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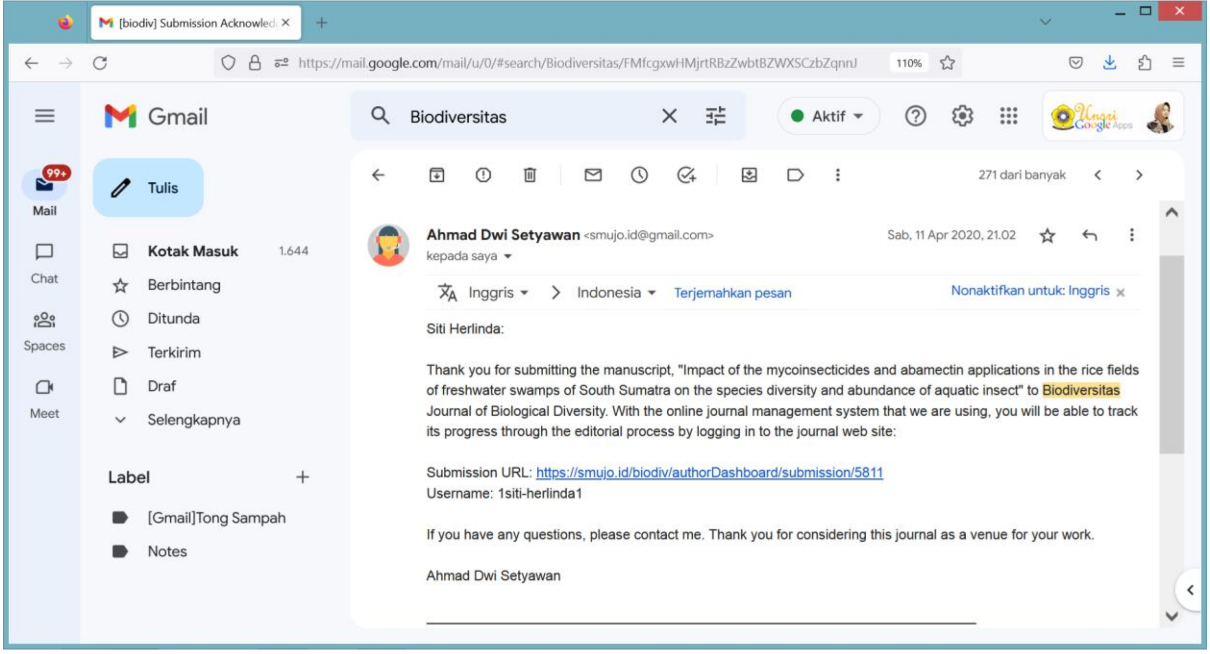

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Dear **Editor-in-Chief,**

I herewith enclosed a research article,

Title:

Impact of the mycoinsecticides and abamectin applications in the rice fields of freshwater swamps of South Sumatra on the species diversity and abundance of aquatic insect

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This study highlights several findings, such as species of aquatic insects found in freshwater swamp rice in South Sumatra, Indonesia are all predatory insects for insect pests of rice. The species diversity and abundance of the aquatic insects inhabiting rice field treated with mycoinsecticides ((active ingredients of conidia *B. bassiana*, *M. anisopliae*, and *Cordycep militaris*) are higher than those on the rice field treated with commercial insecticide (abamectin). So, we found that the three mycoinsecticides are non-lethal against aquatic insects.

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Sincerely yours,

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Impact of the mycoinsecticides and abamectin applications in the rice fields of freshwater swamps of South Sumatra on the species diversity and abundance of aquatic insects

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Abstract. Aquatic insects in rice fields generally act as predators of rice insect pests. The application of insecticides has the opportunity to reduce the abundance and species diversity of these aquatic insect. This study aimed to analyze the effect of the application of mycoinsecticides (active ingredients of conidia *B. bassiana*, *M. anisopliae*, and *Cordycep militaris*) and abamectin on the species diversity and abundance of aquatic insects in rice fields. The experiment was carried out in the rice field applied with the mycoinsecticides of *B. bassiana, M. anisopliae,* and *Cordycep militaris* and abamectin. The eight aquatic insects found were Dytiscidae (unknown species), *Micronecta* sp., *Mesovelia* sp., *Ranatra* sp., *Anisops* sp., *Microvelia* sp., Veliidae (unknown species), and *Orthetrum* sp. The species came from the three orders (Coleoptera, Hemiptera, and Odonata) with 7 families (Dytiscidae, Corixidae, Mesoveliidae, Nepidae, Notonectidae, Veliidae, Libellulidae) and they were predatory insects. The application of the mycoinsecticides did not reduce the abundance and species diversity of the aquatic insect, but the abamectin could reduce them. There were only 5 species of aquatic insects found in the abamectin plot, whereas in *B. bassiana* plot there found 6 species, plot *M. anisopliae* 6 species, and *C. militaris* plot 7 species of aquatic insects. Therefore, the application of *B. bassiana*, *M. anisopliae*, and *C. militaris* to control rice insects is much safer for aquatic insects than that of abamectin.

Key words: *Beauveria bassiana, Cordyceps militaris*, *Metarhizium anisopliae*, predatory insects

Abbreviations (if any): -

Running title: Impact of mycoinsecticides on aquatic insect

INTRODUCTION

There is a swampy area of 39.6 Mha in Indonesia which has the potential for agriculture, some of which (11.9 Mha) is located in Sumatra Island (Margono et al. 2014) which is generally dominated by freshwater swamps. A freshwater swamp is a wetland ecosystem that is flooded with river water or rain throughout the year. The duration of the stagnant water depends on the typology of the land. The results of our observations of the last 7 years in Ogan Ilir District of South Sumatra, Indonesia showed that the deep freshwater swampy typology generally flooded most of the year (October to July), middle freshwater swamps flooded from November to June, and embankment freshwater swamps or shallow freshwater swamps flooded from November to April. Having the condition of stagnant water, the smallholder farmers underutilized their lands and approaching the dry season they started to grow rice or adaptive vegetables.

Crops cultivated in various typologies of the freshwater swamps vary depending on their adaptation to stagnant water. Rice is most widely planted in the South Sumatra frershwater swamps (Lakitan et al. 2018a, b; Prabawati et al. 2019). Besides rice, chilies (Johari et al. 2014, 2016), bitter melon, long beans, eggplants, pumpkins and cucumbers are also cultivated (Siaga et al. 2019). Rice is generally planted once a year (Herlinda et al. 2018a), but currently there are business farmers who have a large piece of land (above 100 ha) starting to plant rice twice to three times a year. There are also smallholder farmers who grow rice twice a year by utilizing the remaining ratoons of the previous season's rice harvest (Lakitan et al. 2018b; Prabawati et al. 2019).

The intensive rice cultivation in the freshwater swamps will increasingly raise the use of insecticides in the rice fields due to the increasing insect population (Prabawati et al. 2019). The commonly used insecticides are synthetic insecticides (Herlinda et al. 2018a) and sometimes bioinsecticides (Karenina et al. 2019; Prabawati et al. 2019). The insecticide which is commonly used in rice fields in Indonesia is abamectin (Luo et al. 2013). Bioinsecticides currently use many active ingredients of entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* (Prabawati et al. 2019), which are referred to as mycoinsecticides.

B. bassiana and *M. anisopliae* are widely used because they have been proven to be easily found in freshwater swamps (Herlinda et al. 2018b; Safitri et al. 2018) and be able to kill important insect pests such as brown planthopper (Sumikarsih et al.. 2019) and *Spodoptera litura* (Ayudya et al. 2019). Although, the entomopathogenic fungi are reported to be host-specific (Farid and Syarief, 2018), it is necessary to monitor the impact of these mycoinsectides on the abundance of non-target arthropods.

There is a lot of information on the impact of synthetic insecticide applications on non-target arthropods has a lot of information (Azod et al. 2016; Khan et al. 2018). However, there is not much information on the impact of synthetic insecticide and mycoinsectides application on aquatic insects. In fact, the rice field water is generally dominated by predatory insects (Thirumalai et al. 2002). The abundance and diversity of non-target insect species in rice field water is high (Settle et al. 1996; Herlinda et al. 2019a,b). The commonly found water insects include *Microvelia douglasi*, *Mesovelia vittigera*, *Anisops barbatus*, *Ranatra elongata*, and *Micronecta scutellaris* (Thirumalai et al. 2002). These water insects play an important role in the aquatic ecosystem (Dunbar et al. 2010) as natural enemies of rice insect pests (Heong et al. 2009). The intensive application of the insecticides and mycoinsectides is likely to have an impact on the abundance and species diversity of the aquatic insect. The high abundance and species diversity of arthropod in water is an indicator of water health (Salachna and Olearczyk 2020). This study aimed to analyze the effect of the application of mycoinsecticides of *B. bassiana, M. anisopliae,* and *Cordycep militaris* and abamectin on the species diversity and abundance of rice water insects.

MATERIALS AND METHODS

Study area

The field experiment was carried out at the freshwater swampy rice field center in the *Pelabuhan Dalam* Village of Pemulutan Subdistrict, Ogan Ilir District of South Sumatra Province. The area of freshwater rice fields in this study site was more than 800 ha. The area of the experimental land was 1 ha made into subplots for 4 treatments and designed using a randomized block design that was repeated 3 times (a total of 12 subplots). The field trials were conducted from May to August 2018 and then it continued with the identification until December 2018. The four treatments were *B. bassiana, M. anisopliae, C. militaris,* and Abamectin as controls. The *B. bassiana* and *M. anisopliae* isolates were based on the result of the exploration of Safitri et al. (2018), while the *C. militaris* was originated from Central Kalimantan. The identification of the three entomopathogenic fungal species used in this experiment was carried out by Dr. Suwandi (a mycologist from Universitas Sriwijaya).

Preparation of land and rice planting

The rice cultivation activities were carried out with tillage, seed preparation, planting and maintenance. Soil processing was carried out using a *singkal* plow machine and then the plowed land is smoothed using a hoe while running water using a pump so the soil becomes muddy. After the soil was cultivated, the rice fields were added with a dose of 1 ton ha^{-1} and flooded with water for 14 days before planting.

Seed preparation followed the habits of local farmers and began with seed preparation, seed treatment, seed curing, and seed sowing. The seeds prepared were the certified seeds with purple color labels and named after Mekongga variety. The dose of rice seeds used was 50 kg ha^{-1}. The seeds were then treated with shrimp shell flour compost at a dose of 0.5 mL L^{-1} water and soaked for 24 hours. The shrimp shell flour compost extract was made following the method of Suwandi et al. (2012). Then, the seeds were cultivated in moist conditions for 24 hours. When the seeds broke, it indicated the beginning of the germination. The seeds were sown by sprinkling them on the rice field dike coated with rice mud and moist manure. This seedling method was called *samir*.

Rice planting and maintenance followed the habits of local farmers, but rice maintenance in this experiment used mycoinsectides and abamectin according to the treatment, while weeding was mechanically without using herbicides. Watering using pumping and fertilizing systems using manure were carried out and every 2 weeks until the milky stage (milk-cooked phase) it was sprayed with shrimp shell flour compost extract at a dose of 2 L ha^{-1} to increase soil fertility. The rice was planted using a *legowo* row of 2:1 planting system with a spacing of 12.5x50x50 cm^{3}. It was conducted by transplantation, i.e. when the seedlings aged 7-10 days in the nursery, and then the seedlings were transplanted to the rice fields with a maximum water level of half of the height of the rice seedlings. The higher the rice plants were, the more water was pumped into the fields. This was carried out to accommodate and maintain the presence of aquatic insects inhabiting the rice field water. The rice field was inundated until nearing the ripe phase of milk and after the grains of rice were at the ripening stage, the fields began to be dried and the experiment was stopped.

Production and spraying of mycoinsectides and abamectin

Mycoinsectides used for the treatment were made in 3 kinds, each of which had active ingredients of conidia *B. bassiana, M. anisopliae,* and *C. militaris* and for the control it used commercial insecticides made from abamectin. The making of the mycoinsecticides was modified using the method of Mascarin et al. (2015) which began with the breeding of entomopathogenic fungi (entomopathogenic fungi) in solid media, and then when it had grown, it was cultured into the liquid media. The culture in this liquid media was used as the active ingredient of mycoinsectides. The cultivation of entomopathogenic fungi in solid media used Sabouraud Dextrose Agar (SDA, Merck) and they were incubated for 10 days. After that, they were transferred to liquid media, Sabouraud Dextrose Broth (SDB, Merck). In SDB media, the entomopathogenic fungi culture was shaken up for 7 days at 120 rpm. The liquid culture of entomopathogenic fungi with a minimum density of $1x10^9$ conidia mL^{-1} was used for the active ingredient of mycoinsecticides. The mycoinsecticides were made from a mixture of 600 mL of liquid entomopathogenic fungal culture, 400 mL carrier made from shrimp shell flour compost extract, 10 mL vegetable oil, and 10 mL of sterile aqueous solution of 0.04% polyoxyethyl-189 ene sorbitan mono-oleate (Tween®). The mycoinsectides were applied at a dose of 2 L ha^{-1} every two weeks, starting on the 14th, 28th , 42nd, and 56th days after transplanting (DAT), while the abamectin dosage was applied based on the instructions on the package and the spraying schedule together with the mycoinsecticides spraying.

Sampling of aquatic insects

The day after the mycoinsectides and abamectin were sprayed on the rice field, the sampling of water insects was carried out, that is, when the rice was on the 15th, 29th, 43rd, and 57th DAT. The first sampling was carried out on the 2nd of June 2018, the second was carried out on 16th June 2018, the third was carried out on 30th June 2018, and the fourth was carried out on 17th July 2018, while the next sampling was not carried out because the rice fields began to dry. The sampling of aquatic insects used the method of Salmah et al. (2017) using a fish net (0.4 mm mesh) and carried out in the morning at 6.00-7.00 a.m.

One sample was carried out by swinging the net in rice field water with a 2x2 m^2 sampling area for each treatment and repeated three times. The obtained insects were put into vial bottles filled with 96% ethanol and then taken to the laboratory for identification. The taxonomic identification of aquatic insects used morphological features using those of Basu et al. (2016) and Miller and Bergsten (2016) and it was performed up to the genus level.

Data analysis

The data of the number of each individual species were used to calculate the abundance and species diversity. The different data of the abundance among the treatments were analyzed using analysis of variance (ANOVA). If the differences among the treatments were found, the analysis continued using the Tukey's Honestly Significant Different (HSD) test at 5%. The calculations in the analysis were aided by the software program of SAS University Edition 2.7 9.4 M5. Analysis of the species diversity was carried out using the Shannon index (H'). The diversity level was also evaluated by the Evenness index (J') derived from the Shannon function and Berger-Parker dominance biodiversity indices.

RESULTS AND DISCUSSION

Aquatic insect species

The aquatic insect species obtained from all experimental plots were 8 species, namely Dytiscidae (unknown species), *Micronecta* sp., *Mesovelia* sp., *Ranatra* sp., *Anisops* sp., *Microvelia* sp., Veliidae (unknown species), *Orthetrum* sp. (Figure 1). These eight species came from 3 orders (Coleoptera, Hemiptera, and Odonata) with 7 families (Dytiscidae, Corixidae, Mesoveliidae, Nepidae, Notonectidae, Veliidae, Libellulidae) (Table 1). The number of species found in the plot applied by *B. bassiana* was 6 species (Dytiscidae the unknown species, *Micronecta* sp., *Mesovelia* sp., *Anisops* sp., *Microvelia* sp., and *Orthetrum* sp.) and in the *M. anisopliae* plot there were 6 species (Dytiscidae the unknown species, *Micronecta* sp., *Mesovelia* sp., *Microvelia* sp., Veliidae the unknown species, and *Orthetrum* sp.). In the *C. militaris* plot there were 7 species (Dytiscidae the unknown species, *Micronecta* sp., *Mesovelia* sp., *Anisops* sp., *Microvelia* sp., Veliidae the unknown species, and *Orthetrum* sp.). While in the abamectin plot there were only 5 species found (*Mesovelia* sp., *Ranatra* sp., *Anisops* sp., *Microvelia* sp. and *Orthetrum* sp.). The guilds of the 8 species found were all predators of rice insect pests.

Abundance and diversity of aquatic insect species

The abundance of aquatic insects tended to increase in plots applied with the mycoinsectides (*B. bassiana, M. anisopliae,* and *C. militaris*), but in the plots applied with abamectin they did not increase (Figure 2). The plots applied with *C. militaris* had the most abundant insects, while the plots applied with the abamectin had the lowest abundant insects. The abundance of each species did not show a significant difference, but the accumulated (total) abundance of each species showed that there were significant differences among the treatments. The abundance of *Micronecta* sp. was the highest found in plot *C. militaris* (37.67 ind 4 m^{-2}) but significantly different from that of plots *B. bassiana* (30.33 ind 4 m^{-2}) and *M. anisopliae* (30.33 ind 4 m^{-2}) and abamectin (0 inv 4 m^{-2}) (Table 2). The plot applied with abamectin showed a significant decrease of *Micronecta* sp.

There were three orders found in the mycoinsecticide plots are 3 orders (Coleoptera, Hemiptera, and Odonata), while in the abamectin plot there were only 2 orders found (Hemiptera, and Odonata) (Figure 3). The most dominant order found in all plots was Hemiptera. There were differences in species composition in the plots applied with mycoinsecticides and abamectin (Figure 4). The species composition in the three mycoinsecticide plots had the same tendency, whereas in the abamectin plot it had a different species composition. The *Micronecta* sp. was not found in the plots applied with abamectin, while in the plots applied with mycoinsectides the *Micronecta* sp. found to be the most dominant. The dominance of an insect species or the absence of an insect species can be an indicator of the health status of water.

During one rice season, an increase in diversity of aquatic insect species coincided with an increase in the age of rice. During one rice season, the species diversity in the plots that were applied with mycoinsecticides was higher than those applied with abamectin (Table 3). In the rice at 15th and 29th DAT, the species diversity was highest in the plots applied with *C. militaris*, and then followed by those applied with *B. bassiana*. In the rice at 43rd – 57th DAT, the species diversity was the highest in the plots applied with *B. bassiana*.

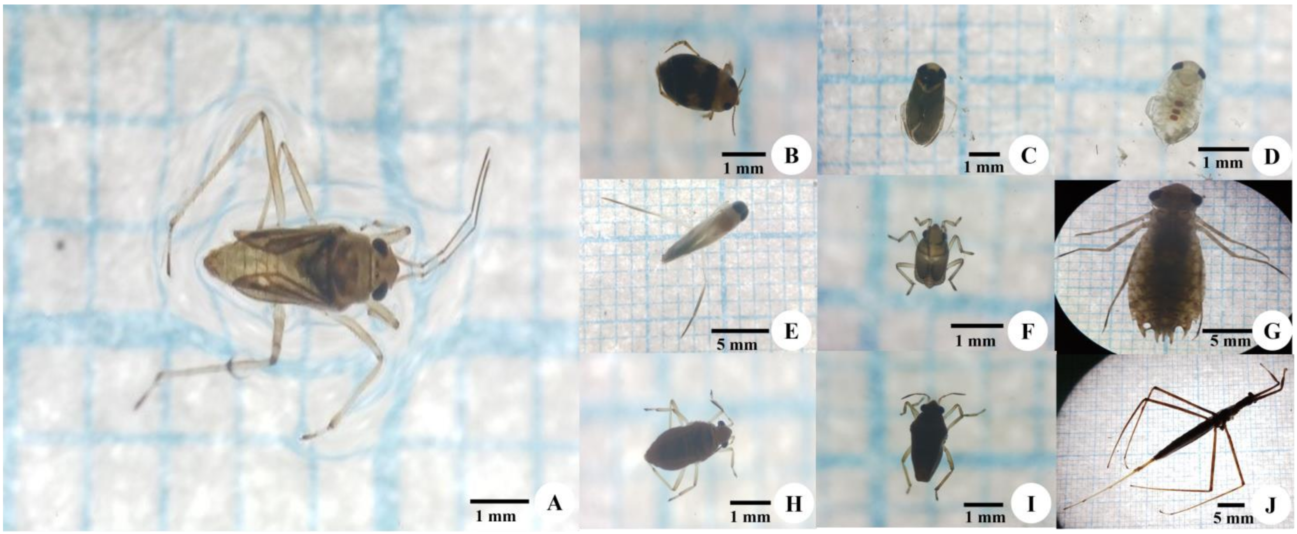

Figure 1. Aquatic insect species found during one planting season of rice: *Mesovelia* sp. (A), Dytiscidae unknown species (B), *Micronecta* sp. adult (C), *Micronecta* sp. nymph (D), *Anisops* sp. (E), Veliidae unknown species (F), *Orthetrum* sp. (G), *Microvelia* sp. (H and I), *Ranatra* sp. (J)

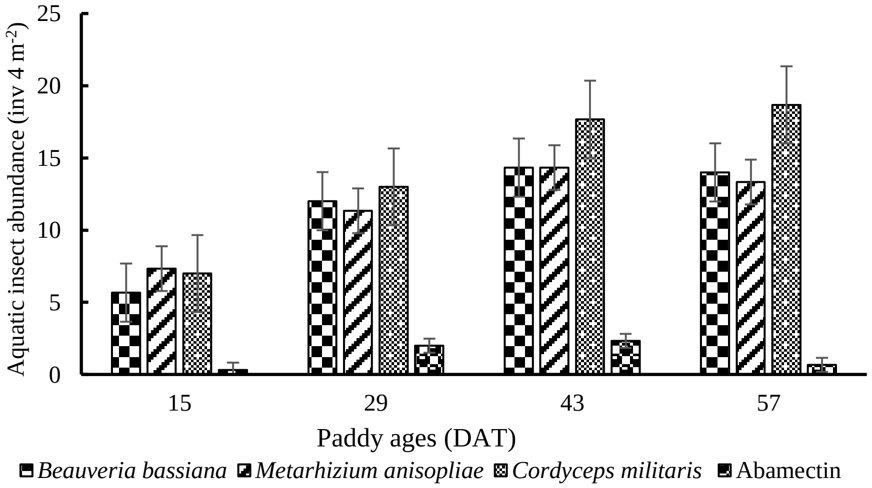

Figure 2. Aquatic insect abundance in rice field applied with bioinsecticides of *Beauveria bassiana, Metarhizium anisopliae, Cordyceps militaris*, and abamectin

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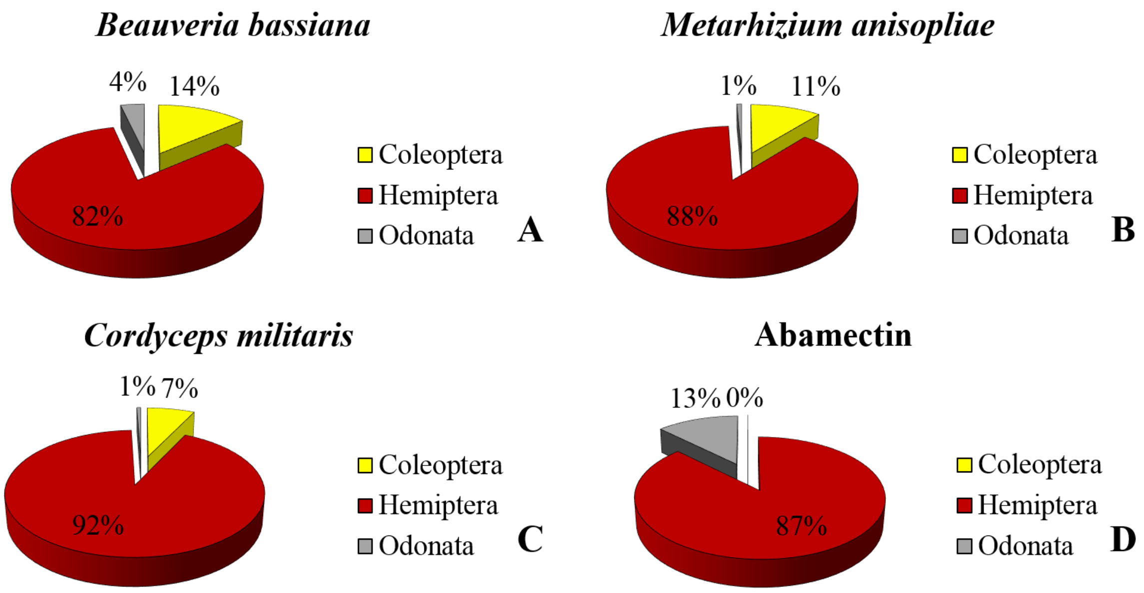

Figure 3. Proportion of aquatic insect in rice field applied with bioinsecticides of *Beauveria bassiana* (A), *Metarhizium anisopliae* (B), *Cordyceps militaris* (C), and abamectin (D) based on their orders

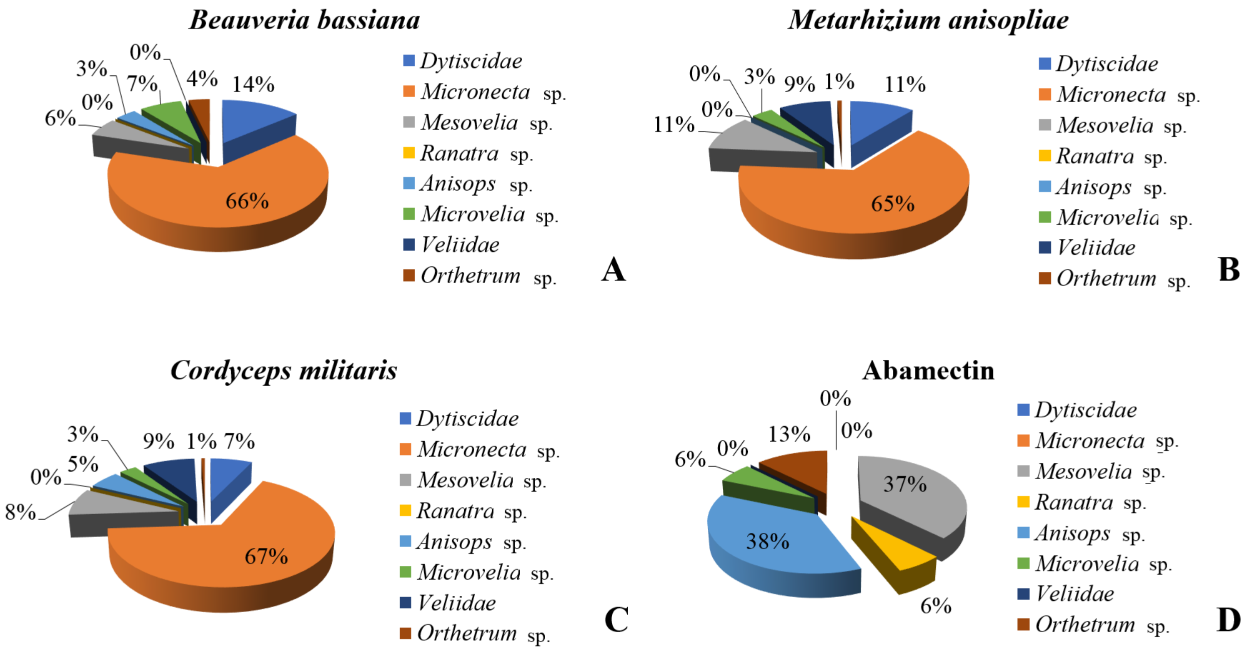

Figure 4. Proportion of aquatic insect in rice field applied with bioinsecticides of *Beauveria bassiana* (A), *Metarhizium anisopliae* (B), *Cordyceps militaris* (C), and abamectin (D) based on their species

Table 1. Order, family, and species of aquatic insect found in rice field applied with bioinsecticides of *Beauveria bassiana*, *Metarhizium anisopliae*, *Cordyceps militaris*, and abamectin

Order	Family	Species	Insecticide treatment			
			Beauveria bassiana	***Metarhizium anisopliae***	***Cordyceps militaris***	**Abamectin**
Coleoptera	Dytiscidae	Unknown species	+	+	+	-
Hemiptera	Corixidae	*Micronecta* sp.	+	+	+	-
	Mesoveliidae	*Mesovelia* sp.	+	+	+	+
	Nepidae	*Ranatra* sp.	-	-	-	+
	Notonectidae	*Anisops* sp.	+	-	+	+
	Veliidae	*Microvelia* sp.	+	+	+	+
		Unknown species	-	+	+	-
Odonata	Libellulidae	*Orthetrum* sp.	+	+	+	+

Note: + insects found, - no insects found

Table 2. Abundance of aquatic insect species found in rice field applied with bioinsecticides of *Beauveria bassiana*, *Metarhizium anisopliae*, *Cordyceps militaris*, and abamectin

Rice age (DAT)	Species of aquatic insect	Abundance of aquatic insect species (inv 4 m^{-2})				F value	P value	Tukey's HSD test
		Beauveria bassiana	***Metarhizium anisopliae***	***Cordyceps militaris***	**Abamectin**			
15 DAT	Dytiscidae	0.00	0.00	0.00	0.00	-	-	-
	Micronecta sp.	5.33	6.33	5.67	0.00	0.25	0.86	-
	Mesovelia sp.	0.00	0.67	0.67	0.00	0.57	0.65	-
	Ranatra sp.	0.00	0.00	0.00	0.00	-	-	-
	Anisops sp.	0.00	0.00	0.00	0.00	-	-	-
	Microvelia sp.	0.00	0.00	0.67	0.33	0.62	0.63	-
	Veliidae	0.00	0.00	0.00	0.00	-	-	-
	Orthetrum sp.	0.33	0.33	0.00	0.00	0.57	0.65	-
29 DAT	Dytiscidae	0.00	0.00	0.00	0.00	-	-	-
	Micronecta sp.	11.67	7.00	6.00	0.00	1.51	0.30	-
	Mesovelia sp.	0.00	4.33	4.00	2.00	0.41	0.75	-
	Ranatra sp.	0.00	0.00	0.00	0.00	-	-	-
	Anisops sp.	0.00	0.00	3.00	0.00	1.00	0.45	-
	Microvelia sp.	0.00	0.00	0.00	0.00	-	-	-
	Veliidae	0.00	0.00	0.00	0.00	-	-	-
	Orthetrum sp.	0.33	0.00	0.00	0.00	1.00	0.45	-
43 DAT	Dytiscidae	3.00	2.67	1.33	0.00	0.78	0.55	-
	Micronecta sp.	7.00	10.00	11.33	0.00	1.80	0.25	-
	Mesovelia sp.	0.00	0.00	0.00	0.00	-	-	-
	Ranatra sp.	0.00	0.00	0.00	0.00	-	-	-
	Anisops sp.	1.67	0.00	0.00	2.00	0.57	0.65	-
	Microvelia sp.	1.67	1.67	0.00	0.00	1.00	0.45	-
	Veliidae	0.00	0.00	5.00	0.00	1.00	0.45	-
	Orthetrum sp.	1.00^{c}	0.00^{a}	0.00^{a}	0.33^{b}	8.00*	0.02	0.22
57 DAT	Dytiscidae	3.33	2.33	2.67	0.00	1.33	0.35	-
	Micronecta sp.	6.33	7.00	14.67	0.00	1.54	0.30	-
	Mesovelia sp.	2.67	0.00	0.00	0.00	1.00	0.45	-
	Ranatra sp.	0.00	0.00	0.00	0.33	1.00	0.45	-
	Anisops sp.	0.00	0.00	0.00	0.00	-	-	-
	Microvelia sp.	1.67	0.00	1.00	0.00	0.59	0.64	-
	Veliidae	0.00	4.00	0.00	0.00	3.50	0.09	-
	Orthetrum sp.	0.00	0.00	0.33	0.33	0.57	0.65	-
Total	Dytiscidae	6.33	5.00	4.00	0.00	2.57	0.15	-
	Micronecta sp.	30.33^{b}	30.33^{b}	37.67^{c}	0.00^{a}	13.91	0.00	1.67
	Mesovelia sp.	2.67	5.00	4.67	2.00	0.11	0.95	-
	Ranatra sp.	0.00	0.00	0.00	0.33	1.00	0.45	-
	Anisops sp.	1.67	0.00	3.00	2.00	0.27	0.84	-
	Microvelia sp.	3.34	1.67	1.67	0.33	0.88	0.50	-
	Veliidae	0.00	4.00	5.00	0.00	1.17	0.40	-
	Orthetrum sp.	1.66	0.33	0.33	0.66	2.60	0.15	-

Note: ns = not significantly different; * = significantly different; values within a row followed by the same letters were not significantly different at $P < 0.05$ according to Tukey's HSD test. Original data were transformed using $\sqrt{}(n_i+0.5)$ transformation prior to statistical analysis

Table 3. Characteristics of aquatic insect communities found in rice field applied with bioinsecticides of *Beauveria bassiana*, *Metarhizium anisopliae*, *Cordyceps militaris*, and abamectin

Rice age (DAT)	**Characteristics communities**	**Insecticides**			
		Beauveria bassiana	***Metarhizium anisopliae***	***Cordyceps militaris***	**Abamectin**
15 DAT	Abundance of aquatic insect species (N) (inv 4 m^{-2})	5.67	7.33	7.00	0.33
	Shanon wiener index (H')	0.22	0.49	0.62	0.00
	Evennes index (E)	0.32	0.44	0.56	0.00
	Dominance index (D)	0.94	0.86	0.81	1.00
29 DAT	Abundance of aquatic insect species (N) (inv 4 m^{-2})	12.00	11.33	13.00	2.00
	Shanon wiener index (H')	0.13	0.67	1.06	0.00
	Evennes index (E)	0.18	0.96	0.96	0.00
	Dominance index (D)	0.97	0.62	0.46	1,00
43 DAT	Abundance of aquatic insect species (N) (inv 4 m^{-2})	14.33	14.33	17.67	2.33
	Shanon wiener index (H')	1.36	0.81	0.84	0.41
	Evennes index (E)	0.85	0.74	0.76	0.59
	Dominance index (D)	0.49	0.70	0.64	0.86
57 DAT	Kelimpahan Serangga (N) (inv 4m^{-2})	14.00	13.33	18.67	0,66
	Indeks Keanekaragaman Jenis (H')	1.27	1.00	0.70	0.69
	Indeks Kemerataan Jenis (E)	0.92	0.91	0.50	1.00
	Indeks Dominansi (D)	0.45	0.53	0.79	0.50
Total	Abundance of aquatic insect species (N) (inv 4 m^{-2})	46,00	46.33	56.33	5.33
	Shanon wiener index (H')	1.14	1,12	1,17	1,34
	Evennes index (E)	0,64	0,63	0,60	0.83
	Dominance index (D)	0,66	0,65	0,67	0,38

Discussion

The existence of the 8 species were found when the experiment was conducted (June 2 to July 17, 2018) in the middle typology of fresh water swamps. When observing after July 17, the rice fields began to dry out and the aquatic insects were no longer found. In the typology, the deep rice fields were still inundated; the water insects generally could still be found in the habitat. The presence of deep fresh water swamp is important for conserving aquatic insects in the dry season.

The aquatic insect species found in this study all acted as predatory insects attacking the rice insect pests. Dytiscidae (unknown species) or diving beetles have species members all acting as predators attacking the insects whose habitat is close to the water surface (Watanabe 2019) such as living on the stems and lower leaves and attacking the rice insect pests, such as *Gryllotalpa orientalis* (Ohba 2009). *Micronecta* sp. and *Mesovelia* sp. are a predatory insects commonly found and can suck the body fluids of the rice insect pests (Wakhid et al. 2020). *Ranatra* sp. are predators playing a role in regulating the balance of insect populations of the rice insect pests (Thongphak and Iwai 2016). *Anisops* sp. is also commonly found in rice field water in Indonesia and acts as a predator for rice insect pests (Wakhid et al. 2020). *Microvelia* sp. and Veliidae (unknown species) were reported by Heong (2009) as predators of brown planthoppers. *Orthetrum* sp. at its immature and adult stages prey on various species of insects of various families (Salmah et al. 2017; Wakhid et al. 2020). These water insects in rice fields generally can attack rice insect pests whose habitat is at the base of the stem, such as brown planthopper (*N. lugens*) (Heong 2009).

In this study, the abundance of aquatic insects decreased significantly in the abamectin plot, but the abundance of aquatic insects remained high in all three plots applied with mycoinsecticides. Abamectin is an insecticide that is commonly applied in rice field ecosystems in Indonesia to control brown planthoppers. This abamectin is widely chosen and applied by local farmers in Indonesia because of its broad spectrum which can kill many species of insect pests and is of contact poison and stomach poison (Luo et al. 2013). Abamectin that flows into the rice field water can cause death of the aquatic insects because abamectin is a stomach poison. The characteristic of abamectin which is stomach poison when flowing into the water it will mix with water and can poison the insects in the water.

In this study, the abundance of aquatic insects remained high in the three plots applied with mycoinsecticides with active ingredients of *B. bassiana*, *M. anisoplia*, and *C. militaris* since the mycoinsecticides did not kill the water insects.

These three entomopathogenic fungal species have specific hosts such as the order of Lepidoptera or Homoptera (Shrestha et al. 2012; Farid and Syarief 2018), while the aquatic insects in this study originated from the orders of Hemiptera, Coleoptera, and Odonata. *B. bassiana* is effective in killing Lepidoptera such as *S. litura* (Ayudya et al. 2019), Homoptera, for example *N. lugens* (Sumikarsih et al. 2019). *M. anisoplia* is also effective when attacking Lepidoptera (Ayudya et al. 2019). *C. militaris* generally attacks Lepidoptera which attacks palm oil leaves (Shrestha et al. 2012). Prabawati et al. (2019) state that *B. bassiana, M. anisoplia,* and *C. militaris* applied in rice fields can reduce insect pest population, but do not harm predatory arthropods inhabiting canopy and rice soil surfaces. Consequently, *B. bassiana, M. anisopliae,* and *C. militaris* applied in the fields are beneficial in reducing rice insect pests and safe by not reducing the abundance of aquatic insects which are predators of rice insect pests.

In the plots applied with the mycoinsecticides, the species diversity and abundance of aquatic insects increasingly soared along with the increasing age of rice. The more complex the structure of plants are, the more complex the species of insects associated with the plants will be (Settle et al. 1996) and the more abundant and complex species of insect pests in the rice canopy with increasing age of the rice plants (Prabawati et al. 2019). These abundant pests in rice are also prey for aquatic insects (Cheong 2009) which increase along with the increasing age of rice.

The diversity of aquatic insect species in the plots applied with the abamectin was lower than the those applied with the mycoinsecticides. There were only 5 species of aquatic insects found in the abamectin plots, while in *B. bassiana* plots there were 6 species found, in *M. anisopliae* plots were found 6 species, and in *C. militaris* plot 7 species of aquatic insects were found. Three species were not found in the Abamectin plots (Dytiscidae, the unknown species, *Micronecta* sp., and Veliidae unknown species). Therefore, these three species were susceptible to the abamectin. The *Micronecta* sp. is generally found in a healthy aquatic ecosystem, but in this study *Micronecta* sp. was not found in the abamectin plots. This is due to the broad spectrum of abamectin characteristic which kills various insect species (Luo et al. 2013) including the aquatic insects in this study. The decrease of the number of aquatic insect species after the application of synthetic insecticides occurs because they kill aquatic insects (Thongphak and Iwai 2016). The decreasing number of species or diversity of aquatic insect species in the rice fields shows a decrease in the water quality of the rice fields (Salmah et al. 2017).

The conclusion of this study is that the obtained eight species of aquatic insects all acted as predators, namely Dytiscidae (unknown species), *Micronecta* sp., *Mesovelia* sp., Ranatra sp., *Anisops* sp., *Microvelia* sp., Veliidae (unknown species), *Orthetrum* sp. originating from 3 orders (Coleoptera, Hemiptera, and Odonata) and 7 families (Dytiscidae, Corixidae, Mesoveliidae, Nepidae, Notonectidae, Veliidae, and Libellulidae). The application of mycoinsecticides *B. bassiana, M. anisopliae,* and *C. militaris* did not reduce the abundance and diversity of aquatic insect species but the application of abamectin could reduce them. Therefore, the application of mycoinsecticides from *B. bassiana, M. anisopliae,* and *C. militaris* to control rice insect pests is safer for aquatic insects than that of abamectin applications.

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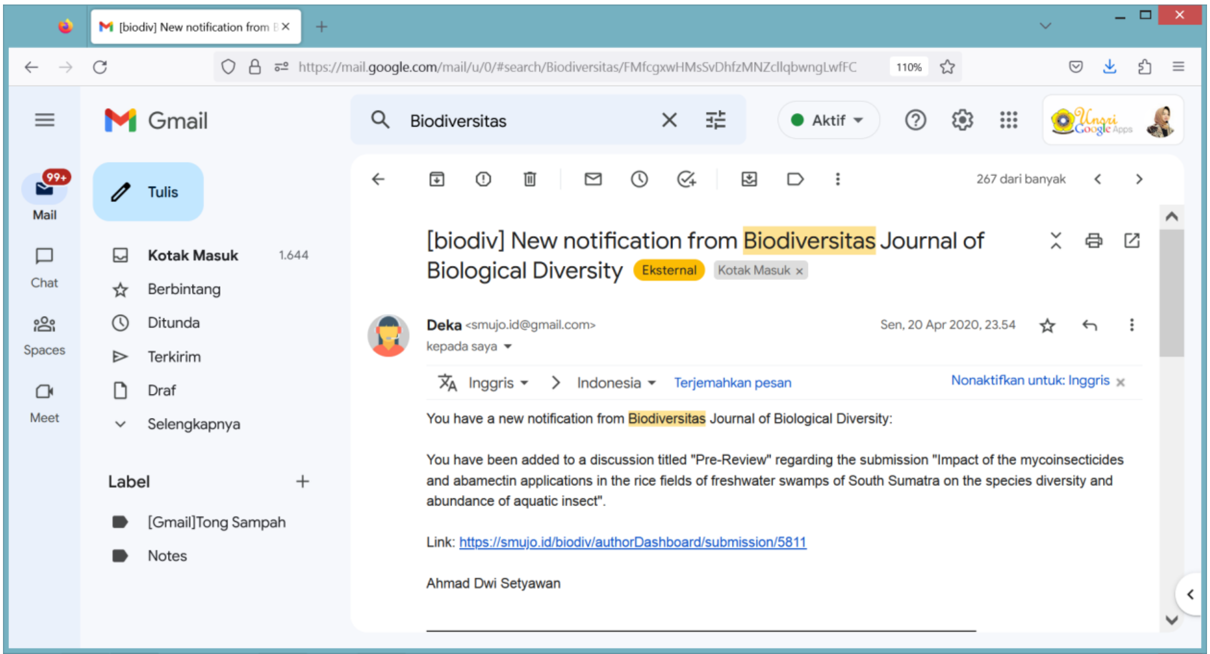

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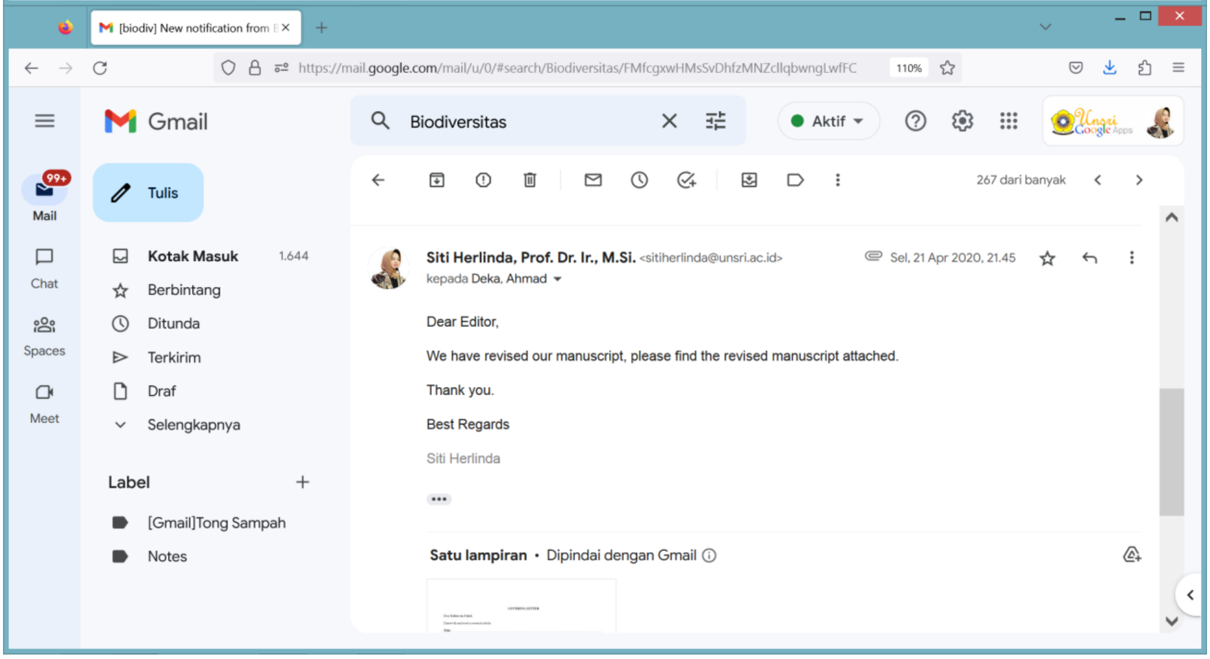

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The impact of mycoinsecticides and abamectin applications in rice fields of freshwater swamps in South Sumatra on species diversity and aquatic insects abundance

Abstract. Aquatic insects in rice fields generally are predators of rice insect pests. The application of insecticides may reduce the abundance and species diversity of aquatic insects. This study aimed to determine the impact of mycoinsecticides and abamectin application on species diversity and abundance of aquatic insects in rice fields. Mycoinsectisedes were derived from *B. bassiana*, *M. anisopliae*, and *Cordycep militaris*). [U1]The results showed there were eight aquatic insects species obtained in this study, i.e. unidentified Dytiscidae, *Micronecta* sp., *Mesovelia* sp., *Ranatra* sp., *Anisops* sp., *Microvelia* sp., unidentified species of Veliidae, and *Orthetrum* sp. belong to 7 families (Dytiscidae, Corixidae, Mesoveliidae, Nepidae, Notonectidae, Veliidae, Libellulidae), and three orders (Coleoptera, Hemiptera, and Odonata). All of the species were predatory insects. The application of mycoinsecticides did not reduce the abundance and species diversity of the aquatic insect, but the application of abamectin reduced the abundance and species diversity of aquatic insects. There were 5 species of aquatic insects in the abamectin plot, while in the plot of *B. bassiana* plot were 6 species, in *M. anisopliae* plot were 6 species, and in *C. militaris* plot were 7 species of aquatic insects. So, the application of mycoinsecticides from *B. bassiana*, *M. anisopliae*, and *C. militaris* is safe for aquatic insects and to control rice insect pests than that of abamectin.

Key words: *Beauveria bassiana, Cordyceps militaris, Metarhizium anisopliae,* mycoinsecticides, aquatic insects

Running title: Impact of mycoinsecticides on aquatic insect

INTRODUCTION

The agricultural potential of the swampy area in Indonesia approximately 39.6 Mha, and 11.9 Mha is located in Sumatra (Margono et al. 2014) which is generally dominated by freshwater swamps. A freshwater swamp is a wetland ecosystem that is flooded with river water or rain throughout the year. The duration of stagnant water depends on the typology of the land. Our observations of the last 7 years in Ogan Ilir District of South Sumatra, Indonesia showed that flooding in the deep freshwater swampy typology is almost all year-round (October to July), middle freshwater swamps from November to June, and embankment freshwater swamps or shallow freshwater swamps from November to April. Due to stagnant water, the smallholder farmers underutilized their lands and they start to plant rice and vegetables approaching the dry season.

Crops cultivated in various typologies of freshwater swamps vary depending on their adaptation to stagnant water. Rice is the most widely planted in freshwater swamps in South Sumatra (Lakitan et al. 2018a, b; Prabawati et al. 2019). Besides rice, chilies (Johari et al. 2014, 2016), bitter melon, long beans, eggplants, pumpkins, and cucumbers are also cultivated (Siaga et al. 2019). Rice is generally planted once a year (Herlinda et al. 2018a). Currently, some farmers earn>100 ha land who start planting rice twice to three times a year, and several smallholder farmers grow rice twice a year by utilizing the remaining ratoons of the previous season's rice harvest (Lakitan et al. 2018b; Prabawati et al. 2019).

Intensive rice cultivation in freshwater swamps will increase the use of insecticides due to the increasing insect population (Prabawati et al. 2019). Commonly used insecticides are synthetic insecticides (Herlinda et al. 2018a) and sometimes bioinsecticides (Karenina et al. 2019; Prabawati et al. 2019). Commonly insecticide used in rice fields in Indonesia is abamectin (Luo et al. 2013), while currently used bioinsecticides are derived from entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* (Prabawati et al. 2019) which are referred as mycoinsecticides. *B. bassiana* and *M. anisopliae* are widely used because they are easily found in freshwater swamps (Herlinda et al. 2018b; Safitri et al. 2018) and able to kill important insect pests such as brown planthopper (Sumikarsih et al.. 2019) and *Spodoptera litura* (Ayudya et al. 2019). Although the entomopathogenic fungi are reported to be host-specific (Farid and Syarief, 2018), it is necessary to monitor the impact of these mycoinsecticides on the abundance of non-target arthropods.

The impact of synthetic insecticide applications on non-target arthropods has been widely studied (Azod et al. 2016; Khan et al. 2018). However, there is not much information on the impact of synthetic insecticide and mycoinsecticides application on aquatic insects. Aquatic insects in the rice field are generally dominated by predatory insects (Thirumalai et al. 2002). The abundance and diversity of non-target aquatic insect species in the rice field are high (Settle et al. 1996; Herlinda et al. 2019a,b). The commonly found of aquatic insects were *Microvelia douglasi*, *Mesovelia vittigera*, *Anisops barbatus*, *Ranatra elongata*, and *Micronecta scutellaris* (Thirumalai et al. 2002). These aquatic insects play an important role in the aquatic ecosystem (Dunbar et al.

2010) as natural enemies of rice insect pests (Heong et al. 2009). The intensive application of insecticides and mycoinsecticides has an impact on the abundance and species diversity of the aquatic insects. The high abundance and species diversity of arthropod in water is an indicator of healthy aquatic ecosystems (Salachna and Olearczyk 2020). This study aimed to determine the effect of the mycoinsecticides application of *B. bassiana*, *M. anisopliae*, and *Cordycep militaris* and abamectin on the species diversity and abundance of aquatic insects in the rice field.

MATERIALS AND METHODS

Study area

The field experiment was carried out in the center of the freshwater swampy rice field in the *Pelabuhan Dalam* Village of Pemulutan Subdistrict, Ogan Ilir District of South Sumatra Province. The area of freshwater rice fields in the study site was more than 800 ha. The experimental plot covers 1 ha of the freshwater swampy rice field that divided into 12 subplots for 4 treatments, each treatment has 3 replicates. This study uses a Randomized Block Design. Field trials were conducted from May to August 2018 and continued with the identification of collected aquatic insects until December 2018. The four treatments used in this study were the application of mycoinsecticides derived from *B. bassiana, M. anisopliae, C. militaris,* and Abamectin as a positive control. *B. bassiana* and *M. anisopliae* isolates were collected from the exploration of Safitri et al. (2018), while *C. militaris* was collected from Central Kalimantan. The identification of three entomopathogenic fungal species used in this experiment was carried out by Dr. Suwandi (a mycologist from Sriwijaya University).

Preparation of land and rice planting

Rice cultivation was carried out through the stages of tillage, seed preparation, planting, and maintenance as carried out by local farmers. Soil processing was carried out using a *singkal* plow machine and then smoothed using a hoe added running water so the soil becomes muddy. After the soil was cultivated, the rice fields were added with a dose of 1 ton ha^{-1} [U2]and flooded with water for 14 days before planting.

Seed sowing by local farmers includes seed preparation, seed treatment, seed curing, and seed sowing. The seeds used were certified Mekongga variety at a dose of 50 kg ha^{-1}. The seeds were soaked for 24 hours with shrimp shell flour compost at a dose of 0.5 mL L^{-1}. Composting of shrimp shell flour was made according to the method of Suwandi et al. (2012). After 24 hr soaking, broadcast the seeds in moist conditions on the rice field dike that has been coated with rice mud and moist manure. The seeds were broadcast by sprinkling called *Samir*.

Insecticides used in this study were mycoinsecticides and abamectin, while weeding was done mechanically without using herbicides. Watering was done by using a pump, and manure was used as fertilizer. Shrimp shell flour compost extract at a dose of 2 L ha^{-1} was applied every 2 weeks until the milky stage (milk-cooked phase) by spraying to increase soil fertility. Rice was planted using a *legowo* row of 2:1 planting system with a spacing of 12.5x50x50 cm^3. It was conducted by transplantation using 7-10 days old seedlings. Seedlings were transplanted with a maximum water level of half of rice seedlings height. The higher the rice plants, the more water was pumped into the fields. This was carried out to accommodate and maintain the presence of aquatic insects inhabiting the rice field water. The rice field was inundated until nearing the ripe phase of milk and after the grains of rice were at the ripening stage, the fields began to be dried and the experiment was stopped.

Production and application of mycoinsecticides and abamectin

Mycoinsectides used as treatments in this study were derived from 3 species of entomopathogenic fungi, i.e. *B. bassiana, M. anisopliae,* and *C. militaris* and commercial insecticides abamectin as a positive control. Production of mycoinsecticides was following the modified method of Mascarin et al. (2015). Entomopathogenic fungi were cultured on Sabouraud Dextrose Agar (SDA, Merck) solid media and incubated for 10 days at….., Entomopathogenic fungi [U3]grew on solid media were transferred into Sabouraud Dextrose Broth (SDB, Merck) liquid media and incubated for 7 days under agitation at 120 rpm. Liquid entomopathogenic fungal culture with a minimum density of 1x10^9 conidia mL^{-1} was used as the active ingredient of mycoinsecticides. The mycoinsecticides were made from a mixture of 600 mL of liquid entomopathogenic fungal culture, 400 mL carrier made from shrimp shell flour compost extract, 10 mL vegetable oil, and 10 mL of a sterile aqueous solution of 0.04% polyoxyethyl-189 ene sorbitan mono-oleate (Tween®). Mycoinsectides were applied at a dose of 2 L ha^{-1} every two weeks, starting on the 14th, 28th, 42nd, and 56th days after transplanting (DAT), while abamectin was applied according to the manufacturer's instructions with the same spraying schedule with the mycoinsecticides.

Samplings of aquatic insects

One day after mycoinsecticides and abamectin were sprayed on the rice field, the sampling of aquatic insects was carried out, which were on the 15th, 29th, 43rd, and 57th DAT,i.e. 2nd of June 2018, 16th June 2018, 30th June 2018, and 17th July 2018, while the next sampling was not carried out because the rice fields began to dry out. The sampling of aquatic insects following the method of Salmah et al. (2017) by using a fishnet (0.4 mm mesh) conducted at 6.00-7.00 a.m.

Sampling was carried out by swinging the net into rice field water with a 2x2 m^2 sampling area for each treatment and repeated three times. The obtained insects were put into a vial containing 96% ethanol and then taken to the laboratory for identification. The taxonomic identification of aquatic insects using morphological features based on Basu et al. (2016) and Miller and Bergsten (2016) up to the genus level.

Data analysis

Data on the number of each species were used to calculate species abundance and diversity. The obtained data on species abundance were subjected to Analysis of variance (ANOVA) followed by Tukey's Honestly Significant Different (HSD) to determine significant differences among treatments at a level of 5% using the software program of SAS University Edition 2.7 9.4 M5. Analysis of the species diversity was carried out using the Shannon index (H'). The diversity level was also evaluated by the Evenness index (J') derived from the Shannon function and Berger-Parker dominance biodiversity indices.

RESULTS AND DISCUSSION

Aquatic insect species

This study successfully collected 8 species of aquatic insects from all experimental plots, namely unidentified species of Dytiscidae, *Micronecta* sp., *Mesovelia* sp., *Ranatra* sp., *Anisops* sp., *Microvelia* sp., unidentified species of Veliidae, *Orthetrum* sp. (Figure 1) belong to 7 families (Dytiscidae, Corixidae, Mesoveliidae, Nepidae, Notonectidae, Veliidae, Libellulidae), 3 orders (Coleoptera, Hemiptera, and Odonata), (Table 1). The number of species found in the plot applied with *B. bassiana* was 6 species (unidentified species of Dytiscidae, *Micronecta* sp., *Mesovelia* sp., *Anisops* sp., *Microvelia* sp., and *Orthetrum* sp.) and in the plot applied with *M. anisopliae* were 6 species (unidentified species of Dytiscidae, *Micronecta* sp., *Mesovelia* sp., *Microvelia* sp., unidentified species of Veliidae, and *Orthetrum* sp.). In the plot applied with *C. militaris* were 7 species (unidentified species of Dytiscidae, *Micronecta* sp., *Mesovelia* sp., *Anisops* sp., *Microvelia* sp., unidentified species of Veliidae, and *Orthetrum* sp.). While in the abamectin plot there were only 5 species found (*Mesovelia* sp., *Ranatra* sp., *Anisops* sp., *Microvelia* sp. and *Orthetrum* sp.). In total, eight aquatic insect species were collected, all of the species were predators of rice insect pests.

Abundance and diversity of aquatic insect species

The abundance of aquatic insects tends to increase in plots applied with the mycoinsecticides (*B. bassiana*, *M. anisopliae*, and *C. militaris*), however, the abundance of the aquatic insect in the abamectin plot was not increased (Figure 2). The plots applied with *C. militaris* had the most abundant insects, while the plots applied with the abamectin had the lowest abundant insects. The abundance of each species was not significantly different, but in total, aquatic insect abundance was significantly different among treatments. The highest abundance of *Micronecta* sp. was in plot applied with *C. militaris* (37.67 ind [U4]4 m^{-2}) but significantly different from that of plots applied with *B. bassiana* (30.33 ind [U5]4 m^{-2}), *M. anisopliae* (30.33 ind [U6]4 m^{-2}) and abamectin (0 inv[U7] 4 m^{-2}) (Table 2). The plot applied with abamectin showed a significant decrease in *Micronecta* sp.

The aquatic insects collected in the mycoinsecticides plots applied were belonging to 3 orders (Coleoptera, Hemiptera, and Odonata), while in the abamectin plot there were only 2 orders of aquatic insects (Hemiptera, and Odonata) (Figure 3). The most dominant order in all plots was Hemiptera. Species composition in the plots applied with mycoinsecticides was different from the abamectin plots (Figure 4). The species composition in the three mycoinsecticide plots had the same tendency. *Micronecta* sp. was not found in the abamectin plots, but *Micronecta* sp. was the most dominant species in the plots applied with mycoinsecticides. The presence and composition of aquatic insect species can be used as an indicator of the water quality.

The results of the study showed that the diversity of aquatic insect species increased with increasing age of rice. The species diversity was higher in plots applied with mycoinsecticides than plots applied with abamectin (Table 3). The highest species diversity on the 15th and 29th DAT was in the plots applied with *C. militaris*, followed by those applied with *B. bassiana*. However, the highest species diversity on the 43rd – 57th DAT was in the plots applied with *B. bassiana*.

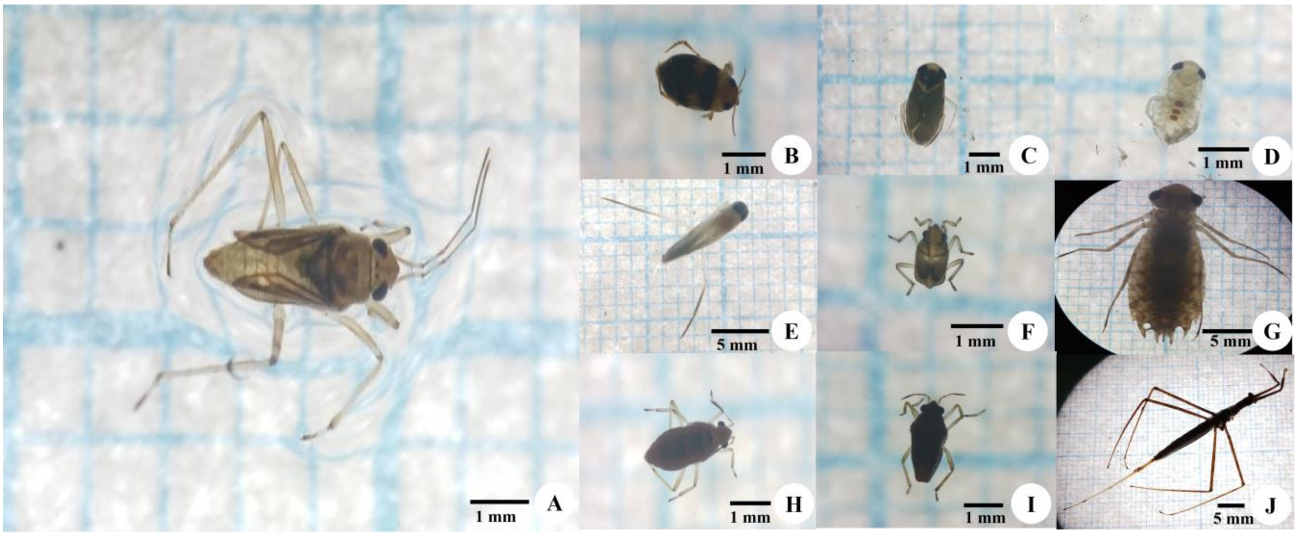

Figure 1. Aquatic insect species obtained in complete rice production cycle: *Mesovelia* sp. (A), unidentified species of Dytiscidae (B), *Micronecta* sp. adult (C), *Micronecta* sp. nymph (D), *Anisops* sp. (E), unidentified species of Veliidae (F), *Orthetrum* sp. (G), *Microvelia* sp. (H and I), *Ranatra* sp. (J)

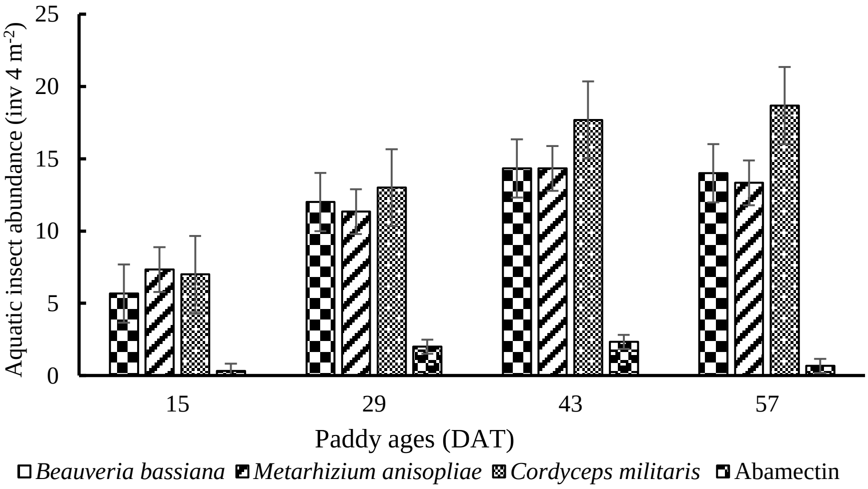

Figure 2. Aquatic insect abundance in rice field applied with bioinsecticides of *B. bassiana, M. anisopliae, C. militaris,* and abamectin

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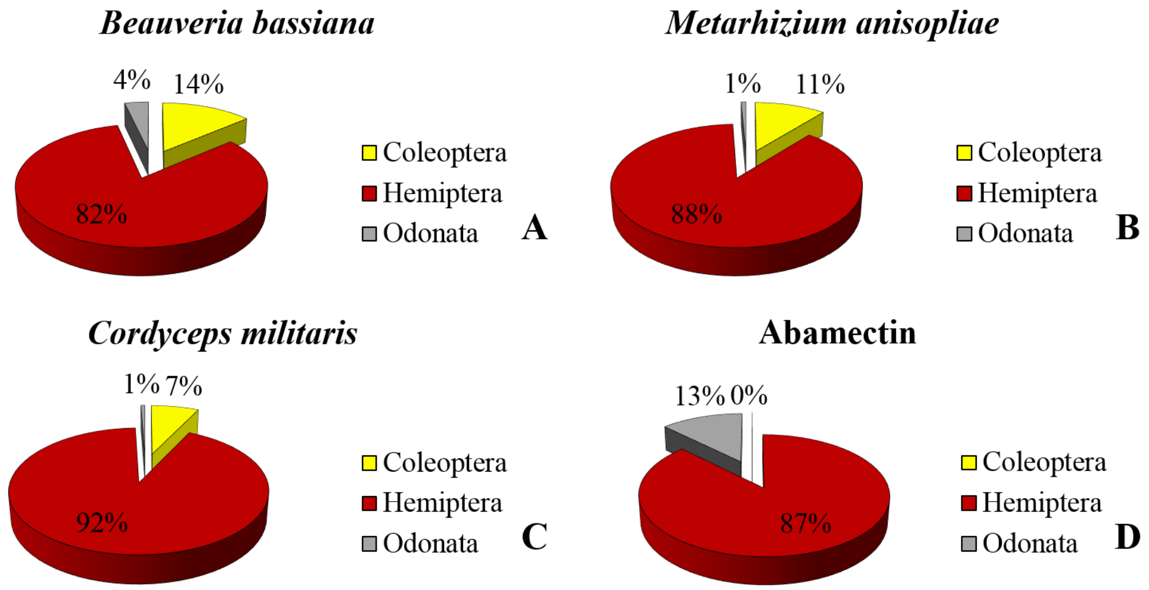

Figure 3. The relative abundance of the order of aquatic insect in rice field applied with bioinsecticides of *B. bassiana* (A), *M. anisopliae* (B), *C. militaris* (C), and abamectin (D)

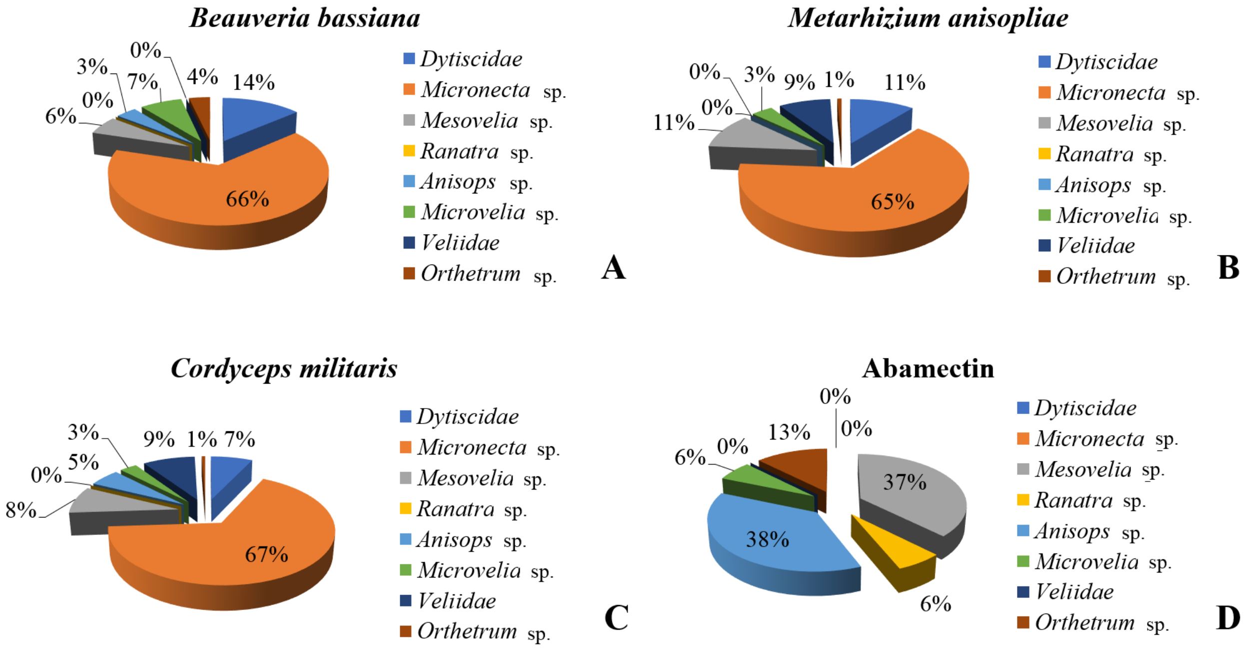

Figure 4. The relative abundance of aquatic insect species in rice field applied with bioinsecticides of *B. bassiana* (A), *M. anisopliae* (B), *C. militaris* (C), and abamectin (D)

Table 1. Aquatic insect in rice field applied with bioinsecticides of *B. bassiana*, *M. anisopliae*, *C. militaris*, and abamectin

Order	**Family**	**Species**	**Insecticide treatment**			
			Beauveria bassiana	***Metarhizium anisopliae***	***Cordyceps militaris***	**Abamectin**
Coleoptera	Dytiscidae	Unknown species	+	+	+	-
Hemiptera	Corixidae	*Micronecta* sp.	+	+	+	-
	Mesoveliidae	*Mesovelia* sp.	+	+	+	+
	Nepidae	*Ranatra* sp.	-	-	-	+
	Notonectidae	*Anisops* sp.	+	-	+	+
	Veliidae	*Microvelia* sp.	+	+	+	+
		Unknown species	-	+	+	-
Odonata	Libellulidae	*Orthetrum* sp.	+	+	+	+

Note: + insects found, - no insects found

Table 2. The abundance of aquatic insect species in rice field applied with bioinsecticides of *B. bassiana*, *M. anisopliae*, *C. militaris*, and abamectin

Rice age (DAT)	**Species of aquatic insect**	**The abundance of aquatic insect species (inv[U8] 4 m^{-2})**				**F value**	**P value**	**Tukey's HSD test**
		Beauveria bassiana	***Metarhizium anisopliae***	***Cordyceps militaris***	**Abamectin**			
15 DAT	Dytiscidae	0.00	0.00	0.00	0.00	-	-	-
	Micronecta sp.	5.33	6.33	5.67	0.00	0.25	0.86	-
	Mesovelia sp.	0.00	0.67	0.67	0.00	0.57	0.65	-
	Ranatra sp.	0.00	0.00	0.00	0.00	-	-	-
	Anisops sp.	0.00	0.00	0.00	0.00	-	-	-
	Microvelia sp.	0.00	0.00	0.67	0.33	0.62	0.63	-
	Veliidae	0.00	0.00	0.00	0.00	-	-	-
	Orthetrum sp.	0.33	0.33	0.00	0.00	0.57	0.65	-
29 DAT	Dytiscidae	0.00	0.00	0.00	0.00	-	-	-
	Micronecta sp.	11.67	7.00	6.00	0.00	1.51	0.30	-
	Mesovelia sp.	0.00	4.33	4.00	2.00	0.41	0.75	-
	Ranatra sp.	0.00	0.00	0.00	0.00	-	-	-
	Anisops sp.	0.00	0.00	3.00	0.00	1.00	0.45	-
	Microvelia sp.	0.00	0.00	0.00	0.00	-	-	-
	Veliidae	0.00	0.00	0.00	0.00	-	-	-
	Orthetrum sp.	0.33	0.00	0.00	0.00	1.00	0.45	-
43 DAT	Dytiscidae	3.00	2.67	1.33	0.00	0.78	0.55	-
	Micronecta sp.	7.00	10.00	11.33	0.00	1.80	0.25	-
	Mesovelia sp.	0.00	0.00	0.00	0.00	-	-	-
	Ranatra sp.	0.00	0.00	0.00	0.00	-	-	-
	Anisops sp.	1.67	0.00	0.00	2.00	0.57	0.65	-
	Microvelia sp.	1.67	1.67	0.00	0.00	1.00	0.45	-
	Veliidae	0.00	0.00	5.00	0.00	1.00	0.45	-
	Orthetrum sp.	1.00^{c}	0.00^{a}	0.00^{a}	0.33^{b}	8.00*	0.02	0.22
57 DAT	Dytiscidae	3.33	2.33	2.67	0.00	1.33	0.35	-
	Micronecta sp.	6.33	7.00	14.67	0.00	1.54	0.30	-
	Mesovelia sp.	2.67	0.00	0.00	0.00	1.00	0.45	-
	Ranatra sp.	0.00	0.00	0.00	0.33	1.00	0.45	-
	Anisops sp.	0.00	0.00	0.00	0.00	-	-	-
	Microvelia sp.	1.67	0.00	1.00	0.00	0.59	0.64	-
	Veliidae	0.00	4.00	0.00	0.00	3.50	0.09	-
	Orthetrum sp.	0.00	0.00	0.33	0.33	0.57	0.65	-
Total	Dytiscidae	6.33	5.00	4.00	0.00	2.57	0.15	-
	Micronecta sp.	30.33^{b}	30.33^{b}	37.67^{c}	0.00^{a}	13.91	0.00	1.67
	Mesovelia sp.	2.67	5.00	4.67	2.00	0.11	0.95	-
	Ranatra sp.	0.00	0.00	0.00	0.33	1.00	0.45	-
	Anisops sp.	1.67	0.00	3.00	2.00	0.27	0.84	-
	Microvelia sp.	3.34	1.67	1.67	0.33	0.88	0.50	-
	Veliidae	0.00	4.00	5.00	0.00	1.17	0.40	-
	Orthetrum sp.	1.66	0.33	0.33	0.66	2.60	0.15	-

Note: ns = not significantly different; * = significantly different; values within a row followed by the same letters were not significantly different at $P < 0.05$ according to Tukey's HSD test. Original data were transformed using $\sqrt{(n_i+0.5)}$ transformation before statistical analysis

Table 3. Characteristics of aquatic insect communities in rice field applied with bioinsecticides of *B.bassiana*, *M.anisopliae*, *C. militaris*, and abamectin

Rice age (DAT)	**Characteristics of communities**	**Insecticides**			
		Beauveria bassiana	***Metarhizium anisopliae***	***Cordyceps militaris***	**Abamectin**
15 DAT	Abundance of aquatic insect species (N) (inv 4 m^{-2})	5.67	7.33	7.00	0.33
	Shanon wiener index (H')	0.22	0.49	0.62	0.00
	Evennes index (E)	0.32	0.44	0.56	0.00
	Dominance index (D)	0.94	0.86	0.81	1.00
29 DAT	Abundance of aquatic insect species (N) (inv 4 m^{-2})	12.00	11.33	13.00	2.00
	Shanon wiener index (H')	0.13	0.67	1.06	0.00
	Evennes index (E)	0.18	0.96	0.96	0.00
	Dominance index (D)	0.97	0.62	0.46	1,00
43 DAT	Abundance of aquatic insect species (N) (inv 4 m^{-2})	14.33	14.33	17.67	2.33
	Shanon wiener index (H')	1.36	0.81	0.84	0.41
	Evennes index (E)	0.85	0.74	0.76	0.59
	Dominance index (D)	0.49	0.70	0.64	0.86
57 DAT	Kelimpahan Serangga (N) (inv 4m^{-2})[U9]	14.00	13.33	18.67	0,66
	Indeks Keanekaragaman Jenis (H')	1.27	1.00	0.70	0.69
	Indeks Kemerataan Jenis (E)	0.92	0.91	0.50	1.00
	Indeks Dominansi (D)[U10]	0.45	0.53	0.79	0.50
Total	Abundance of aquatic insect species (N) (inv 4 m^{-2})	46,00	46.33	56.33	5.33
	Shanon wiener index (H')	1.14	1,12	1,17	1,34
	Evennes index (E)	0,64	0,63	0,60	0.83
	Dominance index (D)	0,66	0,65	0,67	0,38

Discussion

Eight aquatic insect species were obtained in the center of freshwater swamps from June 2nd to July 17th. In the dried rice field after July 17 the aquatic insects were no longer found. However, in the rice fields that still inundated; the aquatic insects still could be found. Therefore, the presence of deep freshwater swamp is important for conserving aquatic insects in the dry season.

All species of aquatic insects collected in this study were predatory insects that attack rice insect pests. All species belonging to Dytiscidae (unidentified species) or diving beetles were predators of insects whose habitat is close to the water surface (Watanabe 2019) such as living on the stems and lower leaves and attacking the rice insect pests, such as *Gryllotalpa orientalis* (Ohba 2009). *Micronecta* sp. and *Mesovelia* sp. are predatory insects commonly found and suck out the internal body fluids of the rice insect pests (Wakhid et al. 2020). *Ranatra* sp. are predators that have a role in regulating the balance of insect populations of the rice insect pests (Thongphak and Iwai 2016). *Anisops* sp. is commonly found in rice field water in Indonesia and also known as a predator for rice insect pests (Wakhid et al. 2020). *Microvelia* sp. and Veliidae (unidentified species) were reported as predators of brown planthoppers (Heong, 2009). Immature and adult stages of *Orthetrum* sp. prey on various insect species from various families (Salmah et al. 2017; Wakhid et al. 2020). The aquatic insects in rice fields obtained in this study generally attack rice insect pests that inhabit the base of the stem, such as brown planthopper (*N. lugens*) (Heong 2009.

The results showed that the abundance of aquatic insects decreased significantly in the abamectin plot, but it remained high in all three plots applied with mycoinsecticides. Abamectin is an insecticide that is commonly applied in the rice field to control brown planthoppers in Indonesia. Abamectin is contact poison and stomach poison and widely chosen and applied by farmers in Indonesia because it has a broad spectrum that kills many species of insect pests (Luo et al. 2013). Abamectin that flows into the rice field water will poison and cause the death of aquatic insects.

The abundance of aquatic insects in the three plots applied with mycoinsecticides from *B. bassiana, M. anisoplia,* and *C. militaris* remain high since these mycoinsecticides did not poison the aquatic insects. These three entomopathogenic fungal species have specific hosts, i.e. the order of Lepidoptera or Homoptera (Shrestha et al. 2012; Farid and Syarief 2018), while the aquatic insects obtained in this study belong to the orders of Hemiptera, Coleoptera, and Odonata. *B. bassiana* is effective in poisoning and killing *S. litura* (Lepidoptera) (Ayudya et al. 2019), and *N. lugens* (Homoptera) (Sumikarsih et al. 2019). *M. anisoplia* effectively attacks Lepidoptera (Ayudya et al. 2019), while *C. militaris* generally attacks insect pests of which attacks palm oil leaves (Lepidoptera) (Shrestha et al. 2012). Prabawati et al. (2019) reported that application of *B. bassiana, M. anisoplia,* and *C. militaris* in rice fields reduce insect pest population, but do not harm predatory arthropods inhabiting the canopy and rice soil surfaces. Therefore, the application of *B. bassiana, M. anisopliae,* and *C. militaris* in rice fields are beneficial in reducing rice insect pests and safe for the aquatic insect as predators of rice insect pests.

Species diversity and aquatic insect abundance are increasing along with the increasing age of rice in the plots treated with mycoinsecticides. The more complex the structure of plants are, the more complex the species of insects associated with the plants (Settle et al. 1996) and the more abundant and diverse insect pests in the rice canopy with increasing age of rice plants (Prabawati et al. 2019). The abundance of pests in rice are prey for aquatic insects (Cheong 2009)

The diversity of aquatic insect species in plots applied with abamectin was lower than those applied with the mycoinsecticides. ~~There were only 5 species of aquatic insects found in the abamectin plots, while in *B. bassiana* plots there were 6 species found, in *M. anisopliae* plots were found 6 species, and in *C. militaris* plot 7 species of aquatic insects were found.~~ [U11]Three species were not found in the Abamectin plots (unidentified species of Dytiscidae, *Micronecta* sp., and unidentified species of Veliidae). This could be due to the broad spectrum of abamectin that can kill various insect species (Luo et al. 2013) including the aquatic insects (Thongphak and Iwai 2016) in this study. These three species were susceptible to abamectin. The *Micronecta* sp. is generally found in a healthy aquatic ecosystem. The decreasing number of species or diversity of aquatic insect species in the rice fields could be an indicator of decreasing water quality of the rice fields (Salmah et al. 2017).

It can be concluded that all eight aquatic insect species obtained in this study were predators of rice insect pests. The eight aquatic insect species were: unidentified species of Dytiscidae, *Micronecta* sp., *Mesovelia* sp., *Ranatra* sp., *Anisops* sp., *Microvelia* sp., unidentified species of Veliidae, and *Orthetrum* sp. belong to 7 families (Dytiscidae, Corixidae, Mesoveliidae, Nepidae, Notonectidae, Veliidae, and Libellulidae), and three orders (Coleoptera, Hemiptera, and Odonata). The application of mycoinsecticides from *B. bassiana, M. anisopliae,* and *C. militaris* did not reduce the abundance and diversity of aquatic insect species, while the application of abamectin reduces the abundance and diversity of aquatic insect species. The application of mycoinsecticides from *B. bassiana, M. anisopliae,* and *C. militaris* is a safe application for aquatic insects, and to control rice insect pests than that of abamectin applications.

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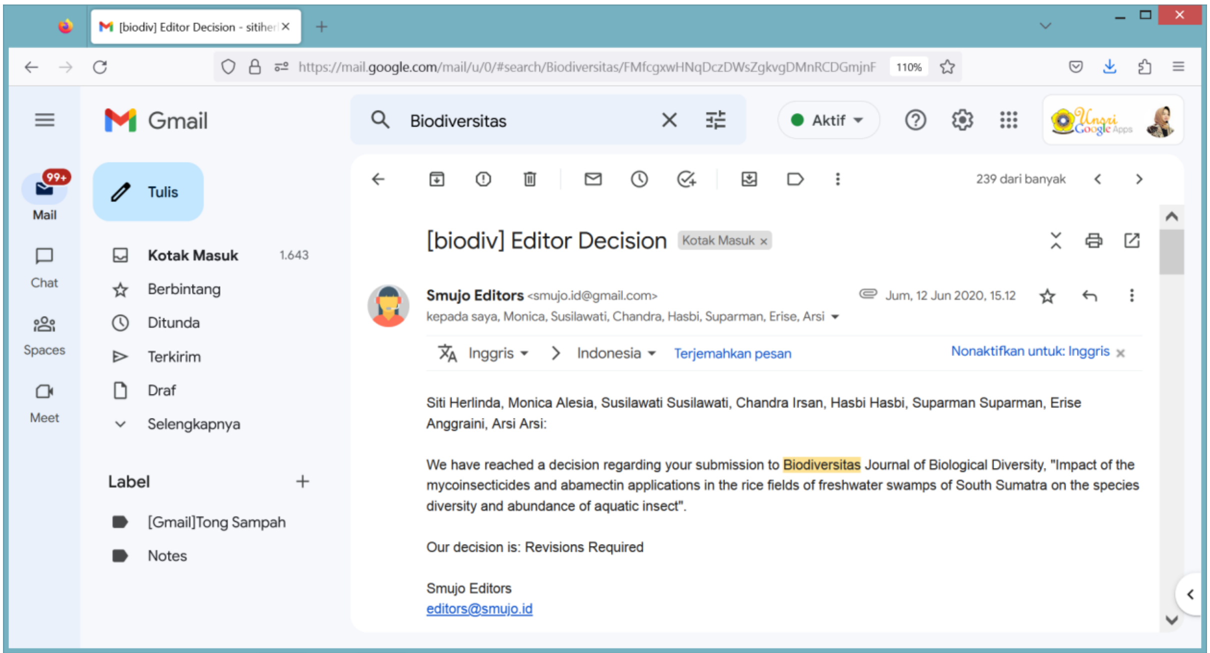
3.Bukti konfirmasi review kedua dan hasil revisi kedua



[biodiv] Editor Decision

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Siti Herlinda, Monica Alesia, Susilawati Susilawati, Chandra Irsan, Hasbi Hasbi, Suparman Suparman, Erise Anggraini, Arsi Arsi:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Impact of the mycoinsecticides and abamectin applications in the rice fields of freshwater swamps of South Sumatra on the species diversity and abundance of aquatic insect".

Our decision is: Revisions Required

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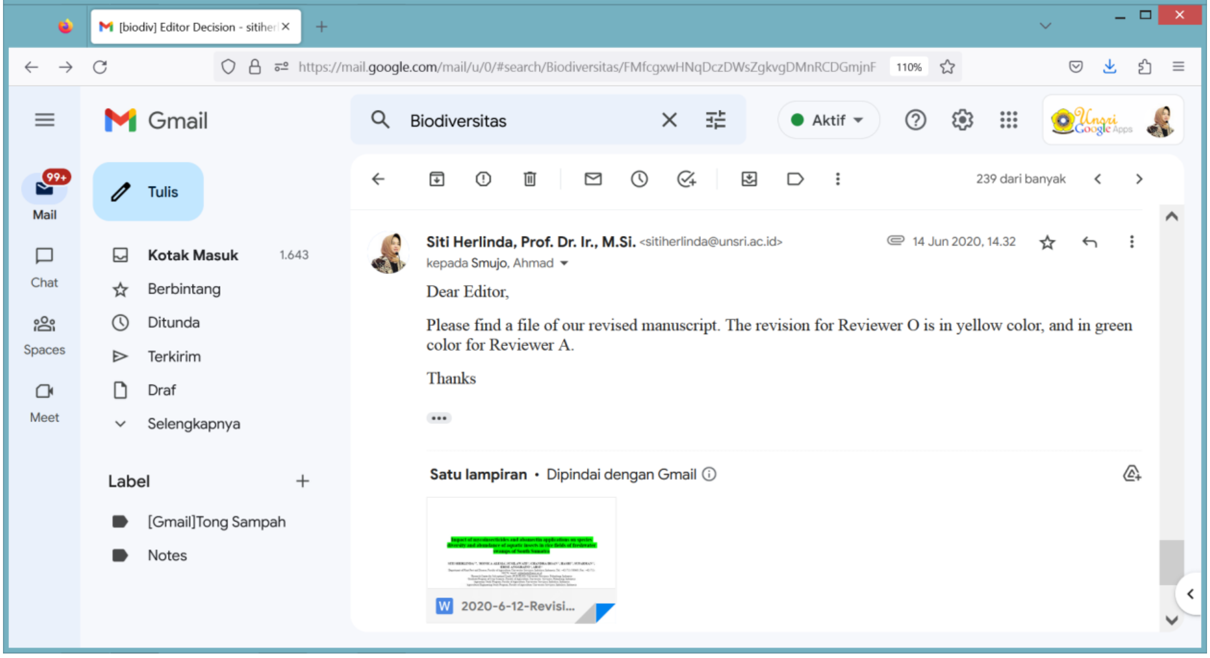

Siti Herlinda, Prof. Dr. Ir., M.Si. <sitiherlinda@unsri.ac.id> 14 Jun 2020, 14.32
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Dear Editor,

Please find a file of our revised manuscript. The revision for Reviewer O is in yellow color, and in green color for Reviewer A.

Thanks

Impact of mycoinsecticides and abamectin applications on species diversity and abundance of aquatic insects in rice fields of freshwater swamps of South Sumatra

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Abstract. Aquatic insects in rice fields generally are predators of rice insect pests. The application of insecticides may reduce the abundance and species diversity of these predators. This study aimed to determine the impact of mycoinsecticides and abamectin application on species diversity and abundance of aquatic insects in rice fields. Mycoinsecticides were made from *Beauveria bassiana* s.l., *Metarhizium anisopliae* s.l. and *Cordyceps militaris* s.l. with carrier from shrimp shell flour compost extract, vegetable oil, and Tween®. The treatments were the mycoinsectides and abamectin. The results showed there were eight aquatic insects species obtained in this study, i.e. unidentified Dytiscidae, *Micronecta* sp., *Mesovelia* sp., *Ranatra* sp., *Anisops* sp., *Microvelia* sp., unidentified species of Veliidae, and *Orthetrum* sp. belong to 7 families (Dytiscidae, Corixidae, Mesoveliidae, Nepidae, Notonectidae, Veliidae, Libellulidae), and three orders (Coleoptera, Hemiptera, and Odonata). All of the species were predatory insects. The application of mycoinsecticides did not reduce the abundance and species diversity of the aquatic predatory insects, but the application of abamectin reduced the abundance and species diversity of the predators. The highest insect species diversity was in the plots applied with *C. militaris* s.l., followed by the *B. bassiana* s.l. and *M. anisopliae* s.l. plots and the lowest one was found in the abamectin plot. So, the application of mycoinsecticides from *B. bassiana* s.l., *M. anisopliae* s.l. and *C. militaris* s.l. is safe for the aquatic predatory insects and to control rice insect pests than that of abamectin.

Key words: *Beauveria bassiana*, *Cordyceps militaris*, *Metarhizium anisopliae*, mycoinsecticides, aquatic predatory insects

Running title: Impact of mycoinsecticides on aquatic insect

INTRODUCTION

The agricultural potential of the swampy area in Indonesia approximately 39.6 Mha, and 11.9 Mha is located in Sumatra (Margono et al. 2014) which is generally dominated by freshwater swamps. A freshwater swamp is a wetland ecosystem that generally floods with river water or rain throughout the year. The duration of stagnant water depends on the typology of the land. Our observation over the past 7 years in Ogan Ilir District of South Sumatra, Indonesia showed that flooding in the deep freshwater swampy typology is almost all year-round (October to July), middle freshwater swamps from November to June, and embankment freshwater swamps or shallow freshwater swamps from November to April. Due to stagnant water, the smallholder farmers underutilize their lands and start to plant rice and vegetables approaching the dry season.

Crops cultivated in various typologies of freshwater swamps vary depending on their adaptation to stagnant water. Rice is the most planted in freshwater swamps in South Sumatra (Lakitan et al. 2018a, b; Prabawati et al. 2019). Besides rice, chilies (Johari et al. 2014, 2016), bitter melon, long beans, eggplants, pumpkins, and cucumbers are also cultivated (Siaga et al. 2019). Rice is generally planted once a year (Hanif et al. 2020). Currently, some farmers earn>100 ha land who start planting rice two to three times a year, and several smallholder farmers grow rice two times a year by utilizing the remaining ratoons of the previous season's rice harvest (Lakitan et al. 2018b; Prabawati et al. 2019).

Intensive rice cultivation in freshwater swamps will increase the use of insecticides due to the increasing insect population (Prabawati et al. 2019). Commonly used insecticides are synthetic insecticides (Hanif et al. 2020) and sometimes bioinsecticides (Karenina et al. 2019; Prabawati et al. 2019; Hanif et al. 2020). Commonly insecticide used in rice fields in Indonesia is abamectin (Luo et al. 2013). Entomopathogenic fungi such as *Beauveria bassiana* (Ascomycota: Hypocreales) and *Metarhizium anisopliae* (Ascomycota: Hypocreales) are the most commonly used bioinsecticides (Prabawati et al. 2019). *B. bassiana* and *M. anisopliae* are widely used because they are easily found in freshwater swamps (Herlinda et al. 2018b; Safitri et al. 2018; Gustianingtyas et al. 2020). and able to kill important insect pests such as brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae) (Sumikarsih et al. 2019) and *Spodoptera litura* (Lepidoptera: Noctuidae) (Ayudya et al. 2019). Although the entomopathogenic fungi are generally reported to be host-specific (Farid and Syarief 2018), it is necessary to monitor the impact of these mycoinsecticides on the abundance of beneficial arthropods.

The impact of synthetic insecticide applications on beneficial arthropods has been widely studied (Azod et al. 2016; Khan et al. 2018). However, there is not much information on the impact of synthetic insecticide and mycoinsecticides application on aquatic insects. Aquatic insects in the rice field are generally dominated by predatory insects (Thirumalai et al. 2002). The abundance and diversity of the beneficial aquatic insect species in the rice field are high (Settle et al. 1996; Herlinda et al. 2019a,b). The commonly found of the aquatic insects were *Microvelia douglasi*, *Mesovelia vittigera*, *Anisops barbatus*, *Ranatra elongata*, and *Micronecta scutellaris*

(Thirumalai et al. 2002). These aquatic insects play an important role in the aquatic ecosystem (Dunbar et al. 2010) as natural enemies of rice insect pests (Heong et al. 2009).

The *B. bassiana*, *M. anisopliae* and *Cordyceps militaris* (Ascomycota: Cordycipitaceae) do not reduce abundance and species diversity of canopy predatory arthropods in the rice field (Prabawati et al. 2019), but the effect of the fungi on the species diversity and abundance of aquatic insects has not been studied. The intensive application of insecticides and mycoinsecticides may have an impact on the abundance and species diversity of the aquatic insects. The high abundance and species diversity of arthropod in water is an indicator of healthy aquatic ecosystems (Salachna and Olearczyk 2020). This study aimed to determine the effect of the mycoinsecticides application of *B. bassiana*, *M. anisopliae* and *C. militaris* and abamectin on the species diversity and abundance of aquatic insects in the rice field.

MATERIALS AND METHODS

Study area

The field experiment was carried out in the center of the freshwater swampy rice field in the *Pelabuhan Dalam* Village of Pemulutan Subdistrict, Ogan Ilir District of South Sumatra Province. The area of freshwater rice fields in the study site was more than 800 ha. The experimental plot covers 1 ha of the freshwater swampy rice field that divided into 12 subplots for 4 treatments, each treatment has 3 replicates. This study uses a Randomized Block Design. Field trials were conducted from May to August 2018 and continued with the identification of collected aquatic insects until December 2018. The four treatments used in this study were the application of mycoinsecticides derived from *B. bassiana, M. anisopliae, C. militaris*, and abamectin as a positive control. *B. bassiana* s.l. (BPCmS isolate) and *M. anisopliae* s.l. (MSwTp1 isolate) were used in this study from the exploration in the South Sumatra (Indonesia) soil by Safitri et al. (2018), while *C. militaris* s.l. (CmKT isolate) was collected from Central Kalimantan. The identification of three entomopathogenic fungal species used in this experiment was carried out by Dr. Suwandi (a mycologist from Universitas Sriwijaya). The fungi were identified based on microscopic and macroscopic characteristics.

Preparation of land and rice planting

Rice cultivation was carried out through the stages of tillage, seed preparation, planting, and maintenance as carried out by local farmers. Soil processing was carried out using a *singkal* plow machine and then smoothed using a hoe added running water so the soil becomes muddy. After the soil was cultivated, the rice was grown and added with a dose of 1 ton ha^{-1} manure and flooded with water for 14 days before planting.

Seed sowing by local farmers includes seed treatment, seed curing, and seed sowing. The seeds used were certified Mekongga variety at a dose of 50 kg ha^{-1}. The seeds were soaked for 24 hours with shrimp shell flour compost at a dose of 0.5 mL L^{-1}. Composting of shrimp shell flour was made according to the method of Suwandi et al. (2012). To facilitate germination after 24 hours soaking, broadcast the seeds in moist conditions on the rice field dike that has been coated with rice mud and moist manure. The seeds were broadcast by sprinkling called *Samir*.

Insecticides used in this study were mycoinsecticides and abamectin, while weeding was done mechanically without using herbicides. Watering was done by using a pump, and manure was used as fertilizer. Shrimp shell flour compost extract at a dose of 2 L ha^{-1} was applied every 2 weeks until the milky stage (milk-cooked phase) by spraying to increase soil fertility. Rice was planted using a *legowo* row of 2:1 planting system with a spacing of 12.5x50x50 cm^3. It was conducted by transplantation using 7-10 days old seedlings. Seedlings were transplanted with a maximum water level of half of rice seedlings height. The higher the rice plants, the more water was pumped into the fields. The plants were watered daily to accommodate and maintain the presence of aquatic insects inhabiting the rice field water. The rice field was inundated until nearing the ripe phase of milk and after the grains of rice were at the ripening stage, the fields began to be dried and the experiment was stopped.

Production and application of mycoinsecticides and abamectin

Mycoinsectides used as treatments in this study were derived from 3 species of entomopathogenic fungi, i.e. *B. bassiana* s.l., *M. anisopliae* s.l. and *C. militaris* s.l. and commercial insecticides abamectin as a positive control. Production of mycoinsecticides was following the modified method of Mascarin et al. (2015). Entomopathogenic fungi were cultured on Sabouraud Dextrose Agar (SDA, Merck) solid media and incubated for 10 days at room temperature. Entomopathogenic fungi grew on solid media were transferred into Sabouraud Dextrose Broth (SDB, Merck) liquid media and incubated for 7 days under agitation at 120 rpm. Liquid fungal culture with a minimum density of 1x10^9 conidia mL^{-1} was used as the active ingredient of mycoinsecticides. The mycoinsecticides were made from a mixture of 600 mL of liquid entomopathogenic fungal culture, 400 mL carrier made from shrimp shell flour compost extract, 10 mL vegetable oil, and 10 mL of a sterile aqueous solution of 0.04% polyoxyethyl-189 ene sorbitan mono-oleate (Tween®). Mycoinsectides were applied at a dose of 2 L ha^{-1} every two weeks, starting on the 14th, 28th, 42nd, and 56th days after transplanting (DAT), while abamectin was applied according to the manufacturer's instructions with the same spraying schedule with the mycoinsecticides.

Samplings of aquatic insects

One day after mycoinsecticides and abamectin were sprayed on the rice field, the sampling of aquatic insects was carried out, which were on the 15th, 29th, 43rd, and 57th DAT, i.e. 2nd of June 2018, 16th June 2018, 30th June 2018, and 17th July 2018, while the next sampling was not carried out because the rice fields began to dry out. The sampling of aquatic insects following the method of Salmah et al. (2017) by using a fishnet (0.4 mm mesh) conducted at 6.00-7.00 a.m.

Sampling was carried out by swinging the net into rice field water with a 2x2 m^2 sampling area for each treatment and repeated three times. The obtained insects were put into a vial containing 96% ethanol and then taken to the laboratory for identification. The taxonomic identification of aquatic insects using morphological features based on Basu et al. (2016) and Miller and Bergsten (2016) up to the genus level.

Statistical analyses

Data on the number of each species were used to calculate species abundance and diversity. The obtained data on species abundance were subjected to Analysis of variance (ANOVA) followed by Tukey's Honestly Significant Different (HSD) to determine significant differences among treatments at a level of 5% using the software program of SAS University Edition 2.7 9.4 M5. Analysis of the species diversity was carried out using the Shannon index (H'). The diversity level was also evaluated by the Evenness index (J') derived from the Shannon function and Berger-Parker dominance biodiversity indices.

RESULTS

Aquatic insect species

This study successfully collected 8 species of aquatic insects from all experimental plots, namely unidentified species of Dytiscidae, *Micronecta* sp., *Mesovelia* sp., *Ranatra* sp., *Anisops* sp., *Microvelia* sp., unidentified species of Veliidae, *Orthetrum* sp. (Figure 1) belong to 7 families (Dytiscidae, Corixidae, Mesoveliidae, Nepidae, Notonectidae, Veliidae, Libellulidae), 3 orders (Coleoptera, Hemiptera, and Odonata), (Table 1). The number of species found in the plot applied with *B. bassiana* s.l. was 6 species (unidentified species of Dytiscidae, *Micronecta* sp., *Mesovelia* sp., *Anisops* sp., *Microvelia* sp., and *Orthetrum* sp.) and in the plot applied with *M. anisopliae* s.l. were 6 species (unidentified species of Dytiscidae, *Micronecta* sp., *Mesovelia* sp., *Microvelia* sp., unidentified species of Veliidae, and *Orthetrum* sp.). In the plot applied with *C. militaris* s.l. were 7 species (unidentified species of Dytiscidae, *Micronecta* sp., *Mesovelia* sp., *Anisops* sp., *Microvelia* sp., unidentified species of Veliidae, and *Orthetrum* sp.). While in the abamectin plot there were only 5 species found (*Mesovelia* sp., *Ranatra* sp., *Anisops* sp., *Microvelia* sp. and *Orthetrum* sp.). In total, eight aquatic insect species were collected, all of the species were predators of rice insect pests.

Abundance and diversity of aquatic insect species

The abundance of aquatic insects tends to increase in plots applied with the mycoinsecticides (*B. bassiana*, *M. anisopliae*, and *C. militaris* s.l.), however, the abundance of the aquatic insect in the abamectin plot was not increased (Figure 2). The plots applied with *C. militaris* s.l. had the most abundant insects, while the plots applied with the abamectin had the lowest abundant insects. The abundance of each species was not significantly different, but in total, aquatic insect abundance was significantly different among treatments. The highest abundance of *Micronecta* sp. was in plot applied with *C. militaris* s.l. (37.67 individual 4 m^{-2}) but significantly different from that of plots applied with *B. bassiana* (30.33 individual 4 m^{-2}), *M. anisopliae* (30.33 individual 4 m^{-2}) and abamectin (0 individual 4 m^{-2}) (Table 2). The plot applied with abamectin showed a significant decrease in *Micronecta* sp.

The aquatic insects collected in the mycoinsecticides plots applied were belonging to 3 orders (Coleoptera, Hemiptera, and Odonata), while in the abamectin plot there were only 2 orders of aquatic insects (Hemiptera, and Odonata) (Figure 3). The most dominant order in all plots was Hemiptera. Species composition in the plots applied with mycoinsecticides was different from the abamectin plots (Figure 4). The species composition in the three mycoinsecticide plots had the same tendency. *Micronecta* sp. was not found in the abamectin plots, but *Micronecta* sp. was the most dominant species in the plots applied with mycoinsecticides. The presence and composition of aquatic insect species can be used as an indicator of the water quality.

The results of the study showed that the diversity of aquatic insect species increased with increasing age of rice. The species diversity was higher in plots applied with mycoinsecticides than plots applied with abamectin

(Table 3). The highest species diversity on the 15th and 29th DAT was in the plots applied with *C. militaris* s.l., followed by those applied with *B. bassiana* s.l. However, the highest species diversity on the 43rd – 57th DAT was in the plots applied with *B. bassiana*.

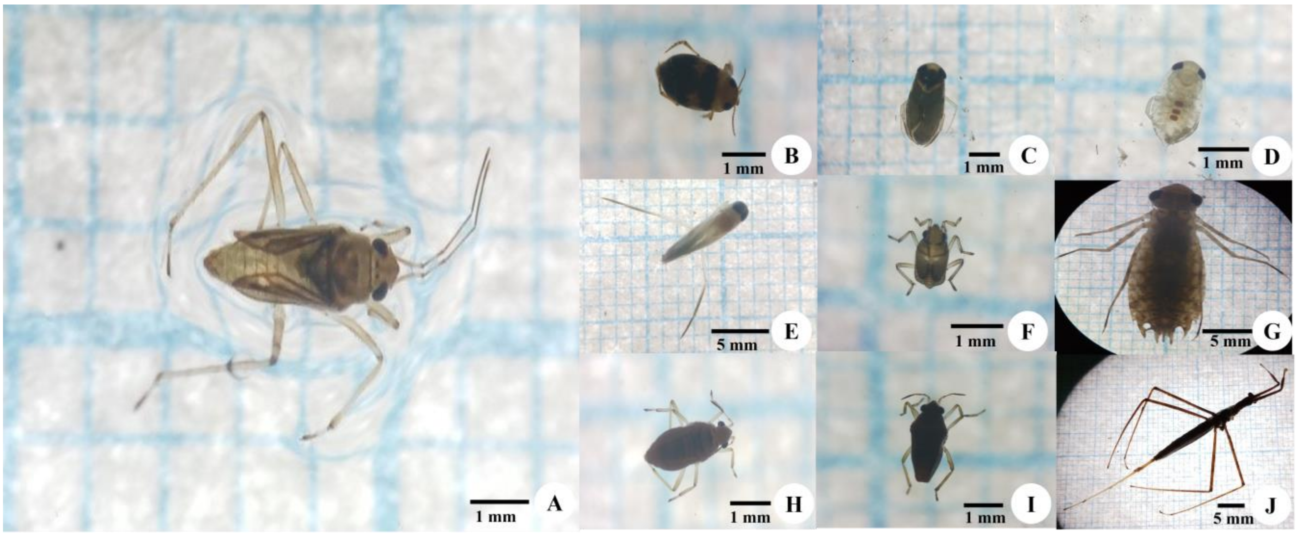

Figure 1. Aquatic insect species obtained in complete rice production cycle: *Mesovelia* sp. (A), unidentified species of Dytiscidae (B), *Micronecta* sp. adult (C), *Micronecta* sp. nymph (D), *Anisops* sp. (E), unidentified species of Veliidae (F), *Orthetrum* sp. (G), *Microvelia* sp. (H and I), *Ranatra* sp. (J)

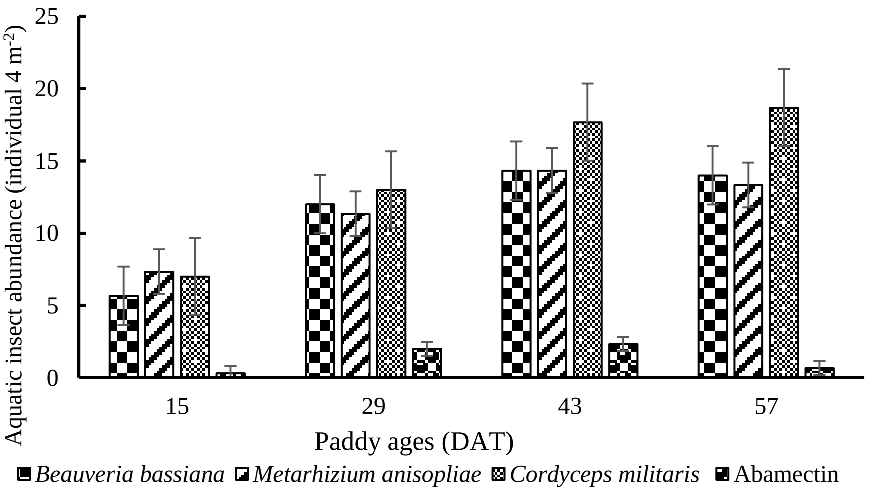

Figure 2. Aquatic insect abundance in rice field applied with bioinsecticides of *Beauveria bassiana* s.l. (A), *Metarhizium anisopliae* s.l. (B) and *Cordyceps militaris* s.l. (C) and abamectin (D)

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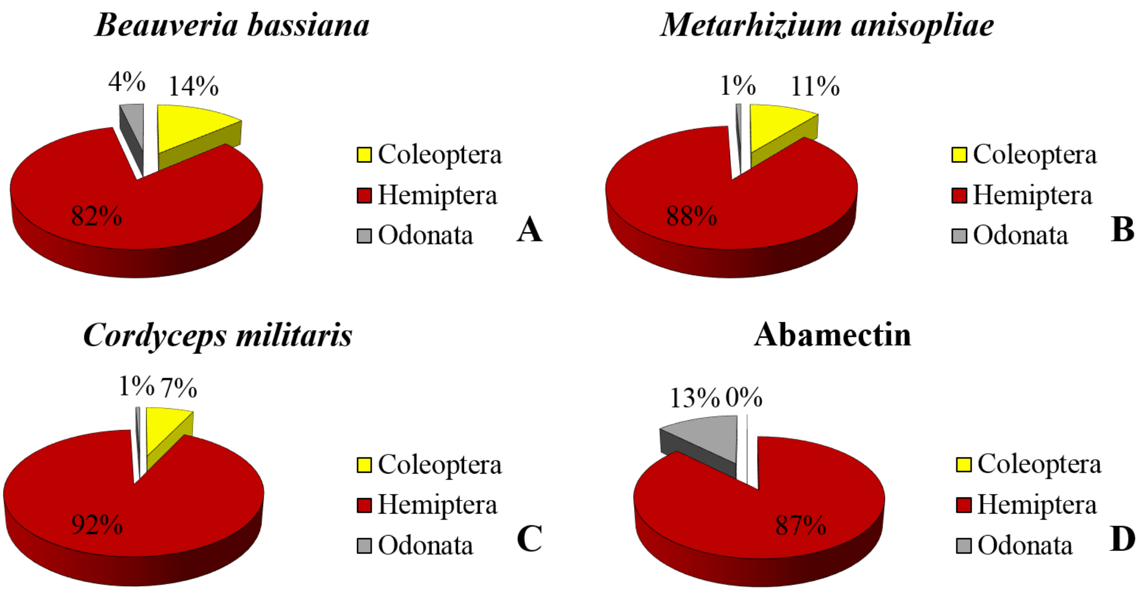

Figure 3. The relative abundance of the order of aquatic insect in rice field applied with bioinsecticides of *Beauveria bassiana* s.l. (A), *Metarhizium anisopliae* s.l. (B) and *Cordyceps militaris* s.l. (C) and abamectin (D)

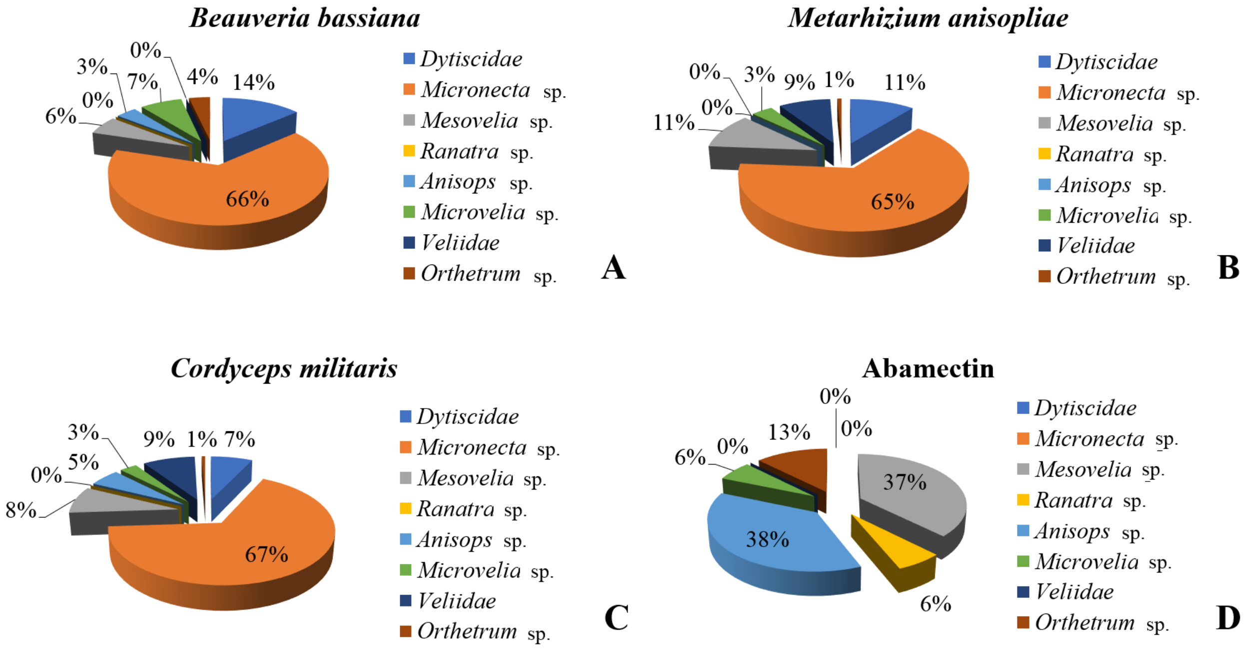

Figure 4. The relative abundance of aquatic insect species in rice field applied with bioinsecticides of *Beauveria bassiana* s.l. (A), *Metarhizium anisopliae* s.l. (B) and *Cordyceps militaris* s.l. (C) and abamectin (D)

Table 1. Aquatic insect in rice field applied with bioinsecticides of *Beauveria bassiana* s.l., *Metarhizium anisopliae* s.l. and *Cordyceps militaris* s.l. and abamectin

Order	**Family**	**Species**	**Insecticide treatment**			
			***Beauveria bassiana* s.l.**	***Metarhizium anisopliae* s.l.**	***Cordyceps militaris* s.l.**	**Abamectin**
Coleoptera	Dytiscidae	Unknown species	+	+	+	-
Hemiptera	Corixidae	*Micronecta* sp.	+	+	+	-
	Mesoveliidae	*Mesovelia* sp.	+	+	+	+
	Nepidae	*Ranatra* sp.	-	-	-	+
	Notonectidae	*Anisops* sp.	+	-	+	+
	Veliidae	*Microvelia* sp.	+	+	+	+
		Unknown species	-	+	+	-
Odonata	Libellulidae	*Orthetrum* sp.	+	+	+	+

Note: + insects found, - no insects found

Table 2. The abundance of aquatic insect species in rice field applied with bioinsecticides of *Beauveria bassiana* s.l., *Metarhizium anisopliae* s.l. and *Cordyceps militaris* s.l. and abamectin

Rice age (DAT)	**Species of aquatic insect**	**The abundance of aquatic insect species (individual 4 m^{-2})**				**F value**	**P value**	**Tukey's HSD test**
		***Beauveria bassiana* s.l.**	***Metarhizium anisopliae* s.l.**	***Cordyceps militaris* s.l.**	**Abamectin**			
15 DAT	Dytiscidae	0.00	0.00	0.00	0.00	-	-	-
	Micronecta sp.	5.33	6.33	5.67	0.00	0.25^{ns}	0.86	-
	Mesovelia sp.	0.00	0.67	0.67	0.00	0.57^{ns}	0.65	-
	Ranatra sp.	0.00	0.00	0.00	0.00	-	-	-
	Anisops sp.	0.00	0.00	0.00	0.00	-	-	-
	Microvelia sp.	0.00	0.00	0.67	0.33	0.62^{ns}	0.63	-
	Veliidae	0.00	0.00	0.00	0.00	-	-	-
	Orthetrum sp.	0.33	0.33	0.00	0.00	0.57^{ns}	0.65	-
29 DAT	Dytiscidae	0.00	0.00	0.00	0.00	-	-	-
	Micronecta sp.	11.67	7.00	6.00	0.00	1.51^{ns}	0.30	-
	Mesovelia sp.	0.00	4.33	4.00	2.00	0.41^{ns}	0.75	-
	Ranatra sp.	0.00	0.00	0.00	0.00	-	-	-
	Anisops sp.	0.00	0.00	3.00	0.00	1.00^{ns}	0.45	-
	Microvelia sp.	0.00	0.00	0.00	0.00	-	-	-
	Veliidae	0.00	0.00	0.00	0.00	-	-	-
	Orthetrum sp.	0.33	0.00	0.00	0.00	1.00^{ns}	0.45	-
43 DAT	Dytiscidae	3.00	2.67	1.33	0.00	0.78^{ns}	0.55	-
	Micronecta sp.	7.00	10.00	11.33	0.00	1.80^{ns}	0.25	-
	Mesovelia sp.	0.00	0.00	0.00	0.00	-	-	-
	Ranatra sp.	0.00	0.00	0.00	0.00	-	-	-
	Anisops sp.	1.67	0.00	0.00	2.00	0.57^{ns}	0.65	-
	Microvelia sp.	1.67	1.67	0.00	0.00	1.00^{ns}	0.45	-
	Veliidae	0.00	0.00	5.00	0.00	1.00^{ns}	0.45	-
	Orthetrum sp.	1.00^{c}	0.00^{a}	0.00^{a}	0.33^{b}	8.00*	0.02	0.22
57 DAT	Dytiscidae	3.33	2.33	2.67	0.00	1.33^{ns}	0.35	-
	Micronecta sp.	6.33	7.00	14.67	0.00	1.54^{ns}	0.30	-
	Mesovelia sp.	2.67	0.00	0.00	0.00	1.00^{ns}	0.45	-
	Ranatra sp.	0.00	0.00	0.00	0.33	1.00^{ns}	0.45	-
	Anisops sp.	0.00	0.00	0.00	0.00	-	-	-
	Microvelia sp.	1.67	0.00	1.00	0.00	0.59^{ns}	0.64	-
	Veliidae	0.00	4.00	0.00	0.00	3.50^{ns}	0.09	-
	Orthetrum sp.	0.00	0.00	0.33	0.33	0.57^{ns}	0.65	-
Total	Dytiscidae	6.33	5.00	4.00	0.00	2.57^{ns}	0.15	-
	Micronecta sp.	30.33^{b}	30.33^{b}	37.67^{c}	0.00^{a}	13.91*	0.00	1.67
	Mesovelia sp.	2.67	5.00	4.67	2.00	0.11^{ns}	0.95	-
	Ranatra sp.	0.00	0.00	0.00	0.33	1.00^{ns}	0.45	-
	Anisops sp.	1.67	0.00	3.00	2.00	0.27^{ns}	0.84	-
	Microvelia sp.	3.34	1.67	1.67	0.33	0.88^{ns}	0.50	-
	Veliidae	0.00	4.00	5.00	0.00	1.17^{ns}	0.40	-
	Orthetrum sp.	1.66	0.33	0.33	0.66	2.60^{ns}	0.15	-

Note: ns = not significantly different; * = significantly different; values within a row followed by the same letters were not significantly different at $P < 0.05$ according to Tukey's HSD test. Original data were transformed using $\sqrt{(n_i+0.5)}$ transformation before statistical analysis

Table 3. Characteristics of aquatic insect communities in rice field applied with bioinsecticides of *Beauveria bassiana* s.l., *Metarhizium anisopliae* s.l. and *Cordyceps militaris* s.l. and abamectin

Rice age (DAT)	**Characteristics of communities**	**Insecticides**			
		***Beauveria bassiana* s.l.**	***Metarhizium anisopliae* s.l.**	***Cordyceps militaris* s.l.**	**Abamectin**
15 DAT	Abundance of aquatic insect species (N) (individual 4 m^{-2})	5.67	7.33	7.00	0.33
	Shanon wiener index (H')	0.22	0.49	0.62	0.00
	Evennes index (E)	0.32	0.44	0.56	0.00
	Dominance index (D)	0.94	0.86	0.81	1.00
29 DAT	Abundance of aquatic insect species (N) (individual 4 m^{-2})	12.00	11.33	13.00	2.00
	Shanon wiener index (H')	0.13	0.67	1.06	0.00
	Evennes index (E)	0.18	0.96	0.96	0.00
	Dominance index (D)	0.97	0.62	0.46	1,00
43 DAT	Abundance of aquatic insect species (N) (individual 4 m^{-2})	14.33	14.33	17.67	2.33
	Shanon wiener index (H')	1.36	0.81	0.84	0.41
	Evennes index (E)	0.85	0.74	0.76	0.59
	Dominance index (D)	0.49	0.70	0.64	0.86
57 DAT	Abundance of aquatic insect species (N) (individual 4 m^{-2})	14.00	13.33	18.67	0,66
	Shanon wiener index (H')	1.27	1.00	0.70	0.69
	Evennes index (E)	0.92	0.91	0.50	1.00
	Dominance index (D)	0.45	0.53	0.79	0.50
Total	Abundance of aquatic insect species (N) (individual 4 m^{-2})	46,00	46.33	56.33	5.33
	Shanon wiener index (H')	1.14	1,12	1,17	1,34
	Evennes index (E)	0,64	0,63	0,60	0.83
	Dominance index (D)	0,66	0,65	0,67	0,38

Discussion

Eight aquatic insect species were obtained in the center of freshwater swamps from June 2nd to July 17th. In the dried rice field after July 17 the aquatic insects were no longer found. However, in the rice fields that still inundated; the aquatic insects still could be found. Therefore, the presence of deep freshwater swamp is important for conserving aquatic insects in the dry season.

All species of aquatic insects collected in this study were predatory insects that attack rice insect pests. All species belonging to Dytiscidae (unidentified species) or diving beetles were predators of insects whose habitat is close to the water surface (Watanabe 2019) such as living on the stems and lower leaves and attacking the rice insect pests, such as *Gryllotalpa orientalis* (Ohba 2009). *Micronecta* sp. and *Mesovelia* sp. are predatory insects commonly found and suck out the internal body fluids of the rice insect pests (Wakhid et al. 2020). *Ranatra* sp. is predators that have a role in regulating the balance of insect populations of the rice insect pests (Thongphak and Iwai 2016). *Anisops* sp. is commonly found in rice field water in Indonesia and also known as a predator for rice insect pests (Wakhid et al. 2020). *Microvelia* sp. and Veliidae (unidentified species) were reported as predators of brown planthoppers (Heong, 2009). Immature and adult stages of *Orthetrum* sp. prey on various insect species from various families (Salmah et al. 2017; Wakhid et al. 2020). The aquatic insects in rice fields obtained in this study generally attack rice insect pests that inhabit the base of the stem, such as brown planthopper (*N. lugens*) (Heong 2009.

The results showed that the abundance of aquatic insects decreased significantly in the abamectin plot, but it remained high in all three plots applied with mycoinsecticides. Abamectin is an insecticide that is commonly applied in the rice field to control brown planthoppers in Indonesia. Abamectin is contact poison and stomach poison and widely chosen and applied by farmers in Indonesia because it has a broad spectrum that kills many species of insect pests (Luo et al. 2013). Abamectin that flows into the rice field water will poison and cause the death of aquatic insects.

The abundance of aquatic insects in the three plots applied with mycoinsecticides from *B. bassiana* s.l., *M. anisoplia* s.l. and *C. militaris* s.l. remain high since these mycoinsecticides did not poison the aquatic insects. These three entomopathogenic fungal species have specific hosts, i.e. the order of Lepidoptera or Homoptera (Shrestha et al. 2012; Farid and Syarief 2018), while the aquatic insects obtained in this study belong to the orders of Hemiptera, Coleoptera, and Odonata. *B. bassiana* is effective in poisoning and killing *S. litura* (Lepidoptera) (Ayudya et al. 2019), and *N. lugens* (Homoptera) (Sumikarsih et al. 2019). *M. anisoplia* effectively attacks Lepidoptera (Ayudya et al. 2019), while *C. militaris* generally attacks insect pests of which attacks palm oil leaves (Lepidoptera) (Shrestha et al. 2012). Prabawati et al. (2019) reported that application of *B. bassiana*, *M. anisoplia*, and *C. militaris* in rice fields reduce insect pest population, but do not harm predatory arthropods inhabiting the canopy and rice soil surfaces. Therefore, the application of *B. bassiana*, *M. anisopliae*, and *C. militaris* in rice fields are beneficial in reducing rice insect pests and safe for the aquatic insect as predators of rice insect pests.

Abundance and species diversity of aquatic insect are increasing along with the increasing age of rice in the plots treated with mycoinsecticides. The more complex the structure of plants are, the more complex the species of insects associated with the plants (Settle et al. 1996) and the more abundant and diverse insect pests in the rice canopy with increasing age of rice plants (Prabawati et al. 2019). The abundance of pests in rice are prey for aquatic insects (Cheong 2009)

The diversity of aquatic insect species in plots applied with abamectin was lower than those applied with the mycoinsecticides. Three species were not found in the abamectin plots (unidentified species of Dytiscidae, *Micronecta* sp., and unidentified species of Veliidae). This could be due to the broad spectrum of abamectin that can kill various insect species (Luo et al. 2013) including the aquatic insects (Thongphak and Iwai 2016) in this study. These, three species were susceptible to abamectin. The *Micronecta* sp. is generally found in a healthy aquatic ecosystem. The decreasing number of species or diversity of aquatic insect species in the rice fields could be an indicator of decreasing water quality of the rice fields (Salmah et al. 2017).

It can be concluded that all eight aquatic insect species obtained in this study were predators of rice insect pests. The eight aquatic insect species were: unidentified species of Dytiscidae, *Micronecta* sp., *Mesovelia* sp., *Ranatra* sp., *Anisops* sp., *Microvelia* sp., unidentified species of Veliidae, and *Orthetrum* sp. belong to 7 families (Dytiscidae, Corixidae, Mesoveliidae, Nepidae, Notonectidae, Veliidae, and Libellulidae), and three orders (Coleoptera, Hemiptera, and Odonata). The application of mycoinsecticides from *B. bassiana* s.l., *M. anisopliae* s.l. and *C. militaris* s.l. did not reduce the abundance and species diversity of the aquatic predatory insects, while the application of abamectin reduces the abundance and species diversity of the predatory insects. The application of mycoinsecticides from *B. bassiana* s.l., *M. anisopliae* s.l. and *C. militaris* s.l. is a safe application for the aquatic predatory insects, and to control rice insect pests than that of abamectin applications.

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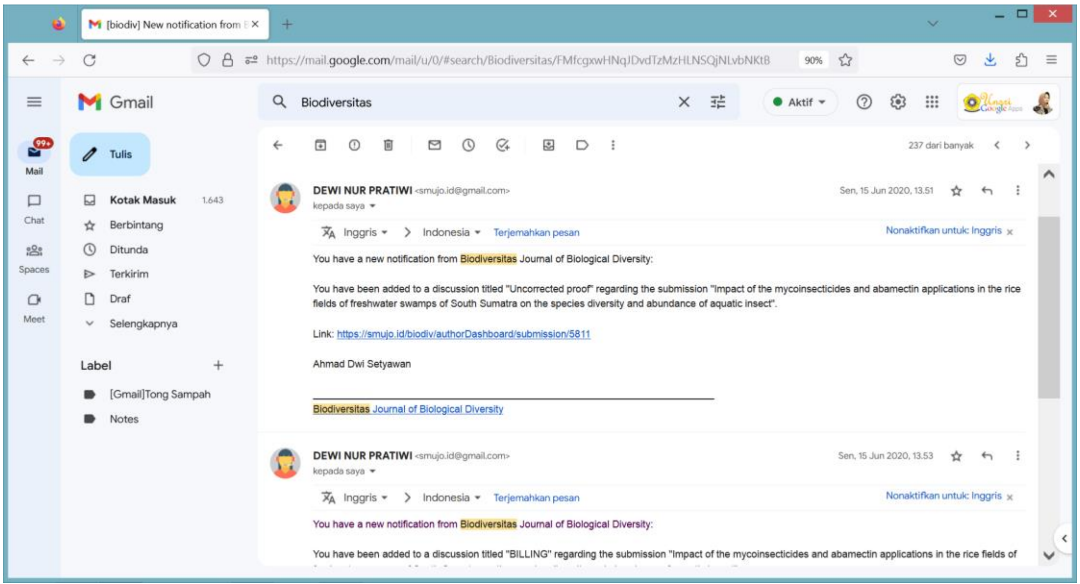

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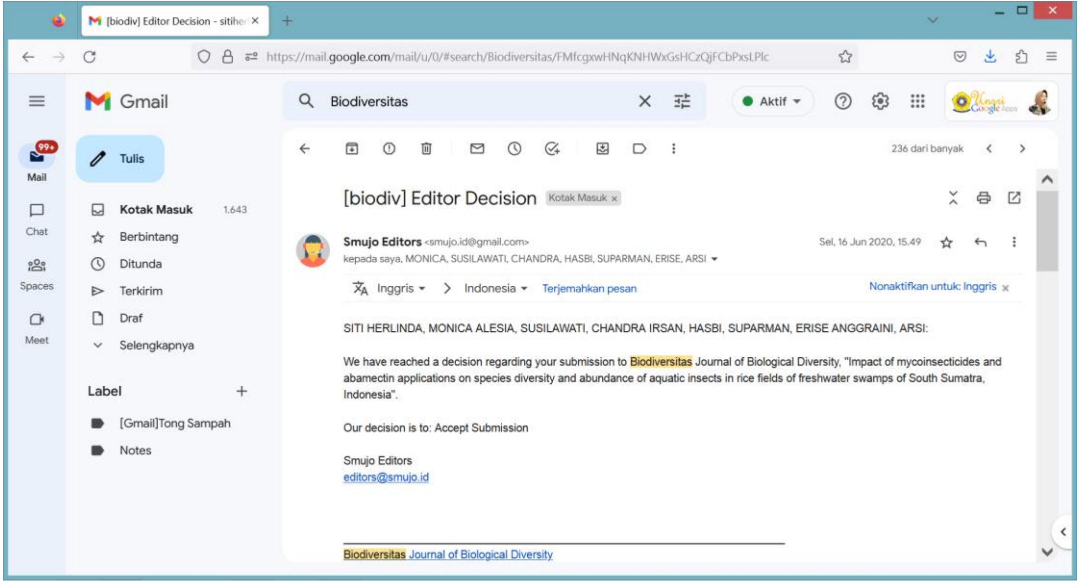

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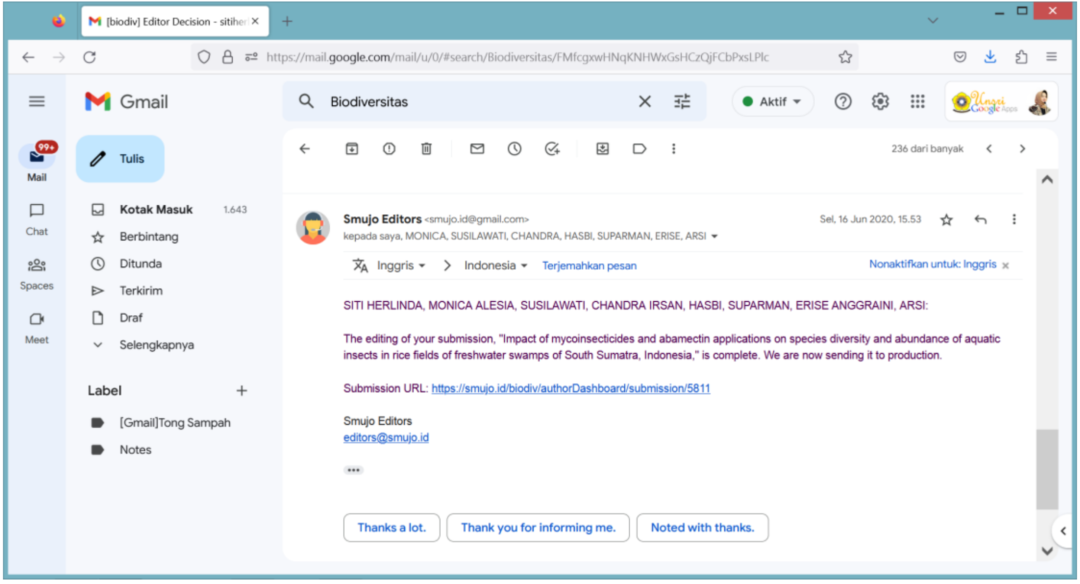

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