Iran	0.83	0.99	7.6	0.81	-0.19	15	Hatami et al. (2014)
Local Sheep (8)	China	0.54	0.59	3.8-5.4	0.49	0.404	10	Zeng et al. (2010)
Albanian Sheep (3)	Albania	0.75	0.72	8.54	0.72	0.041	31	Hoda and Marsan (2012)
Chilean sheep (4)	Chile	0.82	0.696	9-25	0.55-0.90	0.040	9	De la Barra et al. (2010)
Sheep populations (15)	Kenya	0.72	0.65	7.70	NA	0.109	15	Mukhongo et al. (2014)
Nigerian Indigenous Sheep (4)	Nigeria	0.78	0.49	8.64	0.85	0.34	15	Brilliant et al. (2012)
Sheep breeds (3)	Saudi Arabia	0.59-0.82	0.65-0.989	11.47	0.75	0.031	17	Mahmoud et al. (2017)
Trans-caucasian, Asian, European and African sheep breeds (22)	*	0.62-0.81	0.60-0.77	6.71-9.36	NA	*F*ST (0.06-0.10)	14	Hirbo et al. (2006)
Karakul sheep	Iran	0.831	0.989	8.07	0.81	-0.197	15	Nanekarani et al. (2011)
Romanian sheep breeds (4)	Romania	0.740	0.640	9.275	NA	NA	11	Emil and Marieta 2012)
NamaquaAfrikaner (3)	South Africa	0.50	0.49	3.9	0.44	0.019	22	Sithembile (2011)
Sheep breeds (10)	**	0.74	0.59	5.4-6.0	NA	0.060	10	Farid et al. (2000)

Note: MS = Microsatellite; * Azerbajan (5), Armenia (3), Georgia (2), Uzbekistan (1), Pakistan (2), Syria (1), China (1), India (1), Portugal (2), Barbados (1), UK (2) and Senegal (1); ** Canada, Iceland, USA, Denmark, UK and Kenya

Most of the observed heterozygosity values are generally closer to, but lower than, the expected heterozygosity in most of the breeds and loci indicating no overall loss in heterozygosity (allele fixation) (Araujo et al. 2006) and the populations are at Hardy-Weinberg equilibrium (HWE).

ESTIMATION OF POLYMORPHIC INFORMATION CONTENT (PIC)

Polymorphic information content (PIC) depicts the suitability of the markers and their primers used in the study for analyzing the genetic variability of a population. A marker with PIC>0.5 can be considered as highly informative and highly polymorphic, whereas 0.5>PIC>0.25 is recognized as reasonably informative and below 0.25 is measured as slightly informative (Marshall et al. 1998). In line with this, highly polymorphic markers were employed for most of the sheep populations studied (Table 1) except the local sheep breeds in China PIC=0.492. In fact, PIC is determined by heterozygosity and number of alleles (Aljumaah et al. 2012) and this makes microsatellite markers the choice for genetic characterization and diversity studies.

LEVEL OF INBREEDING (*F*IS)

*F*IS is estimated for populations that show significant deviation from the HWE and are significant for significant HWE estimation (Ojango et al. 2011). A high positive *F*IS indicates a high degree of homozygosity and vice versa, while negative values indicate low level of inbreeding (Dorji et al. 2012). Taking this background information into consideration, moderate to high inbreeding levels were reported by various scholars for different sheep populations; for instance, three sheep breeds of Ethiopia (*F*IS=0.236) (Nigussie 2015), Vembur (*F*IS=0.29) (Pramod et al. 2011), Magra (*F*IS=0.159) (Arora and Bhatia 2006) and Kheri (*F*IS=0.128) (Arora and Bhatia 2006)sheep breeds of India, some Merino derived sheep breeds of Italy (*F*IS=0.048-0.118) (Ceccobelli et al. 2009), some Turkish sheep breeds (*F*IS=0.09-0.16) (Evren 2004) eight local sheep breeds of China (*F*IS=0.404) (Zeng et al. 2010), fifteen sheep populations of Kenya (*F*IS=0.109) (Mukhongo et al. 2014) and Nigerian indigenous sheep (*F*IS=0.34) (Brilliant et al. 2012). This might be because of the small sheep population size, closed breeding system and very limited number of breeding rams used for many consecutive years. The lowest heterozygosity and MNA estimates indicated in table 1 above strengthen this justification.

However, tolerable mean values of *F*IS (0.087) for Ganjam (Arora et al. 2010), (0.0525) for Kail (Ahmed et al. 2014) and (0.0786) for Tamil Nadu (Kavitha et al. 2010) sheep breeds of India and *F*IS (0.07) for Turkish breeds (Yilmaz et al. 2015) were reported by scholars. These moderate levels of inbreeding may be a result of moderate levels of mating between closely related individuals under field conditions and maybe the uncontrolled and unplanned mating that caused high levels of inbreeding (Mekuriaw et al. 2016). On the contrary, *F*IS (-0.19) (Hatami et al. 2014) and *F*IS (-0.197) (Nanekarani et al. 2011) depict low levels of inbreeding and an excess of heterozygotes was reported for three Iranian sheep breeds and Karakul sheep breed of Iran, respectively.

GENETIC DISTANCE AND VARIATION AMONG POPULATIONS

Kalinowski (2004) had suggested that the highest genetic distance (*F*ST) be higher than 0.25, moderate to be between 0.05 and 0.25 and the lowest estimate below 0.05. In relative to many reports, the genetic distance among most of the populations obtained by many of the scholars (Farid et al. 2000 (*F*ST=0.163); Evren 2004 (*F*ST=0.002-0.146); Hirbo et al. 2006 (*F*ST=-0.001-0.183); Sithembile 2011 (*F*ST=0.105); Brilliant et al. 2012 (*F*ST=0.088); Hoda and Marsan 2012 (*F*ST=0.011); Hatami et al. 2014 (*F*ST=0.018); Mukhongo et al. 2014 (*F*ST=0.101) and Mahmoud et al. 2017 (*F*ST=0.042)) is almost negligible (<0.05) and/or moderate (0.05<*F*ST<0.25) values. Some of the authors revealed significant genetic distance estimates among populations. This implies that there is relatively low to moderate genetic sub-differentiation among the sheep populations. A fixation index (*F*ST) of about 0.15 is an indication of significant differentiation among populations (Frankham et al. 2002).

The average *F*ST value overall microsatellite loci in the sheep populations in Ethiopia was reported to be 0.046, indicating a 4.6% of total genetic variation among populations and a 95.4% difference among individuals (Gizaw 2008). The same author reported that lack of differentiation in those phenotypically different sub-populations could be due to gene flow between the areas having close geographical distance and similar ecology. Similarly, Nigussie (2015) noted that 3% of the total variation occurred due to population subdivision, while 97% of the variation existed among individuals within the sheep populations, which might be due to migration of individuals from one sub-population to the other (Nigussie 2015). Hailu et al. (2008) and Halima et al. (2012b) also confirmed that the low genetic differentiation between sub-populations might be due to traditional uncontrolled mating practices and policies that facilitated or led to uncontrolled movement of animals through various market routes and agricultural extension systems in Ethiopia.

IDENTIFIED GAPS, THEIR IMPLICATIONS AND FUTURE PROSPECTS

One of the gaps, identified so far, is related to the expected and observed heterozygosity estimates and microsatellite loci. It is generally suggested that microsatellite loci showing *H*e and *H*o estimates of less than 0.5 were not appropriate for heterozygosity evaluation. However, microsatellite loci with heterozygosity estimate less than 0.5 or close to that were used in some of the studies (Table 1).

Similarly, though FAO (2011) recommended the genetic diversity studies of livestock using all the 30 microsatellite markers, most of the sheep genetic diversity studies were undertaken by using a subset of the markers. For example, De la Barra et al. (2010) used only 9 microsatellites to study four Chilean sheep breeds and Farid et al. (2000) used only 10 microsatellites to study ten sheep populations in Canada, Iceland, USA, Denmark, UK, and Kenya. Hence, studying a greater number of microsatellite markers to reveal more information on the population structure is suggested in future sheep genetic diversity studies. If less than 30 microsatellites are to be used, it is important to be keen in selecting microsatellites to bring an appropriate recommendation that can support sustainable breeding strategies.

The mean number of alleles (MNA) in sheep genetic diversity studies in Ethiopia, China, and South Africa (Zeng et al. 2010; Sithembile 2011; Nigussie 2015) were below the recommended value, the microsatellite loci for genetic diversity studies should have more than four alleles (FAO 2011). This indicated that some of the microsatellite loci were not sufficiently polymorphic and were not appropriate for genetic diversity analysis.

Some of the diversity studies used not only very small number of sheep which is by far lower than the recommendation of FAO for microsatellite marker analysis (FAO 2011), e.g., Hirbo et al. 2006 used only 9 animals to represent a population, but also, they used unequal sample size. This may lead to biasedness in estimating genetic parameters such as the MNA, there was not any technique indicated in the papers which were employed to handle such a limitation.

All these gaps point out that the microsatellites which were not sufficiently polymorphic could be dropped out and it is very important to be ardent in selecting them to bring the right recommendation that can support appropriate and sustainable sheep breeding programs.

CONCLUDING REMARK

The results from this review indicated that the within population genetic diversity, in all sheep populations, is extremely higher than between population variation which might be due to the uncontrolled and random mating practiced among the breeding flocks having close geographical distance and similar ecology. There was also poor level of population differentiations, high levels of inbreeding, low estimates of heterozygosity and MNA and markers which were not sufficiently polymorphic in most of the studies. All these results demand further works to reveal more information on the sheep population structures and help to start sustainable breeding programs and policies involving the decision on pure or crossbreeding. Moreover, appropriate conservation activities on breeding farms must be taken to avoid losses of genetic diversity and thereby to support the breeding programs. It is also suggested to set up an improvement scheme for the frequent exchange of rams among farms or flocks rearing the same breed, aimed to increase genetic diversity.

ACKNOWLEDGEMENTS

The first author gratefully acknowledges the Federal Ministry of Education, Ethiopia, Assosa and Bahirdar Universities for the Ph.D. fellowship award.

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