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BIODIVERSITAS
Volume 21, Number 8, August 2020
Pages: 3755-3768

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d210843

Herbivore insects and predatory arthropods in freshwater swamp rice field in South Sumatra, Indonesia sprayed with bioinsecticides of entomopathogenic fungi and abamectin

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Manuscript received: 6 June 2020. Revision accepted: 25 July 2020.

Abstract. *Herlinda S, Prabawati G, Pujiastuti Y, Susilawati, Karenia T, Hasbi, Irsan C. 2020. Herbivore insects and predatory arthropods in freshwater swamp rice field in South Sumatra, Indonesia sprayed with bioinsecticides of entomopathogenic fungi and abamectin. Biodiversitas 21: 3755-3768.* Herbivore insect population and predatory arthropods in rice field may be effected by the application entomopathogenic fungi or synthetic insecticide. The objective of this research was to analyze individual quantity of herbivore insects and predatory arthropod inhabiting freshwater swamp rice fields treated with bioinsecticides and abamectin (commercial insecticide). This research was conducted in the freshwater swamp rice field located in Village Pelabuhan Dalam, Sub District Pemulutan, District Ogan Ilir, and South Sumatra. The experiment was arranged in a Completely Randomized Block Design consisted of four plots of treatment**.** The research used the bioinsecticides made from entomopathogenic fungi (*Beauveria bassiana*, *Metarhizium anisopliae*, and *Cordyceps military*), and abamectin. Sampling to collect arthropods inhabiting rice canopy was also conducted using an entomological net in the study locations. This research found 12 families of herbivore insects with 22 species dominated by *Nilaparvata lugens* and *Leptocorisa acuta* and 32 species of spider belonged to 8 families dominated by *Tetragnatha virescens* and *Oxyopes matiensis*. The species diversity of spider was higher in the plots of the bioinsecticide compared to that of the abamectin. Predatory insects found belonged to 14 species belonged to 8 families dominated by species of *Ophionea nigrofasciata, Verania discolor,* and *Paedorus fuscipe*. The abundance of predatory insects in plots sprayed with the bioinsecticides was higher compared to that of the abamectin plot. The abundance and species diversity of predatory arthropod sprayed with bioinsecticide of *B. bassiana*, *M. anisopliae*, and *C. militaris* did not decrease, while the population of herbivore insect tended to decrease.

Keywords: *Beauveria bassiana*; *Metarhizium anisopliae*; *Cordyceps militaris*; neutral insect; parasitoid

INTRODUCTION

Rice cultivation in freshwater swamps of South Sumatra has specific characteristics making it different from rice cultivation in other ecosystems in Indonesia (Karenina et al. 2020). Rice cultivation is affected by climate conditions, especially rainfall and tidal flush (Herlinda et al. 2020). From November to April, freshwater swamps are generally inundated so that local farmers cultivate rice only in the period of May to September (Herlinda et al. 2018; Herlinda et al. 2019a). Due to difficulties in managing water, farmers grow rice once a year using the transplanting method. In maintaining their rice cultivations, the local farmers scarcely apply synthetic insecticide (Herlinda et al. 2019b).

The population of insect pests attacking freshwater swamp rice plants is increasing and starting to result in yield loss, especially rice bug (*Leptocorixa acuta*), while brown planthoppers (*Nilavarpata lugens*) that have not appeared for a long time start attacking (Hanif et al. 2020). Pests that attack rice plants have been controlled using various entomopathogenic fungi including *Beauveria bassiana* (Li et al. 2012; Li et al. 2014; Lee et al. 2015), *Metarizhium anisopliae* (Girish and Balikai, 2015; Chinniah et al. 2016) and *Cordyceps militaris* (Prabawati et al. 2019). *C. militaris* is reported to produce beauvericin that is toxic to insects (Rachmawati et al. 2018) and *B. bassiana* has been reported to be effective against homopteran (Li et al. 2012; Li et al. 2014; Lopez et al. 2014; Lee et al. 2015; Sumikarsih et al. 2019), coleopteran (Kavallieratos et al. 2015), hemipteran (Girish and Balikai 2015), and lepidopteran insects (Ayudya et al. 2019; Ma et al. 2019; Gustianingtyas et al. 2020). *M. anisopliae* has also been demonstrated to be effective against homopteran (Mweke et al. 2019), lepidopteran (Gustianingtyas et al. 2020), and coleopteran insects (Kavallieratos et al. 2015). *M.*

anisopliae could be used in multiple roles, ranging from controlling insect pests and promoting plant growth (Liu et al. 2017).

Being effective against various orders of insects, including the coleopteran insects (Kavallieratos et al. 2015), the rice field ecosystem which is dominated by species of predatory insects, it is important to be very careful in the application of entomopathogenic fungi in ecosystem to avoid negative impact on non-target arthropods (Scorsetti et al. 2017). High species diversity and abundance of arthropod in freshwater swamp rice field (Herlinda et al. 2018) might be disturbed by the application of the entomopathogenic fungi. However, there have been some reports suggested that entomopathogenic fungi did not affect abundance and species diversity of arthropods (Prabawati et al. 2019; Hanif et al. 2020). For example, predatory insect in paddy field (*Andrallus spinidens*) was reported to be resistant to *B. bassiana* (Firouzbakht et al. 2015; Gholamzadeh-Chitgar et al. 2017; Scorsetti et al. 2017). Parasitoid tends to avoid host insect infected by entomopathogenic fungi (Emami et al. 2013). Coccinellidae, the family of generalist predatory insects, was reported to be resistant to *M. anisopliae* (Bayissa et al. 2016). This research aimed to analyze individual quantity of herbivore insects and predatory arthropod inhabiting freshwater swamp rice field treated with bioinsecticides containing *Beauveria bassiana*, *Metarhizium anisopliae*, and *Cordyceps militaris* and the abamectin.

MATERIALS AND METHODS

Study area

The research was conducted from April to August 2018 in the freshwater swamp rice field located in Village Pelabuhan Dalam, Sub District Pemulutan, District Ogan Ilir, and South Sumatra, Indonesia. The experiment was arranged in a Completely Randomized Block Design consisted of four plots of treatment. The first three plots were treated with bioinsecticide containing *B. bassiana*, *M. anisopliae*, and *C. military,* and the fourth plot was treated with the abamectin (the commercial insecticide) as control. The plot area was 120 m^2 for treatment and each treatment was replicated five times. Distance between plots was 10 m.

Land preparation, transplanting, and crop maintenance

Land preparation was conducted using a moldboard plow and was continued by using harrow plow. Before being plowed, the land was cleared using a hoe and a long knife. The excessive water was pumped out until the soil was slightly watery, and the dung compost was added at a dosage of 1 ton ha^{-1}. Rice seed used was certified purple labeled seed of Mekongga variety at a dosage of 50 kg/ha. Rice was cultivated using a transplanting system from which the seedlings were prepared in 34 x 26 x 5 cm^3 plastic trays filled with 3 cm high soil. The seeds were soaked in water for 24 hours before being planted in the prepared plastic trays. The soaked seeds were spread evenly in each tray at a dosage of 150 g per tray. The trays were then covered with thick plastic for seven days. The seven-day-old seedlings were then transferred to places receiving enough sunlight. Fourteen-day old seedlings were transplanted to all prepared plots at a density of 2 seedlings per hole in a 2:1 (12.5 x 25 x 50 cm^3) "jajar legowo" planting system (Figure 1).

Crop maintenance was conducted in the form of replanting, irrigating, weeding, and fertilizing. Replanting was conducted seven days after transplanting to replace the dead seedlings. Irrigating was conducted by making use of water flush from the river under high tide and pumping system. Weeding was conducted both at vegetative and generative stages using a sickle. Fertilizer used was shrimp shell extract compost prepared according to the method by Suwandi et al. (2012). The compost was applied every two weeks started from 14 days after planting until harvesting (84 days) at a dosage of 2 L ha^{-1}. The extract compost was a fermentation result of shrimp shell meal containing chitinolytic bacteria, cellulolytic bacteria, and sulfate diluter. Nitrogen fertilizer was applied 30 days after planting at a dosage of 100 kg ha^{-1}.

A B C D

Figure 1. Two-day old rice seedling in a plastic tray (A), 7-day old seedlings (B), rice transplanting (D) rice planted in "jajar legowo" planting system (E)

Formulation and application of bioinsecticides

All species of fungi have been explored from soil following the modified method of Kin et al. (2017). *B. bassiana* and *M. anisopliae* used in this research were explored from freshwater swamp soil of South Sumatra, while *C. militaris* was explored from the soil in Central Kalimantan, Indonesia. *B. bassiana*, *M. anisopliae*, and *C. militaris* were cultured in solid media of Sabouraud Dextrose Agar (SDA, Merck) made from 16.2 g SDA in 250 mL aquadest. The cultures were incubated for 10 x 24 hours. The 10 days old fungal solid cultures were then cultured in liquid media (Figure 2) of Sabouraud Dextrose Broth (SDB, *Merck*) made from 30 g SDB in 1000 ml aquadest and were incubated in a 120 rpm shaker for 7 x 24 hours. The liquid culture of the entomopathogenic fungi was used to make bioinsecticide. Seven days after incubation, the density of fungal spores of the bioinsecticide was counted, the counting was stopped when the spore density reached 1 x 10^9 conidia mL^{-1}. One liter of bioinsecticide was made by adding 400 mL shrimp shell meal extract compost as a carrier and active ingredient consisting of 600 mL liquid culture of entomopathogenic fungi and 10 mL vegetable oil. Compost extract used to make bioinsecticide was previously autoclaved under 1 atm for 2 hours.

The bioinsecticides were applied at a dosage of 2 L ha^{-1} per application. The applications were conducted at 13, 27, 41, 55, 69, and 83 days after transplanting (DAT). The bioinsecticide applications were conducted in the evening (at 5 pm) to avoid spores damaged by ultraviolet. The control plot was sprayed with abamectin at a dosage of 0.5 L ha^{-1}. The insecticide contained abamectin active ingredient and was sprayed at the same time as bioinsecticide applications. The control plot was 50 m away from treatment plots to avoid contamination in the treatment plots, and there was 10 m distance between bioinsecticide treatment plots. A day after application, arthropod samplings were carried out to collect arthropods inhabiting rice canopy.

Arthropod sampling in rice canopy

Sampling to collect arthropods inhabiting rice canopy was conducted using an entomological net (75 cm length, 30 cm diameter, and 100 cm length of handle) (Lami et al. 2016; Ivantsova et al. 2017). Sampling was conducted in every treatment subplot and was repeated five times. Samplings were conducted at 14, 28, 42, 56, 70, and 84 DAT. Arthropod samplings were conducted in the morning, at 06.00-07.00 am. During sampling, the sweep net was swept 30 cm depth into rice canopy to collect representative samples.

Sampled arthropods were cleaned and placed in the labeled plastic bottles (330 mL) containing 100 mL absolute ethanol. The samples were brought into the Laboratory of Entomology, Department of Plant Pest and Disease, Faculty of Agriculture, Universitas Sriwijaya for identification. Spider identification was conducted using the reference of Barrion and Litsinger (1995), while for insect identification we used reference of Gullan and Cranston (2014) and Heinrichs et al. (2017).

Data analysis

Data of arthropods species were grouped based on their guild, comprised of herbivore insects, predators, parasitoids, and neutral insects, and was presented in the form of a graphic. The difference of individual quantity of the herbivore insects, spiders, and predatory insects amongst treatments was analyzed using Analysis of Variance (ANOVA). If there was a significant difference among treatments, the analysis was continued with Tukey's HSD (Honesty Significant Difference) test at 5% degree of significance. Analyses data was conducted using Software of SAS University Edition 2.7 9.4 M5. Data of abundance were used to analyze species diversity by using the Shannon index (H'). Degree of diversity was counted using the Evenness index (J') derived from the Shannon function, and Berger-Parker dominance biodiversity indices. The coefficient of Sorensen was counted to measure the degree of similarity of the spider or predatory insect among treatments (Magurran 2004).

A B C D E F

Figure 2. Cultures of *Beauveria bassiana* (A), *Metarhizium anisopliae* (B), and *Cordyceps militaris* (C) on SDB; liquid bioinsecticide of *Beauveria bassiana* (D), *Metarhizium anisopliae* (E), and *Cordyceps militaris* (F)

RESULTS AND DISCUSSION

Abundance of herbivore insects in one rice cropping season

In one rice cropping season, it was found 22 species of herbivore insects belonged to 12 families. Dominant herbivore species were *Nilaparvata lugens, Leptocorisa acuta, Acrida turrita, Valanga nigricornis,* and *Dicladispa armigera* (Figure 3). The population of the herbivore was not significantly different among treatments, except for Family Cecidomyiidae (Table 1). Therefore, the capacity of bioinsecticide in suppressing the population of the herbivore insects has generally almost similar to the capacity of the abamectin.

During field observations, insect died due to infection by entomopathogenic fungi were documented and showed specific symptoms of each fungus. The larvae of *S. litura* found infected by *B. bassiana* in the rice field showed white mycelia covering its body, the dried, and not smelly (Figure 4). Larvae of *S. litura* infected by *M. anisopliae* showed symptoms of the dried body covered by greenish white mycelia and was not smelly, while larvae of *S. litura* infected by *C. militari*s showed similar symptoms to those of *B. bassiana* infection, larval body drying, shriveled, covered by white mycelia, and not smelly. Entomopathogenic fungi infected sampled insects were isolated and purified in the laboratory. Color, structure, and spore of the isolated fungi were as presented in Figure 5.

Abundance of spider and predatory insect in one rice cropping season

This research found 32 species of spiders belonged to eight families. The dominant species of them were *Tetragnatha virescens, T. virescens*, *T. maxilosa*, *Argiope catenulata*, and *Oxyopes matiensis* (Figure 6). The total abundance of spiders in the plot treated with the abamectin was significantly lower than that of plots treated with bioinsecticide (Table 2). The abundance of *Bathyphantes* sp. in abamectin plot decreased. The highest abundance of *Araneus inustus* was found in the plot treated with bioinsecticide *C. militaris* and was different from that of other treatments. The abundance of Tetragnathidae was significantly decreasing in the plot treated with insecticide was found to be lower compared to plots treated with bioinsecticide.

Predatory insects found in this research belonged to 14 species and eight families. The dominant species were *Formicomus* sp., *Ophionea nigrofasciata, Verania discolor, Verania lineata, Menochilus sexmaculatus, Micrapis inop, Paedorus fuscipe, Cyrtorhinus lividipennis*, and *Orthotylus* sp. (Figure 7). The abundance of predatory insects was not significantly different among treatments (Table 3).

Characteristic of spider and predatory insect community in one rice cropping season

The number of spiders increased with increasing rice age (Table 4). The highest number of spiders occurred when the rice was at a mature grain ripening stage (84 DAT). The abundance of spider in the plot treated with abamectin tend to be lower compared to that of plots treated with the bioinsecticides. The total number of spiders in one rice cropping season was higher in the plots treated with the bioinsecticide than that in the plot treated with the abamectin. The species diversity of spiders was also higher in the plots treated with entomopathogenic fungal bioinsecticide compared to that in the abamectin plot. The high species diversity in plots treated with entomopathogenic fungal bioinsecticide was also followed by the high species evenness and low species dominance.

Number of predatory insects decreased with increasing stages of rice, but at approaching harvest (84 DAT), the number of predatory insects drastically decreased (Table 5). The highest abundance of predatory insects occurred at the milk grain ripening stage (70 DAT). The phenomenon of abundance fluctuation of predatory insects was slightly different from that of spider, which its peak occurred at a mature grain ripening stage (84 DAT). The abundance of the predatory insects in plots treated with abamectin was generally lower than that of plots treated with the entomopathogenic fungal bioinsecticide. Total abundance of predatory insects in one rice cropping season of plots sprayed with entomopathogenic fungal bioinsecticide was higher compared to that of abamectin plot. However, species diversity, evenness, and species dominance tend to be similar among treatments.

Spider community in plot sprayed with the bioinsecticide of *B. bassiana* tends to be more similar to that of plots sprayed with bioinsecticide (*M. anisopliae* and *C. militaris*) than that of abamectin plot. The total of one rice cropping season, spider community in plot sprayed with bioinsecticide *B. bassiana* was more similar to that of plot of *C. militaris* (0.77), followed by plot *M. anisopliae* (0.76), and least similar to that of the abamectin plot (0.49) (Table 6).

Predatory insect community in plot sprayed with bioinsecticide *B. bassiana* tent to be more similar to that of plots sprayed with bioinsecticide *M. anisopliae* and *C. militaris* than that of the abamectin plot (Table 7). The total of one rice cropping season, predatory insect community in plots sprayed with bioinsecticide *B. bassiana* was more similar to that of plot *M. anisopliae* (0.91), followed by plot of *C. militaris* (0.85), and least similar to that of insecticide plot (0.66).

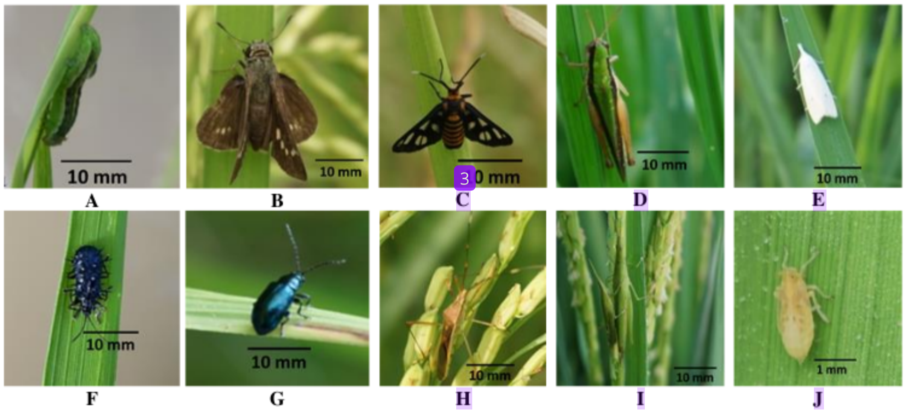

Figure 3. Herbivore insects found in rice canopy: *Spodoptera* sp. (A), *Pelopidas* sp. (B), *Amata nigriceps* (C), *Oxya chinensis* (D), *Scirpophaga* sp. (E), *Dicladispa armigera* (F), *Chrysolina coerulans* (G), *Leptocorisa acuta* (H), *Acrida turita* (I), *Nilaparvata lugens* (J)

Table 1. Population of herbivore insects in rice canopy sprayed with bioinsecticides (*B. bassiana, M. anisopliae, C. militaris)* and abamectin

Ordo / family/ species	Mean of herbivore abundance (individuals/nets)				F-value	P-value (0.05)	Tukey's HSD test
	B. bassiana	*M. anisopliae*	*C. militaris*	Abamectin			
COLEOPTERA	0.2	0.13	0.17	0.23	0.26	0.85	
Chrysomelidae	0.2	0.13	0.17	0.23	0.26	0.85	
Chrysolina coerulans	0.07	0	0.07	0.2	2.11	0.15	
Dicladispa armigera	0.13	0.13	0.1	0.03	0.53	0.67	
DIPTERA	0.4^{b}	0.03^{ab}	0.07^{ab}	0.00^{a}	4.40*	0.03	0.20
Cecidomyiidae	0.4^{b}	0.03^{ab}	0.07^{ab}	0.00^{a}	4.40*	0.03	0.20
Cecidomyiidae sp.	0.33	0	0	0	2.55	0.1	
Orseolia sp.	0.07	0.03	0.07	0	0.81	0.51	
HEMIPTERA	1.67	1.1	1.7	0.97	2.42	0.12	
Alydidae	0.57	0.4	0.73	0.5	2.02	0.17	
Leptocorisa acuta	0.57	0.4	0.73	0.5	2.02	0.17	
Cicadellidae	0.17	0.1	0	0.1	0.42	0.74	
Nephotettix cincticeps	0	0	0	0.03	1.00	0.43	
Nephotettix virescen	0	0.1	0	0.07	1.04	0.41	
Recilia dorsalis	0.17	0	0	0	1.00	0.43	
Delphacidae	0.93	0.6	0.97	0.37	2.59	0.1	
Nilaparvata lugens	0.77	0.53	0.9	0.27	2.23	0.14	
Sogatella furcifera	0.17	0.07	0.07	0.1	0.50	0.69	
LEPIDOPTERA	0.37	0.3	0.5	0.17	2.61	0.1	
Pyralidae	0.33	0.23	0.33	0.17	0.99	0.43	
Cnaplocrosis medinalis	0	0	0	0.03	1.00	0.43	
Scirpophaga incertulas	0.17	0.13	0.17	0.07	1.31	0.32	
Scirpophaga sp.	0.17	0.1	0.17	0.07	0.66	0.59	
Erebidae	0	0	0.07	0	1.00	0.43	
Amata nigriceps	0	0	0.07	0	1.00	0.43	
Hesperiidae	0	0	0.03	0	1.00	0.43	
Pelopidas mathias	0	0	0.03	0	1.00	0.43	
Noctudae	0.03	0.07	0.07	0	0.47	0.71	
Spodoptera sp.	0.03	0.07	0.07	0	0.47	0.71	
ORTHOPTERA	0.63	0.83	0.67	0.8	0.58	0.64	
Acrididae	0.63	0.83	0.67	0.77	0.48	0.7	
Acrida turrita	0.2	0.17	0.1	0.4	2.68	0.09	
Gesonula mundata	0.03	0.1	0.03	0.1	0.35	0.79	
Oxya chinensis	0.07	0.1	0.1	0.1	0.07	0.97	
Valanga nigricornis	0.33	0.47	0.43	0.17	2.02	0.17	
Pyrgomorphidae	0	0	0	0.03	1.00	0.43	
Atractomorpha crenulata	0	0	0	0.03	1.00	0.43	
THYSANOPTERA	0.1	0.1	0.1	0.07	0.11	0.95	
Thripidae	0.1	0.1	0.1	0.07	0.11	0.95	
Liothrips sp.	0.1	0.1	0.1	0.07	0.11	0.95	
Total	3.37	2.5	3.2	2.23	2.77	0.09	

Note: *: 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

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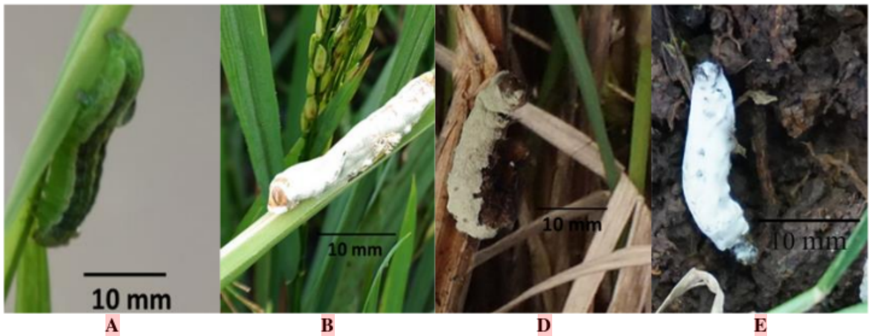

Figure 4. Herbivore insects infected by the entomopathogenic fungal of bioinsecticide in rice field: healthy larvae of *Spodoptera* sp. (A), infected by *B. bassiana* (B), infected by *M. anisopliae* (C), and infected by *C. militaris* (D)

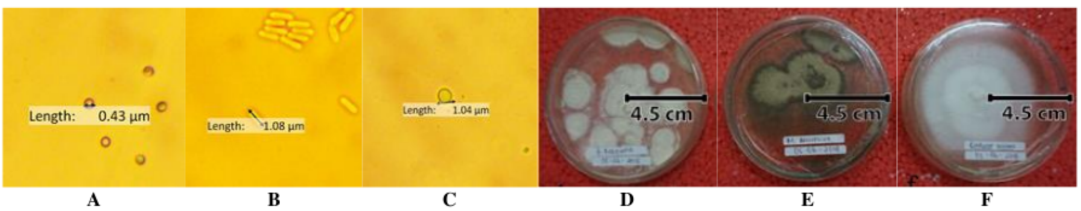

Figure 5. Spores of *Beauveria bassiana* (A), *Metarhizium anisopliae* (B), and *Cordyceps militaris* (C); colonies of *Beauveria bassiana* (d), *Metarhizium anisopliae* (E), and *Cordyceps militaris* (F)

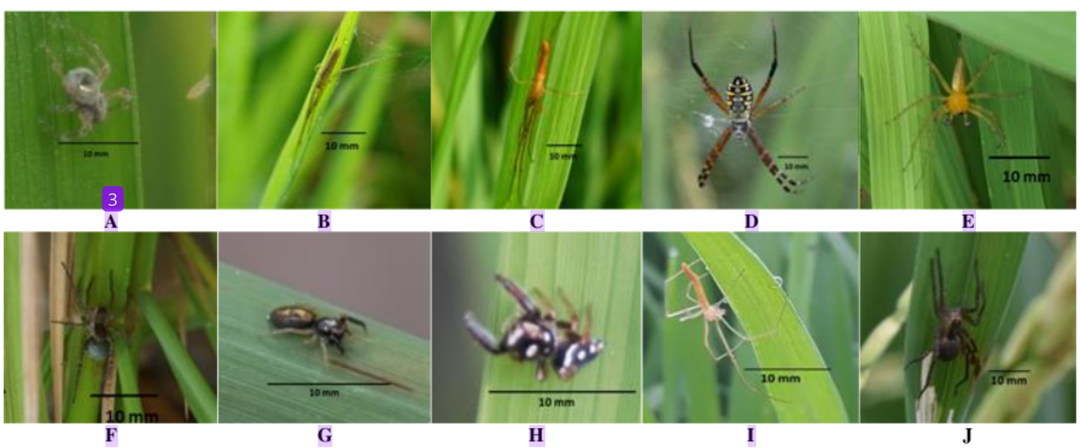

Figure 6. Spiders found in rice canopy: Linyphiidae (A), *Tetragnatha virescens* (B), *Tetragnatha maxilosa* (C), *Argiope catenulate* (D), *Oxyopes matiensis* (E), Lycosidae (F), Salticidae sp. A (G), Salticidae sp. B (H), *Oxyopes javanus* (I), *Pardosa* sp. (J)

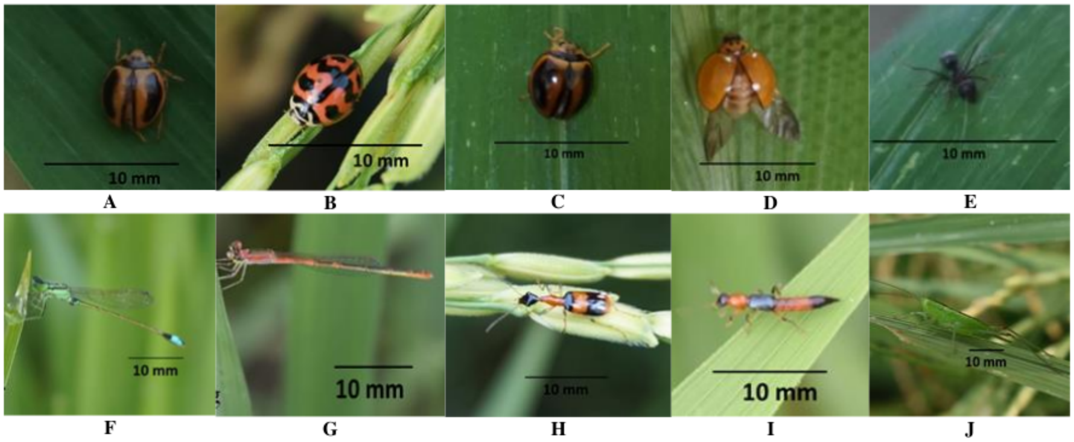

Figure 7. Predatory insect in rice field: *Verania lineata* (A), *Menochilus sexmaculatus* (B), *Micrapis inops* (C), *Formica* sp. (D), *Odontoponera transversa* (E), *Argia* sp. (F), *Agriocnemis* sp. (G), *Ophionea nigrofasciata* (H), *Paederus fuscipes* (I), and *Conocephalus longipennis* (J)

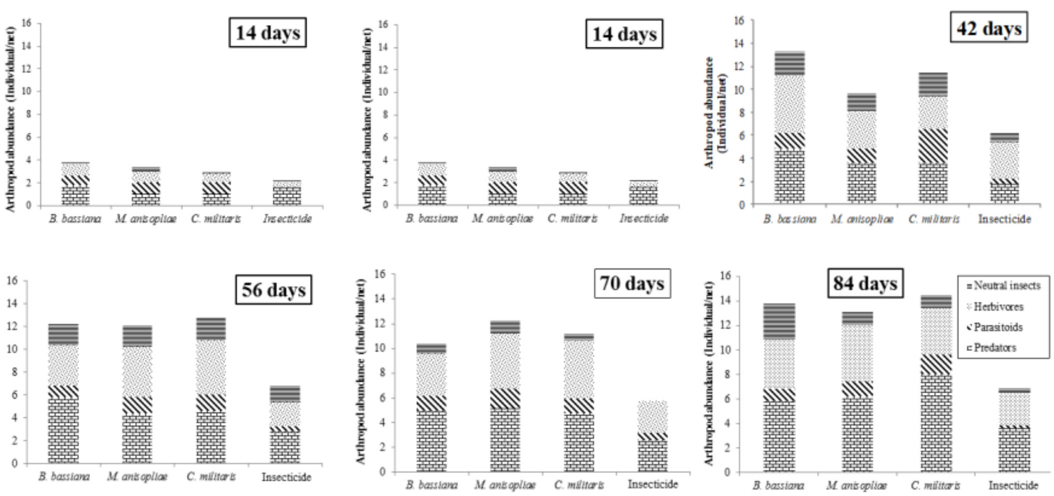

Figure 8. Arthropod abundance on rice field treated with bioinsecticides and abamectin (insecticide)

Table 2. The abundance of spider inhabiting rice canopy sprayed with bioinsecticides (*B.bassiana, M. anisopliae*, and *C. militaris*) and abamectin

Ordo / family/ species	Mean of spider abundance (individuals/nets)				F-value	P-value (0.05)	Tukey's HSD test
	B. bassiana	*M. anisopliae*	*C. militaris*	Abamectin			
ARACHINIDA							
Araneidae	0.03	0.07	0.27	0.13	2.02	0.17	
Araneus inustus	0.00[a]	0.03[ab]	0.2[b]	0.03[ab]	4.82*	0.02	0.11
Araneidae sp.	0.03	0.03	0.07	0.07	0.35	0.79	
Gea subarmata	0.00	0.00	0.00	0.03	1.00	0.43	
Linyphiidae	0.47	0.30	0.20	0.10	2.76	0.09	
Bathyphantes tagalogensis	0.00	0.00	0.07	0.00	2.67	0.10	
Bathyphantes sp. A	0.07	0.00	0.00	0.07	0.89	0.48	
Bathyphantes sp. B	0.13[ab]	0.2[b]	0.07[ab]	0[a]	3.74*	0.04	0.12
Atypena adelinae	0.03	0.03	0.03	0.03	0.00	1.00	
Erigone bifurca	0.03	0.00	0.00	0.00	1.00	0.43	
Linyphiidae sp. A	0.20	0.03	0.03	0.00	2.46	0.11	
Atypena formosana	0.00	0.03	0.00	0.00	0.98	0.44	
Lycosidae	0.10	0.13	0.37	0.10	1.90	0.18	
Arctosa sp.	0.00	0.03	0.03	0.00	0.60	0.63	
Pardosa birmanica	0.03	0.00	0.00	0.00	1.00	0.43	
Pardosa pseudoannulata	0.03	0.00	0.27	0.07	2.10	0.15	
Pardosa apostoli	0.00	0.03	0.00	0.00	1.00	0.43	
Pardosa pullata	0.03	0.03	0.03	0.00	0.37	0.77	
Hogna rizali	0.00	0.00	0.03	0.00	1.00	0.43	
Pirata luzonensis	0.00	0.03	0.00	0.03	0.62	0.62	
Oxyopidae	0.03	0.03	0.10	0.03	0.51	0.68	
Oxyopes javanus	0.00	0.03	0.07	0.03	0.76	0.54	
Oxyopes matiensis	0.00	0.00	0.03	0.00	1.00	0.43	
Oxyopes pingasus	0.03	0.00	0.00	0.00	1.00	0.43	
Salticidae	0.07	0.07	0.07	0.07	0.00	1.00	
Salticidae sp. A	0.03	0.00	0.03	0.03	0.38	0.77	
Salticidae sp. B	0.03	0.07	0.03	0.03	0.37	0.77	
Theridiidae	0.07	0.03	0.03	0.00	0.62	0.62	
Theridiidae sp.	0.07	0.03	0.03	0.00	0.62	0.62	
Tetragnathidae	1.33[b]	1.2[ab]	1.2[ab]	0.63[a]	4.16*	0.03	0.28
Tetragnatha javana	0.33	0.40	0.43	0.20	2.40	0.12	
Tetragnatha maxillosa	0.07	0.07	0.03	0.03	0.24	0.87	
Tetragnatha montana	0.07	0.00	0.03	0.03	0.37	0.77	
Tetragnatha virescens	0.47	0.33	0.37	0.23	0.76	0.54	
Tetragnatha nitens	0.00	0.03	0.03	0.03	0.38	0.77	
Tetragnatha okumae	0.33	0.13	0.10	0.10	1.88	0.19	
Tetragnatha mandibulata	0.03	0.07	0.10	0.00	0.84	0.50	
Tetragnatha vermiformis	0.00	0.10	0.07	0.00	1.27	0.33	
Tetragnatha iwahigensis	0.03	0.07	0.07	0.00	0.76	0.54	
Theridiosomatidae	0.00	0.00	0.07	0.00	1.00	0.43	
Wendilgarda sp.	0.00	0.00	0.07	0.00	1.00	0.43	
Total Abundance (N)	2.1[b]	1.83[ab]	2.3[b]	1.07[a]	7.29*	0.00	0.30

Note: *: 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

The abundance of associated arthropod with rice in one cropping season

The abundance of associated arthropods tends to increase with increasing rice growth stage (Figure 8). In all observations, the abundance of predatory insect was found to be higher compared to the abundance of other guilds (neutral insect, herbivore, and parasitoid). The abundance of predator was generally higher in plots sprayed entomopathogenic fungal bioinsecticide compared to that of abamectin plot. The abundance of all guilds of arthropod in the plot of abamectin was always lower compared to that of bioinsecticide plots. When rice was 70 days old, the abundance of herbivore reached its peak, then decreased at a mature grain stage. The abundance of predators reached its peak when rice was 84 days old.

Table 3. The abundance of predatory insect in rice field sprayed with bioinsecticides (*B. bassiana, M. anisopliae*, and *C. militaris*) and abamectin

Ordo / family/ species	Mean of predatory insect abundance (individuals/nets)				F value	P value
	B. bassiana	*M. anisopliae*	*C. militaris*	Abamectin	0.05	(0.05)
COLEOPTERA	0.47	0.63	0.40	0.63	0.64	0.60
Anthicidae	0.07	0.07	0.07	0.00	0.86	0.49
Formicomus sp.	0.07	0.07	0.07	0.00	0.86	0.49
Carabidae	0.10	0.17	0.17	0.07	1.07	0.40
Ophionea nigrofasciata	0.10	0.17	0.17	0.07	1.07	0.40
Coccinellidae	0.67	0.57	0.57	0.40	1.41	0.29
Verania discolor	0.03	0.07	0.03	0.10	0.19	0.90
Verania lineata	0.00	0.00	0.00	0.07	1.00	0.44
Menochilus sexmaculatus	0.13	0.04	0.07	0.07	0.24	0.86
Micraspis inops	0.50	0.47	0.47	0.17	3.14	0.07
Staphylidae	0.27	0.30	0.33	0.27	0.36	0.78
Paederus fuscipes	0.27	0.30	0.33	0.27	0.36	0.78
HEMIPTERA	0.13	0.10	0.00	0.03	1.49	0.27
Miridae	0.13	0.10	0.00	0.03	1.49	0.27
Cyrtorhinus lividipennis	0.13	0.03	0.00	0.00	2.81	0.09
Orthotylus sp.	0.00	0.07	0.00	0.03	0.74	0.58
HYMENOPTERA	0.30	0.30	0.20	0.10	0.87	0.48
Formicidae	0.30	0.30	0.20	0.00	0.87	0.48
Formica sp.	0.07	0.13	0.03	0.00	0.61	0.66
Odontoponera transversa	0.23	0.17	0.17	0.10	0.27	0.85
ODONATA	0.33	0.20	0.20	0.20	0.34	0.80
Coenagrionidae	0.33	0.20	0.20	0.20	0.34	0.80
Pyrrhosoma sp.	0.20	0.07	0.10	0.10	1.00	0.43
Agriocnemis pygmaea	0.20	0.07	0.10	0.10	0.10	0.96
ORTHOPTERA	0.33	0.27	0.27	0.17	0.56	0.65
Tettigoniidae	0.33	0.27	0.27	0.17	0.56	0.65
Conocephalus longipennis	0.33	0.27	0.27	0.17	0.56	0.65
Total Abundance (N)	2.20	1.97	1.80	1.23	1.25	0.34

Note: values within a row followed by the same letters were not significantly different at $P<0.05$ according to Tukey's HSD test

Discussion

From this study, the population of herbivore insects in plots sprayed with the entomopathogenic fungal bioinsecticides was not significantly different from the population of the herbivore in the abamectin plot. This was because the entomopathogenic fungi applied in this research was effective and had capacity equal to the abamectin in reducing the population of herbivore insects. *B. bassiana* was proved to be effective in killing pest insects of rice, such as brown planthopper (Lee et al. 2015) and rice bug (Girish and Balikai 2015). In this research, *S. litura* found was infected by *B. bassiana*, *M. anisopliae*, and *C. militaris*. Gustianingtyas et al. (2020) also reported that *S. litura* subjected to infection by *M. anisopliae*. *C. militaris* could suppress the population of lepidopteran (Shrestha et al. 2016) and coleopteran pest insects (Kryukov et al. 2014).

Based on this study, symptoms developed on *S. litura* larvae infected by *B. bassiana* in the rice field were similar to the symptoms reported by Gustianingtyas et al. (2019) that host insect infected by *B. bassiana* was covered by white mycelia and its body was shriveled. Morphological characteristics of *B. bassiana* colony were in accordance with the result of isolation in the laboratory, white mycelia with hyaline 1-celled cylindrical and septate conidia (Safitri et al. 2018). *M. anisopliae* infecting *S. litura* in this research showed symptom as reported by Humber (2012) that the integument of infected insect turned to white to dark green, green to yellow conidia, 1-celled and cylindrical conidia, and septate hypha. Larvae of *S. litura* infected by *C. militaris* in the rice field showed symptoms similar to those of *B. bassiana* infection, and in accordance to symptoms characteristics reported by Kryukov et al. (2014) that infected insect was white in color with fruiting bodies appear from the insect, the colony was yellowish-white in color, and conidia were globular in shape (Zheng et al. 2011).

In this research, the abundance of hunting spiders, such as species of Lycosidae did not decrease after being sprayed with the entomopathogenic fungal bioinsecticides well as after being sprayed with abamectin. The abundance of a web spider, such as *Bathyphantes*, *A. inustus,* and Family of Tetragnathidae, decreased after application of the abamectin, while application of the bioinsecticide could only decrease the population of *A. inustus*. Therefore, the entomopathogenic fungal bioinsecticides were relatively safer than the abamectin. Furthermore, spiders generally prey on healthy herbivore insects, while the herbivore insects infected by entomopathogen or the unhealthy ones would not be preyed (Chaubey and Yadav 2017). Web spider abundance decreased after the application of the abamectin and the bioinsecticides because the spiders were moving less in rice canopy, making the insecticide as well as the bioinsecticide easy to reach the spider body. Therefore, it would better to stop the application of bioinsecticide when the abundance of web spiders is high.

Table 4. Characteristic of spider community in rice canopy sprayed with bioinsecticides (*B. bassiana, M. anisopliae*, and *C. militaris*) and abamectin

Characteristic of spider community	*B. bassiana*	*M. anisopliae*	*C. militaris*	Abamectin
14 days				
Abundance (individual/hill)	0.80	0.40	0.80	0.80
Biodiversity index (H')	1.03	0.69	1.38	1.38
Dominance index (D)	0.50	0.50	0.25	0.25
Evenness index (E)	0.94	1.00	1.00	1.00
28 days				
Abundance (individual/hill)	1.00	1.20	1.20	0.40
Biodiversity index (H')	1.33	1.24	1.79	0.69
Dominance index (D)	0.40	0.50	0.16	0.50
Evenness index (E)	0.96	0.89	1.00	1.00
42 days				
Abundance (individual/hill)	2.2	1.80	2.00	0.80
Biodiversity index (H')	1.03	1.67	1.74	1.03
Dominance index (D)	0.45	0.33	0.40	0.50
Evenness index (E)	0.94	0.93	0.89	0.94
56 days				
Abundance (individual/hill)	3.00	1.80	2.20	1.00
Biodiversity index (H')	1.70	1.30	1.12	1.33
Dominance index (D)	0.33	0.55	0.54	0.40
Evenness index (E)	0.87	0.80	0.80	0.96
70 days				
Abundance (individual/hill)	2.40	2.40	2.60	1.00
Biodiversity index (H')	1.97	1.81	2.35	1.79
Dominance index (D)	0.25	0.41	0.15	0.16
Evenness index (E)	0.95	0.87	0.98	1.00
84 days				
Abundance (individual/hill)	3.20	3.40	5.00	2.20
Biodiversity index (H')	1.53	1.39	2.07	1.66
Dominance index (D)	0.56	0.58	0.40	0.45
Evenness index (E)	0.73	0.71	0.80	0.85
TOTAL				
Abundance (individual/hill)	12.6	11	13.8	6.2
Biodiversity index (H')	2.48	2.59	2.75	2.45
Dominance index (D)	0.22	0.21	0.18	0.22
Evenness index (E)	0.82	0.83	0.85	0.88

The abundance of spiders increased, followed by the increase of rice stage, and the highest abundance occurred just before harvesting. The abundance of spiders continued to high until harvesting because spider likes complex habitat (Amzah et al. 2018) and inhabited by various insect species. Spider is also a generalist predator, prey on various insect species, include neutral insects, which are generally abundant before harvesting (Karenina et al. 2019). The species diversity of spiders in this research was also higher in plots sprayed with the entomopathogenic fungal bioinsecticide compared to that of the abamectin plot. The higher abundance and species diversity of spiders indicated that the entomopathogenic fungal bioinsecticide was not dangerous to their life. Many reports showed that entomopathogenic fungi did not kill spiders, especially hunting spiders (Prabawati et al. 2019; Hanif et al. 2020).

The abundance of predatory insects did not decrease after the application of the entomopathogenic fungal bioinsecticide or abamectin. The predatory insect has high mobility which differs them from web spiders so that predatory insect could escape from being exposed to bioinsecticide and abamectin. Furthermore, there is evidence that the avoidance behavior in predatory insects from *B. bassiana*-infected insect preys (Seiedy et al. 2015). Therefore, the application of entomopathogenic fungi was relatively safe for predatory insects.

The abundance of the predatory insect was increasing until 70 DAT and was decreased at 84 DAT. The predatory insects were generally more specialists in terms of prey species compared to spiders. Therefore, at approaching to harvest when the number of herbivore insects decreased, the predatory insects also decreased. Such a phenomenon frequently happened in specialist predatory arthropod because the population of the predator depended on the fluctuated population of their prey which is called as a functional response (Karenina et al. 2019).

Table 5. Characteristic of predatory insect community in rice field sprayed with bioinsecticides (. *B. bassiana, M. anisopliae*, and *C. militaris)* and abamectin

Characteristic of spider community	***B. bassiana***	***M. anisopliae***	***C. militaris***	**Abamectin**
14 days old rice				
Abundance (individual/hill)	1.00	0.60	0.20	0.80
Biodiversity index (H')	1.33	1.09	0.00	0.00
Dominance index (D)	0.40	0.33	1.00	0.25
Evenness index (E)	0.96	1.00	0.00	0.00
28 days old rice				
Abundance (individual/hill)	1.60	1.20	1.40	0.80
Biodiversity index (H')	1.21	1.56	0.55	1.38
Dominance index (D)	0.25	0.33	0.28	0.25
Evenness index (E)	0.67	0.96	0.34	1.00
42 days old rice				
Abundance (individual/hill)	2.60	1.80	1.60	1.00
Biodiversity index (H')	1.94	1.88	1.66	1.66
Dominance index (D)	0.15	0.22	0.37	0.40
Evenness index (E)	0.88	0.97	0.93	1.20
56 days old rice				
Abundance (individual/hill)	2.80	2.60	2.40	1.80
Biodiversity index (H')	1.94	1.81	1.86	1.83
Dominance index (D)	0.28	0.30	0.25	0.33
Evenness index (E)	0.93	0.93	0.95	0.94
70 days old rice				
Abundance (individual/hill)	2.60	2.80	2.20	1.60
Biodiversity index (H')	1.60	1.90	1.67	1.49
Dominance index (D)	0.30	0.28	0.27	0.37
Evenness index (E)	0.89	0.91	0.93	0.92
84 days old rice				
Abundance (individual/hill)	0.20	2.80	3.00	1.40
Biodiversity index (H')	1.95	2.06	1.69	1.54
Dominance index (D)	0.23	0.21	0.33	0.28
Evenness index (E)	0.93	0.94	0.87	0.96
Total				
Abundance (individual/hill)	13.2	11.8	9.8	7.4
Biodiversity index (H')	2.25	2.27	2.11	2.25
Dominance index (D)	0.91	0.89	0.88	0.94
Evenness index (E)	0.22	0.23	0.28	0.21

The community of spider and predatory insects in the plot of *B. bassiana* tent to more similar to the community in plots of bioinsecticide *M. anisopliae* and *C. militaris* compared to that in the abamectin plot. This showed that the effect of the application of three entomopathogenic fungal bioinsecticides on the predator communities was not significantly different. The low community similarity between the bioinsecticide and the abamectin because the abamectin could reduce the abundance of spiders and predatory insects. There have been a lot of reports of the decrease of abundance and species diversity of spiders (Preetha et al. 2010) and predatory insects (Salachna et al. 2020) due to the application of abamectin containing organophosphate (Bai and Ogbourne 2016). In this research, abamectin as an active ingredient is a stomach poison and toxic against insect species of *Menochilus sexmaculatus* (Azod et al. 2016) and spider species of *P. pseudoannulata* (Baehaki et al. 2017).

Guild group made based on function correspondence of arthropods i.e. herbivore insects, neural insects, predators, and parasitoids. For one rice cropping season, there was a tendency that the abundance of all guilds group were lower in the abamectin plot. Interestingly, the abundance of predator was always more dominant in plots sprayed with the entomopathogenic fungal bioinsecticide compared to those of herbivore insects, neutral insects, and parasitoid. Neutral insects were not found in the synthetic insecticide plot. Thus phenomenon showed that predator guild was more tolerant to the entomopathogenic fungal bioinsecticide (Firouzbakht et al. 2015; Bayissa et al. 2016; Gholamzadeh-Chitgar et al. 2017), while abamectin tent to reduce the abundance of predators, herbivore insects, neutral insects and parasitoids (Prabawati et al. 2019; Hanif et al. 2020). Among the three entomopathogenic fungi, there was a tendency that the abundance of the predators in plot sprayed with the bioinsecticide of *C. militaris* was higher than that in plots of *B. bassiana* and *M. anisopliae*. Therefore, predator arthropods tend to be more tolerant of *C. militaris* than of *B. bassiana* and *M. anisopliae*.

Table 6. Matrix of similarity (Index Sorensen) of spider community in rice field sprayed with bioinsecticides (*B. bassiana, M. anisopliae*, and *C. militaris*) and abamectin

Rice ages	Treatments	*B. bassiana*	*M. anisopliae*	*C. militaris.*	Abamectin
14 days	*B. bassiana*	1			
	M. anisopliae	0.33	1		
	C. militaris.	0.25	0.00	1	
	Abamectin	0.25	0.00	0.50	1
28 days	*B. bassiana*	1			
	M. anisopliae	0.18	1		
	C. militaris.	0.55	0.17	1	
	Abamectin	0.29	0.25	0.25	1
42 days	*B. bassiana*	1			
	M. anisopliae	0.40	1		
	C. militaris.	0.48	0.42	1	
	Abamectin	0.40	0.31	0.43	1
56 days	*B. bassiana*	1			
	M. anisopliae	0.58	1		
	C. militaris.	0.31	0.40	1	
	Abamectin	0.40	0.43	0.50	1
70 days	*B. bassiana*	1			
	M. anisopliae	0.67	1		
	C. militaris.	0.32	0.64	1	
	Abamectin	0.22	0.33	0.32	1
84 days	*B. bassiana*	1			
	M. anisopliae	0.73	1		
	C. militaris.	0.54	0.67	1	
	Abamectin	0.59	0.50	0.44	1
TOTAL	*B. bassiana*	1			
	M. anisopliae	0.76	1		
	C. militaris.	0.77	0.84	1	
	Abamectin	0.49	0.60	0.56	1

Table 7. Similarity of predatory insect community in rice field sprayed with bioinsecticides (*B. bassiana, M. anisopliae*, and *C. militaris*) and abamectin

Rice ages	Treatments	*B. bassiana*	*M. anisopliae*	*C. militaris.*	Abamectin
14 days	*B. bassiana*	1			
	M. anisopliae	0.50	1		
	C. militaris.	0.33	0.50	1	
	Abamectin	0.22	0.00	0.00	1
28 days	*B. bassiana*	1			
	M. anisopliae	0.57	1		
	C. militaris.	0.53	0.31	1	
	Abamectin	0.33	0.20	0.18	1
42 days	*B. bassiana*	1			
	M. anisopliae	0.73	1		
	C. militaris.	0.76	0.82	1	
	Abamectin	0.44	0.57	0.46	1
56 days	*B. bassiana*	1			
	M. anisopliae	0.89	1		
	C. militaris.	0.69	0.88	1	
	Abamectin	0.70	0.64	0.76	1
70 days	*B. bassiana*	1			
	M. anisopliae	0.74	1		
	C. militaris.	0.75	0.72	1	
	Insecticide	0.57	0.55	0.74	1
84 days	*B. bassiana*	1			
	M. anisopliae	0.89	1		
	C. militaris.	0.71	0.76	1	
	Abamectin	0.50	0.67	0.64	1
Total	*B. bassiana*	1			
	M. anisopliae	0.91	1		
	C. militaris.	0.85	0.91	1	
	Abamectin	0.66	0.77	0.79	1

It could be concluded that the abundance and species diversity of arthropod predators inhabiting freshwater swamp sprayed with the bioinsecticides of *B. bassiana*, *M. anisopliae*, and *C. militaris* did not decrease, while the population of herbivore insects tent to decrease to the same level as its decrease in the abamectin plot. So that, the bioinsecticides of *B. bassiana*, *M. anisopliae*, and *C. militaris* did not decrease the abundance and species diversity of predatory arthropods (non-target arthropods) but could decrease the herbivore insect population.

ACKNOWLEDGEMENTS

This research was funded by Program of Research Grant Based on Competence (HIKOM) with the budget year of 2018 according to the Director of Research and Community Service, Directorate of Research and Community Service (DRPM), Directorate-General for Research and Development, Ministry of Research, Technology, and Higher Education, Republic of Indonesia (Grant number: 093/SP2H/LT/DRPM/IV/2018) chaired by SH. Special thanks to Dr. Suwandi, a microbiologist from the Department of Plant Pest and Disease, Faculty of Agriculture, Universitas Sriwijaya, Palembang, Indonesia for the identification of the fungi.

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