

Growth of *Beauveria bassiana* Combined with MIPC Insecticide and Its Efficacy to Control the Brown Planthopper *Nilaparvata lugens*

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KEYWORDS

Biopesticide Carbamate Compatibility test Entomopathogen **Abstract** The objectives of this study were to evaluate the efficacy of MIPC to control the brown planthopper *Nilaparvata lugens*, one of the most damaging pests of rice in the field and determine the effect of various of its doses on the growth of the fungus *B. bassiana*. The research was carried out in a rice field and in the Biological Agents Development Laboratory, Department of Pests and Plant Diseases, Faculty of Agriculture, Brawijaya University, Indonesia. The field efficacy test of the MIPC insecticide was conducted by applying 0.5-, 1.0-, 1.5-, and 2.0-kg/ha doses. The insecticidal compatibility test of MIPC with *B. bassiana* was conducted in 0.5-, 1.0-, 1.5-, and 2.0-g/L doses of MIPC. Application of MIPC insecticide at 0.5-, 1.0-, 1.5-, and 2.0-kg/ha doses had no significant difference in reducing *N. lugens* population in the vegetative phase (28 to 70 days after planting). The MIPC doses of 1.0, 1.5, and 2.0 g/L were highly toxic or incompatible with the growth of *B. bassiana*. Meanwhile, a sublethal concentration of MIPC insecticide of 0.5 g/L was non-toxic to *B. bassiana* fungi.

Introduction

The brown planthopper Nilaparvata lugens is one of the most destructive pests of rice throughout Asia, causing economic damage to the rice crops directly by feeding and also indirectly by transmitting plant viruses, including rice grassy stunt virus and rice ragged stunt virus (Jia et al. 2012; Zheng et al., 2014). The use of synthetic insecticides is often needed in controlling the population of pests, including Nilaparvata lugens (Martono, 1999). 2-Isopropylphenyl methyl carbamate (MIPC) has been widely used to control N. lugens in paddy fields in Indonesia (Putra et al., 2002). This insecticide inhibits the AChE enzyme and causes overstimulation of the nervous system. MIPC also has a broad spectrum of target pests. However, MIPC is easily degraded, which affects its ability to control pests (Hasibuan, 2012; Sutrisno, 2014).

The application of synthetic insecticides is generally carried out in accordance with the recommended dosage or concentration. Sometimes, farmers apply insecticides at subrecommended or sublethal doses. The application of insecticides at sublethal doses may inhibit the feeding activity, reduce carbohydrate reserves, fecundity, and the length of life of pests. Besides that, the application also affects the growth rate of target insects, feeding rates, and fertility imago (Lee, 2000; Ratna *et al.*, 2009).

The effect of MIPC insecticide in reducing *N*. *lugens* population can be determined through efficacy tests. The efficacy test is a method for assessing the ability of synthetic insecticides to decrease the target pest population in the field. This efficacy test was carried out by comparing the target pest population in the treatment plot with that in the control plot after the application of the

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insecticide (Martono, 1999). However, the

effect of MIPC decreases with its continued

application. Long-term use of MIPC may cause

N. lugens to become resistant, thereby reducing

the ability of the insecticide to control it (Chung

reduce target pest populations can be increased

when associated with entomopathogenic fungi. Beauveria bassiana and Metarhizium anisopliae

are entomopathogenic fungi that are widely used to control various pests in rice plants

(Gentz et al., 2010). The B. bassiana fungus can

control N. lugens because of its facultative and

specific host range to the target pest (Alizadeh

et al., 2007; Iswanto et al., 2016). Synthetic

insecticides associated with pathogenic fungi

The ability of synthetic insecticides to

et al., 1983).

may increase control effectiveness, reduce the number of applied insecticides, minimize the danger of environmental pollution, and minimize pest resistance (de Oliveira et al., 2003; Usha et al., 2014). Before field application, compatibility tests are performed to evaluate the effect of synthetic insecticides on the growth of the fungus B. bassiana (Fauziah and Rohdiana, 2016). The amitraz,

flufenoxuron. imidacloprid, endosulfan, teflubenzuron, phuzalon, acetamiprid, thiamethoxam, spinosad, and indoxacarb insecticides have been reported to be highly compatible with B. bassiana, M. anisopliae, and Paecilomyces sp. (Neves et al., 2001; Alizadeh et al., 2007; Akbar et al., 2012; Hasyim et al., 2016). However, no compatibility test of MIPC with B. bassiana fungi has been performed. This study aimed to evaluate the efficacy of MIPC to control N. lugens in the field and determine the effect of various of its doses on the growth of the fungus B. bassiana.

Materials and methods

The research was carried out in lowland rice fields located in the Prigen Subdistrict, Pasuruan City, and in the Laboratory of

Afandhi et al. Growth of Beauveria bassiana Combined with.....

The rice (IR64 var.) was planted as much as two seedlings per hole with a distance of 25 × 25 cm. Soil fertilization was performed at the planting time by spreading urea fertilizer at 40 kg/ha and P₂O₅ fertilizer at 40 kg/ha. Further fertilization was carried out in 25 days after planting (DAP) and at 50 DAP by spreading urea at 40 kg/ha (Rugaya et al., 2013).

The MIPC insecticide efficacy test uses application. various dosages of The recommended dosage of MIPC for controlling N. lugens pests in rice plants is 1 kg/ha. Determination of the treatment dose based on the method of Fauziah and Rohdiana (2016) that is 0.5, 1.0, 1.5, and 2.0 times the recommended dose. Thus, the treatment doses used in the efficacy test were 0.5, 1.0, 1.5, and 2.0 kg/ha. The MIPC insecticide efficacy test was carried out using a completely randomized design with five treatments and five replications.

The insecticide was applied using a knapsack sprayer with a recommended spray volume of 500 L/ha (Rugaya et al., 2013). The first application was made a week after the discovery of nymphs or when imago N. lugens had reached the economic threshold in each treatment plot. The application of the insecticide was done every two weeks. Preliminary observations were made on rice plants aged 7 DAP. Subsequent observations were made once a week after applying the insecticide until two weeks before harvesting. The observations were conducted in 20 clusters in the rice field separated into five plots. Each plot consisted of four rice plant clumps. The distance between the sample plot and the plot edge was a meter. The data on MIPC efficacy against N. lugens were calculated using the Abbott formula, as follows:

$$IE = (Ca - Ta)/Ca \times 100\%$$
 (1)

where IE is the efficacy of the tested insecticide (%), Ta is the target pest population in the insecticide treatment plot tested after application and Ca is the target pest population in the control plot after insecticide application.

The MIPC insecticide is effective if more than half of observations frequency possessing efficacy value at (EI) \geq 70% (Indiati, 2012; Rugaya *et al.*, 2013). The data obtained were calculated using analysis of variance followed by LSD Tukey post hoc test.

For the compatibility tests, the MIPC was used in four different insecticide concentrations. The recommended concentration for MIPC is 2 g/L. We determined the treatment concentrations based on the method of Usha et al. (2014), which were 0.25, 0.5, 0.75, and 1.0 times the recommended concentration; thus, the MIPC insecticide concentration treatment used for the compatibility tests were 0.5, 1.0, 1.5, and 2.0 g/L. The MIPC insecticide compatibility test against the growth of the B. bassiana fungi was carried out using a completely randomized with five treatments and design five replications.

Data of the compatibility of MIPC with the growth of B. bassiana were obtained from observations on the growth of the colony, inhibition of growth, conidia production, germination, and decreased conidia mold of B. bassiana. The insecticidal toxicity level was calculated using Alves et al. (1998) formula, which was based on BI factor calculated by comparing germination data of conidia, vegetative growth, and sporulation with control data (%). The BI scores are classified into the following categories: very toxic (0-30); toxic (31-45); less toxic (46-60); and are not toxic or compatible (>60). The data obtained were analyzed using analysis of variance (ANOVA) followed by LSD Tukey's multiple range test (P<0.05) using SPSS 20.

Result and Discussion

Afandhi et al. Growth of Beauveria bassiana Combined with.....

Efficacy of MIPC insecticide on N. lugens

The efficacy of MIPC was determined by the population of *N. lugens* after application. The *N. lugens* pest was found to have reached the economic threshold at 14 DAP, and so the first application of MIPC was carried out at 21 DAP. In accordance with a statement from Nurbaeti *et al.* (2010), this pest came to rice plantations aged 10–20 DAP. The application of the insecticide was carried out four times by observing the population of *N. lugens* on five times. Observations were made for two weeks before harvesting. The last observation was made on the 77 DAP.

The population of N. lugens after MIPC insecticide application from the first to the fifth observation decreased (Table 1). Based on the results of recent observations, treatment doses of 1.0, 1.5, and 2.0 kg/ha each showed a population of N. lugens that was not significantly different between treatments. The highest average population at the last observation was found in the treatment plot with a dose of 0.5 kg/ha of one animal per family. According to Hasibuan (2012), MIPC insecticide formulations can paralyze the nervous system of pest insects. Thus, four times the application of MIPC cause N. lugens population decline. Hasibuan (2004) revealed that the population of shield lice pests (Aulacaspis tegalensis) on sugar cane plants after the application of MIPC decreased with time.

The mode of action of the active compound contained in the MIPC insecticide formulation was able to inhibit and bind the work of the enzyme acetylcholine esterase (AChE). The acetylcholine esterase enzymes in the body of an insect function to hydrolyze acetylcholine, and if this enzyme is bound, it will cause an accumulation and an increase in acetylcholine enzyme. The acetylcholine enzyme functions to transmit nerve impulses to the central nervous

28 DAP

1.67

1.69

Doses (kg/ha)

0.5

1.0

Doses (kg/ha)

	-			-	
	$(\overline{x} \pm SD)$				
0	12.0 ± 0.63	10.6 ± 0.49 c	8.0 ± 0.63 b	4.6 ± 0.49 c	1.8 ± 0.40 c
0.5	11.8 ± 0.75	8 6 ± 0.49 ab	6.2 ±0.75 a	1.8 ± 0.40 b	1.0 ± 0.00 b
1.0	11.8 ± 0.98	8.8 ± 0.40 ab	6.0 ± 0.49 a	1.4 ± 0.49 ab	0.0 ± 0.00 a
1.5	11.8 ± 0.75	9.2 ± 0.40 b	6.0 ± 0.00 a	1.2 ± 0.40 a	0.0 ± 0.00 a
2.0	11.6 ± 0.49	8.2 ± 0.40 a	5.8 ± 0.40 a	1.0 ± 0.00 a	0.0 ± 0.00 a
Note: The numbers	in the column follo	owed by the same let	tter indicate no sigr	nificant difference in	the LSD test with an
error rate of 5%.					

 Table 1. Average Population of N. lugens in the Rice Clump in Each Treatment Plot

42 DAP

Population (number of individuals) of N. lugens observed at

56 DAP

Efficacy Value (%)

56 DAP

22.02

19.80

The population of N. lugens at the first observation after MIPC insecticide application was not significantly different from that of the control. Hasibuan (2004) revealed that the population of shield lice pests (A. tegalensis) on sugarcane plantations which MIPC insecticide had been applied decreased with time but was not significantly different from the population in the control plants. However, starting from the second observation until the last observation showed that the population of *N. lugens* was significantly different and lower than in the control. Differences in the numbers of N. lugens in control plots that are higher than in treatment plots can occur due to the effect of insecticides on the reproduction of target pests. Trisnaningsih (2016) revealed that the population of N. lugens that are lower than in the control due to synthetic insecticides also affect reducing egg production and causing the eggs to not hatch. Thus, the number of new individuals that hatch also decreases.

28 DAP

A decline in the population of N. lugens in each plot treated with MIPC also occurred with the increasing age of rice plants. This pest population gradually decreased from the first observation after application, namely when the rice was 28 DAP until the last observation when the rice was 77 DAP. At the fourth observation, the rice had entered its generative phase. Nurbaeti et al. (2010) stated that the N. lugens pest enters young rice plantations 10-20 DAP. When the rice plant has entered the generative phase or matured phase, N. lugens usually moves to other fields to find young rice plants. Sianipar et al. (2015) revealed that temperature, humidity, and rainfall did not have a major effect on the decline in the population of N. lugens, so that changes in the population of N. *lugens* were more influenced by the availability of host plants.

The efficacy value of the MIPC insecticide during the five observations tended to increase (Table 2). The treatments of 1.5 and 2.0 kg/ha at the fourth observation (70 DAP) showed efficacy values of \geq 70%, 73.00% and 78.00%, respectively. The treatment doses of 1.0, 1,5, and 2.0 kg/ha showed an efficacy value of 100% in the fifth observation (77 DAP).

70 DAP

70 DAP

60.00

69.00

77 DAP

77 DAP

40.00

100.00

system of the insect. This results in nerve impulses to be continuously stimulated, causing the occurrence of tremor symptoms or uncontrolled movements in pest insects and death (Hasibuan, 2012; Sutrisno 2014).

Table 2. Efficacy Value of MIPC Insecticide on *N. lugens* (%)

42 DAP

13.91

16.73

Doses		E	fficacy Value (%)	
(kg/ha)	28 DAP	42 DAP	56 DAP	70 DAP	77 DAP
1.5	1.26	12.91	24.52	73.00	100.00
2.0	3.21	22.36	27.02	78.00	100.00

This is in contrast to the results of the study by Indiati (2012) showing that the insecticides Imidacloprid, Fipronil, and Emamectin benzoate have an efficacy value of 100% in decreasing the Trips pest population on green beans starting from 2 to 6 weeks after planting. Rugaya *et al.* (2013) also reported that the Poksindo insecticide at a dose of 0.5, 0.75, or 1 kg/ha has an efficacy value of \geq 70% as much as five observations out of a total of six observations in reducing the population of slender black ladybugs on rice plants.

The effect of MIPC in reducing *N. lugens* population can be considered effective if a minimum of four observations out of five the efficacy value is \geq 70%. Thus, application of MIPC insecticide doses of 1.5 and 2.0 kg/ha were effective in reducing the population of *N. lugens*, with an efficacy value greater than 70% when the rice was 70 DAP. That meant that to control *N. lugens*, a high MIPC dose was necessary.

MIPC Insecticide Compatibility with B. bassiana Fungus Growth

The level of suitability of MIPC insecticides for fungal growth *B. bassiana* is determined from the results of measurements of colony growth diameter, conidia production, and conidia germination, as follows: The fungi colony growth of *B. bassiana* in each treatment increased up to 7 days of inoculation (Table 3). The fungus colony of *B. bassiana* 1 days of inoculation still did not experience growth in all treatments because there is still a period of adaptation to the environment in which it grows. The colony growth at each MIPC treatment began to occur at 2 days of inoculation. The growth of fungal colonies can occur and increase when growth media is added to the insecticide because of the metabolic response of the fungal body. The metabolism of the fungal body is able to utilize components of the insecticide formulation as a source of secondary nutrients (Fauziah and Rohdiana, 2016). However, the more concentrated the treatment the more the growth of the fungal colonies decreases.

Colony growth produced by *B. bassiana* fungus after 7 days of inoculation at the MIPC concentrations of 0.5, 1,0, 1,5, and 2.0 g/L were significantly different from those of the controls. Treatment doses of 1.0, 1,5, and 2.0 g/L produced growth of *B. bassiana* fungi colonies that were not significantly different, namely 10.3, 9.1, and 5.8 mm after 7 days of inoculation. Research from Alizadeh *et al.* (2007) also concluded that the Endosulfan insecticide in the treatment of 0.5 and 1.0 times the recommended concentration was added to the media PDAs are not significantly different in producing *B. bassiana* fungal colony growth that is equal to 16 and 14.64 mm.

The greater the concentration of MIPC insecticide the further the reduction in the growth of fungal colonies. According to Faraji *et al.* (2016), any synthetic pesticide has the potential to influence various stages of development of entomopathogenic fungi, including colony growth.

Treatment with higher concentrations also increases the percentage of colon growth inhibition shown from 3 to 7 days of inoculation. Treatment doses of 1.0, 1.5, and 2.0 g/L produced an inhibition greater than 70%.

This is similar to the results of the research by Alizadeh *et al.* (2007), who showed that the higher concentration of insecticide in the mushroom growing media, the more the inhibition of *B. bassiana* mushroom colony growth. The various types of pesticides tested (Amitraz, Flufenoxuron, Teflubenzuron + Phuzalon) at 0.5, 1, and 2 times the recommended concentration can inhibit the growth of *B. bassiana* fungi colonies by more than 70%.

Conc	Diameter	Day of inoculation					
entra tion (g/L)	(mm) and inhibition (%)	2 (፳ ± SD)	3 (x ± SD)	4 (፳ ± SD)	5 (፳ ± SD)	6 (፳±SD)	7 (x ± SD)
0	Diameter	4.0 ± 1.00 c	10.5 ± 3.08 c	14.9 ± 3.68 c	20.5 ± 4.82 c	47.1 ± 4.82 d	54.3 ± 6.70 c
	Inhibition	0	0	0	0	0	0
0.5	Diameter	2.5 ± 0.71 b	5.1 ± 1.16 b	8.0 ± 1.91 ab	11.1 ± 1.91 b	14.1 ± 1.93 c	17.8 ± 1.69 b
	Inhibition	35	49.6	37.2	42.7	65.6	67.0
1	Diameter	1.5 ± 0.71 ab	2.3 ± 0.40 ab	4.0 ± 0.70 a	5.5 ± 1.38 a	9.3 ± 1.38 b	10.3 ± 1.44 a
	Inhibition	60.0	68.1	63.9	64.2	67.8	71.9
1.5	Diameter	1.8 ± 0.51 ab	3.2 ± 0.24 a	4.4 ± 0.66 a	5.0 ± 0.49 a	7.1 ± 0.45 ab	9.1 ± 1.71 a
	Inhibition	51.2	66.2	69.4	74.1	82.6	83.2
2	Diameter Inhibition	1.3 ± 0.40 a 67.8	1.6 ± 0.49 a 83.1	2.9 ± 1.07 a 79.0	3.3 ± 1.17 a 83.6	4.7 ± 1.17 a 88.4	5.8 ± 1.89 a 89.1

Table 3. Growth Diameter of <i>B. bassiana</i> Fungi Colony on Sabouraud Dextrose Agar Yeast Media with
MIPC Insecticide Appropriate Treatment

Note: The numbers in the column followed by the same letter indicate no significant difference in the LSD test with an error rate of 5%.

The production of conidia by the fungus *B. bassiana* after 7 days of inoculation decreased with increasing concentrations of the insecticide showed in the treatment (Table 4). In free of MIPC media (control), the conidia production was $(27.24 \pm 10.51) \times 10^6$ per ml. The conidia production at a MIPC concentration of 0.5 g/L, was 17.82×10^6 conidia/ml with a reduction percentage of 27.61%. Meanwhile, the conidia production at MIPC concentrations of 1.0, 1,5, and 2.0 g/L produced less than 10 x 10^6 conidia/mL, with a reduction percentage of more than 60%. Further statistical analyses showed that the number of conidia produced by media plus MIPC 1 to 2 g/L was significantly lower than those of control and MIPC 0.5 g/L. The same was observed in the study of Raj et al. (2011), showing that chlorpyrifos insecticide at 0.5, 1.0, and even 2.0 times the recommended concentration produce less than 10 x 106 *B. bassiana* conidia/mL. In addition to the growth of the colony, synthetic insecticides can also affect the amount of conidia produced through compatibility tests (Usha *et al.*, 2014).

Table 4. Number of Conidia Produced by <i>B. bassiana</i> Fungi After 7 Days of Inoculation on Sabouraud
Dextrose Agar Yeast Media with MIPC Insecticide

Concentration (g/L)	Number of conidia (x10 ⁶ conidia/mL) $(\overline{x} \pm SD)$	% sporulation reduction
0	27.24 ± 10.51 c	0
0.5	17.82 ± 3.41 b	27.61
1.0	9.32 ± 0.53 a	62.13

Concentration (g/L)	Number of conidia (x10 ⁶ conidia/mL) $(\overline{x} \pm SD)$	% sporulation reduction
1.5	6.25 ± 0.77 a	74.41
2.0	5.86 ± 1.85 a	73.79

Note: The numbers in the column followed by the same letter indicate no significant difference in the LSD test with an error rate of 5%.

The percentage of *B. bassiana* conidia germination after incubation for 24 hours was not significantly different from control (Table 5). The treatment concentration of 0.5 g/L produced a percentage of germination that was not significantly different from that of the control. The percentage of germination at a concentration of 0.5 g/L was 69.63% while that of the control was 71.01%. The percentage of conidia germination at the treatment concentrations of 1.5 and 2.0 g/L tended to be greater than that in the control. Usha *et al.* (2014) revealed that the insecticide Imidacloprid at 0.1, 0.5, and 1 time the recommended concentration tested with *B. bassiana* produced a conidia germination percentage of more than 90%.

Table 5. Germination Percentage of <i>B. bassiana</i> Fungus Conidia after 7 days of Inoculation on Saboura	ud
Dextrose Agar Yeast Media to which MIPC Insecticide Had Been Added	

Concentration	% germination of conidia	% germination of conidia
(g/L)	$(\overline{x} \pm SD)$	reduction
0	71.01 ± 8.17 ab	0
0.5	69.63 ± 6.43 ab	0.27
1.0	62.70 ± 4.60 a	10.61
1.5	76.27 ± 3.82 b	0
2.0	75.98 ± 4.76 b	0

Note: The numbers in the column followed by the same letter indicate no significant difference in the LSD test with an error rate of 5%.

Conidia germination is an important factor in considering the level of compatibility between synthetic insecticides and the growth of entomopathogenic fungi. Neves *et al.* (2001) stated that in vitro compatibility tests must consider whether conidia germination is inhibited or not when mixed with insecticide formulations. Conidia germination is an important stage in the life cycle of entomopathogenic fungi before penetrating the body of insect pests. Therefore. the success of an entomopathogenic fungus depends on its ability to germinate (Alizadeh *et al.*, 2007; Usha *et al.*, 2014).

The compatibility score of *B. bassiana* combine with 0.5 g/L was 65.5, which was classified as not toxic, while the others fall in the score of less than 40%. Those were classified as very toxic or incompatible with the growth of the fungus *B. bassiana* (Table 8). Meanwhile, research from de Oliveira *et al.* (2003) reported that the insecticide triazophos, chlorpyrifos, and endosulfan at 0.5, 1.0, and 2.0 times the recommended concentration can inhibit the germination of the fungus *B. bassiana*. Usha *et al.* (2014) also revealed that the chlorpyrifos and monocrotophos insecticides at 0.5 and 1.0 times the recommended concentrations inhibited the growth of all isolates of *B. bassiana* tested.

Concentration	Compatibility	Category
(g/L)	score	
0.5	64.5	Not toxic
1.0	34.1	Very toxic
1.5	23.8	Very toxic
2.0	21.5	Very toxic

Table 6. Compatibility Value of *B. bassiana* Fungus after 7 Days of Inoculation on SDAY Media with MIPC Insecticide Addition

Note: The numbers in the column followed by the same letter indicate no significant difference in the LSD test with an error rate of 5%.

The 0.5 g/L concentration treatment was compatible with the growth of B. bassiana fungus because it produced a higher colony growth diameter (17.8 mm) compared to the other treatments. In addition, the conidia germination capacity was also not significantly different from that in the control (69.63%). The amount of conidia produced in the 0.25 kg/ha dose treatment was also higher when compared to those of higher doses. Research from Neves et al. (2001) and Akbar et al. (2012) reported that the insecticides acetamiprid, imidacloprid, thiamethoxam, spinosad, and indoxacarb were compatible with B. bassiana, M. anisopliae, and Paecilomyces sp. because they do not have a negative impact on colony growth, germination capacity, and conidia production.

Synthetic pesticides have the potential to affect various stages of development of entomopathogenic fungi such as colony growth, conidia production, and conidia germination of entomopathogenic fungi. The compatibility test in this research shows that MIPC insecticide concentration which is higher until it reaches the recommended concentration is not compatible to B. bassiana growth. The mechanism of decreased sporulation and decreased colony growth can occur due to the active elements of the insecticides which interfere with cell membrane directly

permeability, enzyme synthesis, and metabolic processes of entomopathogenic fungi (Fauziah and Rohdiana, 2016). The level of suitability of synthetic insecticides to entomopathogenic growth is determined by the ability of fungal colonies to grow and conidia to germinate under conditions of the media mixed with the insecticide. Conidia germination is an important stage in the life cycle of entomopathogenic fungi before penetrating the body of insect pests (Alizadeh *et al.*, 2007).

Conclusion

Application of MIPC insecticide at 0.5, 1.0, 1.5, and 2.0 kg/ha had no significant difference in reducing *N. lugens* population in the vegetative phase (28 to 70 DAP). The MIPC doses of 1.0, 1.5, and 2.0 g/L were highly toxic or incompatible with the growth of the *B. bassiana*. Meanwhile, a sublethal concentration of MIPC of 0.5 g/L was non-toxic to *B. bassiana*. Therefore, this study recommends the use of a 0.5 g/L MIPC dose combined with the fungus *B. bassiana*.

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