INSECTICIDE COMPATIBILITY TO THE ENTOMOPATHOGENIC FUNGI *Beauveria bassiana* AND *Metarhizium anisopliae*

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ABSTRACT

Insecticide use has produced negative impact by affecting the non-target predatory organisms in nature, one of which is the entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae*. Interactions occur, however, between insecticides and the entomopathogens. The combination of insecticides at a low dose and an entomopathogenic fungus can work synergistically to increase pest insect mortality. This combination is particularly advantageous because it decreases the insecticide dose applied, reduces environmental contamination, and decreases pest resistance. The study purpose was to determine the compatible working insecticide dose and the entomopathogenic fungi *B. basiana* and *M. anisopliae*. The experimental design applied completely randomized design consisting of 15 treatments and four replicates. There were five types of insecticides with three different doses each (0.5 × Dose of Field (DF), 1 × DF, and 2 × DF), whereas the fungal isolates included *B. basiana* and *M. anisopliae*. The parameters observed were the germination percentage of conidia, the percentage of inhibition, and the number of conidia.ml^{-1}. Data were analyzed using ANOVA (5% error rate). The mean values were analyzed by DMRT $p < 0.05$. Deltamethrin 0.5 x DF, and imidacloprid 0.5 x DF demonstrated the highest conidial germination in *B. basiana* and *M. anisopli*, respectively. Insecticides showing the highest vegetative growth on *B. basiana* and *M. anisopliae* at 7 DAI was imidacloprid 0.5 x DF, while at 14 DAI was imidacloprid 0.5 x DF and chlorphyrifos 2 x DF, respectively. The highest conidial production of the fungi was triggered by imidacloprid 0.5 x DF. Based on compatibility calculation, imidacloprid 0,5 x DF worked with *B. basiana* (BI: 67.77) and *M. anisopliae* (BI: 67.16).

KEY WORDS: *B. basiana*, compatibility, entomopathogenic fungi, insecticide, *M. Anisopliae*

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INTRODUCTION

The insecticides use is a common practice in agriculture because of their immediate effects in controlling the pests, and requires to suppress rapidly expanding pest insect populations. Traditionally, insecticides have been used to protect agricultural products from arthropod pests, but the indiscriminate use of these compounds can cause serious problems (Golshan *et al.*, 2013).

Problems of using insecticides are environmental contamination, insects resistance, and harmful to nontarget organisms. Almost all types of insecticides are not selective, broad spectrum and have adverse effects due to its toxicity (Ambethgar, 2009). One of the adverse effects of insecticides is killing the non-target organisms which also feed on the pests, such as entomopathogenic fungi. However, it is very likely that interaction might have occurred between insecticides and entomopathogenic fungi (Akbar *et al.*, 2012).

Entomopathogenic fungi are biological agents that can be used to control pest insects (Golshan *et al.*, 2013). The fungal species of *Beauveria bassiana* and *Metarhizium anisopliae* have been reported to very efficiently affect some insect pests, especially Lepidoptera, Hemiptera, Homoptera and Coleoptera (Herdatiarni *et al.*, 2014). The use of entomopathogenic fungi as biological agents has shown some advantages such as increase disease control efficiency; reduce the insecticides applied, minimize environmental contamination hazards, and decrease pest resistance (Ambethgar, 2009).

According to Akbar *et al.* (2012), a compatible combination of insecticides at sublethal doses and entomopathogenic fungi can work synergistically to increase insect mortality. This is particularly advantageous because it will decrease the dose of insecticide application, reduce environmental contamination, and decrease the likelihood of resistance. Many experiments have been carried out to investigate effects of insecticides on various entomopathogenic fungi. Karnataka (2007) examined the effect of five insecticides on vegetative growth of *M. anisopliae*. The results showed the lowest inhibitory effect achieved by imidacloprid (11.1%), followed by deltamethrin (36.7%), cypermethrin (36.7%), thiodicarb (53.5%), and andchlorphyrifos (69.2%). Alizadeh *et al.* (2007) tested the effects of three doses of imidacloprid (0.5 x Dose of Field, 1 x Dose of Field, 2 x Dose of Field) on *B. basiana* and showed low inhibitory effect (< 5%) at the lowest filed dose. Asi *et al.* (2010) observed the effects of thiodicarb on vegetative growth of *M. anisopliae*. Their results showed highly inhibitory effect (> 60%). Based on this problem, research is required to examine which insecticides compatible with entomopathogenic fungus *B. basiana* and *M. anisopliae*. Thus, the purpose of this study was to determine the compatibility of five insecticides at particular doses to entomopathogenic fungus *B. basiana* and *M. anisopliae*.

METHODS

The research run from May to October 2017. The tests for fungal conidia germination, vegetative growth, and conidia production were done at Mycology and Phytopathology Laboratory, Faculty of Biology, Universitas Jenderal Soedirman, Purwokerto.

B. basiana was from Balai Besar Perbenihan dan Proteksi Tanaman Perkebunan (BBPPTP) Surabaya, and *M.*

anisopliae was obtained from Balai Besar Penelitian Veteriner (BBalitvet) Bogor. They were subcultured from the stock. The fungi, then, were cultured on sterile PDA medium on laminar air flow and incubated for ten days.

Insecticides used in this study were thiodicarb, chlorphyrifos, imidacloprid, deltamethrinand, and cypermethrin, with three doses each, i.e., 0.5 x Dose of Field (DF), 1 x DF, and 2 x DF. The doses were obtained by mixing given volume/grams of insecticides in 1 L of distilled water. DF was calculated based on the instructions on the packaging label and adjusted to the volume of the medium.

Conidia germination was tested with modified methods of Alizadeh *et al.* (2007). Each dose of insecticide was poured into a test tube containing 10 ml of warm sterile PDB (± 45 °C). Conidial suspension (1 ml each) of *B. basiana* and *M. anisopliae* (the standard concentration of 10^6 conidia.ml^{-1}) and 0.05% Tween 80 were added into the tube.

The medium without insecticides was inoculated with the fungal conidia suspension as the control. Each medium was incubated at room temperature (24–30 °C) for 24 hours. A total of 1 ml of medium was dropped on Hemocytometer. Conidial germination was observed in 5 medium-sized Hemocytometers under a light microscope, then taken on average. Each treatment was replicated four times. The germinated conidia were characterized by a germ tube. Treatment data were compared to those of the control to obtain the percentage of conidia germination for *B. basiana* and *M. anisopliae*.

Test vegetative growth was measured by poisoned food technique (Moorhouse *et al.*, 1992). A total of 10 ml warm sterile PDA medium (± 45 °C) was poured into a petri dish, then the given dose of insecticide was added aseptically under laminar air flow. The mixture was stirred and poured into a Petri dish (diameter 9 cm), allowed to solidify under laminar air flow.

The 5-mm diameter of 10-day-old *B. basiana* and *M. anisopliae* isolates were transferred to insecticide-PDA medium. Non-insecticide PDA medium was inoculated with *B. basiana* and *M. anisopliae* as the control. The plates were sealed with parafilm and incubated at room temperature for 14 days. This treatment was replicated four times. The colony diameter was measured with a ruler (cm) at day 7 after inoculation (DAI) and 14 DAI. Treatment data were compared to those of the control to determine the percentage value of inhibitory growth.

The fungal mycelium from the vegetative growth test (14 DAI) was taken with ose needle. The mycelium was placed into a test tube containing ten mL distilled water plus 0.05% Tween 80. The suspension was homogenized in a centrifuge for 10 minutes. The part of the natant containing the mycelium debris was discarded, while the supernatant containing the conidia was taken. A total of 1 mL conidial suspension was taken and placed on a hemocytometer. Conidia were counted directly at five medium square on Hemocytometer, then the average value was calculated. It was replicated four times. The data obtained were compared to the control data. The data was standardized into 1 x 10^6 conidia.mL^{-1}.

Calculation of Insecticidal Compatibility used the formula below:

$$BI = \frac{10\ (\mathrm{GR})\ + 47\ (\mathrm{VG})\ +\ 43\ (\mathrm{SPR})}{100}$$

Notes: BI = Biological index (level of insecticide toxicity against entomopathogenic fungi in vitro), GR = Comparison of conidia germination treatment with control, VG = Comparison of vegetative growth treatment with control, SPR = Comparison of conidia numbers of treatment with control.

Insecticidal toxicity level followed Alves *et al.* (2007) formula which was based on BI factor calculated by comparing germination data of conidia (GR), vegetative growth (VG) and sporulation (SPR) with control data (%). BI classification was < 42 = toxic, 42–60 = moderately toxic, and > 60 = compatible.

The experimental design was Completely Randomized Design (CRD). The parameters consisted of germination percentage of conidia, the percentage of inhibition, and the number of entomopathogenic fungi conidia.ml^{-1}. The total treatments enlisted below were 15 treatments and a control, each with four replicates.

MP0 = The control (untreated check)
MPC0 = PDA + chlorphyrifos 0.5 x DF
MPC1 = PDA + chlorphyrifos 1 x DF
MPC2 = PDA + chlorphyrifos 2 x DF
MPD0 = PDA + deltamethrin 0.5 x DF
MPD1 = PDA + deltamethrin 1 x DF
MPD2 = PDA + deltamethrin 2 x DF
MPI0 = PDA + imidacloprid 0.5 x DF
MPI1 = PDA + imidacloprid 1 x DF
MPI2 = PDA + imidacloprid 2 x DF
MPT0 = PDA + thiodicarb 0,5 x DF
MPT1 = PDA + thiodicarb 1 x DF
MPT2 = PDA + thiodicarb 2 x DF
MPY0 = PDA + cypermethrin 0.5 x DF
MPY1 = PDA + cypermethrin 1 x DF
MPY2 = PDA + cypermethrin 2 x DF

The data were analyzed with Analysis of Variance (ANOVA) at 5% error rate. The average was tested using Duncans Multiple Range Test (DMRT) $p < 0.05$ (IBM® SPSS® 20 software).

RESULTS AND DISCUSSION

Conidial germination of *B. basiana* and *M. anisopliae* were significantly affected by all insecticides used. The *B. basiana* germination was inhibited the least by deltamethrin 0.5 x DF (87.50%), followed by imidacloprid 0.5 x DF (77.22%), chlorpyrifos 0.5 x DF (67.71%), and thiodicarb 0.5 x DF (61.35%). All insecticides at 2 x DF were highly repressed *B. basiana*. de Olivera *et al.* (2003) reported that deltamethrin at the concentration of 0.5 x DF showed quite a high percentage (73.4%) conidial germination in *B. basiana*.

The results differed for *M. anisopliae*. Insecticide demonstrating the least effect on conidial germination was imidacloprid 0.5 x DF (77.72%), followed by deltamethrin 0.5 x DF (76.02%), thiodicarb 0.5 x DF (73.12%), and chlorphyrifos 0.5 x DF (60.00%). The Moderate inhibitory insecticides included cypermethrin 0.5 x DF (51.44%), deltamethrin 1x DF (46.36%), imidacloprid 1 x DF (42.49%), chlorphyrifos 1 x DF (35.25%), and cypermethrin 0.5 x DF (31.81%). High inhibitory insecticides were imidacloprid 2 x DF (1.25%), deltamethrin 2 x DF (1.25%), cypermethrin 2 x DF (0.25%), chlorphyrifos 2 x DF (0%), and thiodicarb 2 x DF(0%). Shumacher & Poehling (2012) tested three doses of imidacloprid (0.7 x DF, 1 x DF, and 1.3 x DF) to *M. anisopliae*, and concluded that the lowest dossage of imidacloprid causes highest percentage of conidial germination (> 90%) in the study.

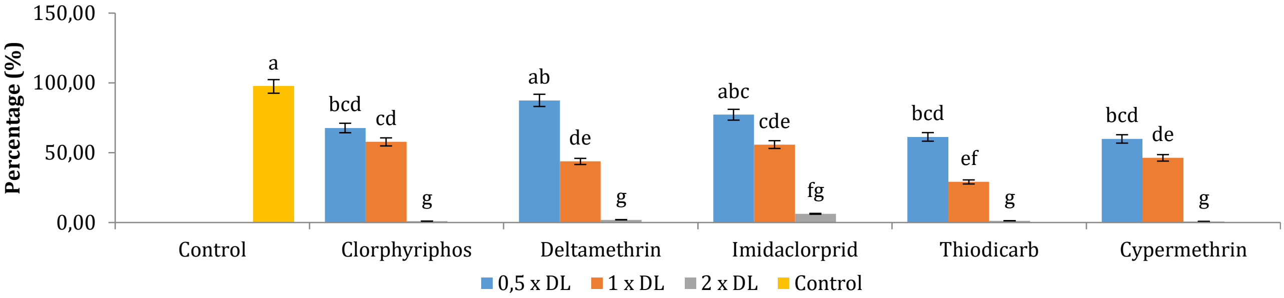

Figure 1. Germination percentage of *B. basiana* conidia.
Mean followed by different letters indicate significant differences at DMRT p <0.05 (Mean±SD)

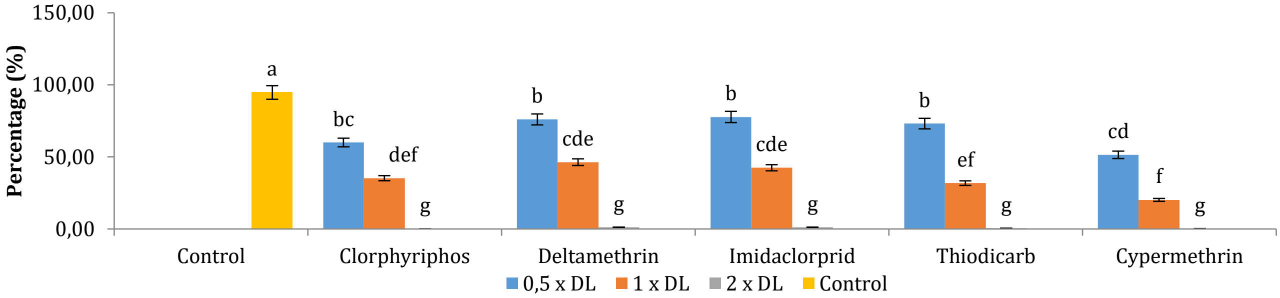

Figure 2. Germination percentage of *M. Anisopliae* conidia.
Mean followed by different letters indicate significant differences at DMRT p <0.05 (Mean±SD)

Vegetative growth was observed after 14 days, but the data were taken at 7 and 14 DAI to examine the growth and adaptation of entomopathogenic fungi against insecticides. Insecticides that inhibited the growth of entomopathogenic fungi at 7 DAI was not necessarily same as 14 DAI. This was determined by the adaptability of entomopathogenic fungi, insecticidal half-life, and insecticide degrading rate by the fungus.

The vegetative growth of *B. basiana* after 7 and 14 DAI were significantly different from the controls. Insecticide showing the highest vegetative growth of *B. basiana* at 7 DAI was imidacloprid 0.5 x DF (64.41%), followed by thiodicarb 0.5 x DL (59.26%), cypermethrin 0.5 x DL (58.82%), chlorphyrifos 0.5 x DL (56.76), imidacloprid 1 x DL (49.26%), cypermethrin 1 x DL (43.53%), thiodicarb 1 x DL (42.50%), deltamethrin 0.5 x DL (42.21%), thiodicarb 2 x DL (38.82%), chlorphyrifos 2 x DL (38.24%), chlorphyrifos 1 x DL (35.74%), deltamethrin 1 x DL (35.10%), imidacloprid 2 x DL (33.24%), cypermethrin 2 x DL (30.29%), and deltamethrin 2 x DL (10.00%).

It was different when compared to *B. basiana* vegetative growth data at 14 DAI. Insecticide that showed the highest vegetative growth was imidacloprid 0.5 x DF (85.22%) and imidacloprid 1 x DF (85.48%), followed by thiodicarb 0.5 x DL (71.57%), cypermethrin 1 x DL (64.61%), chlorphyrifos 0.5 x DL (66.96%), thiodicarb 1 x DL (63.35%), deltamethrin 0.5 x DL (58.35%), cypermethrin 0.5 x DL (60.35%), chlorphyrifos 1 x DL (52.87%), chlorphyrifos 2 x DL (51.30%), imidacloprid 2 x DL (43.48%), thiodicarb 2 x DL (42.09%), cypermethrin 2 x DL (41.91%), deltamethrin 1 x DL (39.30%), anddeltamethrin 2 x DL (17.22%). Alizadeh *et al.*, (2007) investigated the effect of three doses of imidacloprid (0.5 x DF, 1 x DF, and 2 x DF) against *B. basiana* and showed that all three doses of imidacloprid had lowest inhibitory effect (< 22%).

Compared to the control, insecticide that demonstrated high vegetative growth of *M. anisopliae* at 7 DAI was imidacloprid 0.5 x DF (115.70%), followed by chlorphyrifos 2 x DL (99.45%), cypermethrin 0.5 x DL (97.80%), cypermethrin 1 x DL (96.14%), deltamethrin 1 x DL (85.12%), deltamethrin 0.5 x DL (84.30%), thiodicarb 0.5 x DL (82.09%), deltamethrin 2 x DL (76.86%), imidacloprid 2 x DL (75.21%), chlorphyrifos 1 x DL (74.38%), chlorphyrifos 0.5 x DL (73.55%), imidacloprid 1 x DL (73.28%), cypermethrin 2 x DL (60.88%), thiodicarb 1 x DL (55.37%), and thiodicarb 2 x DL (50.41%).

This results were different with vegetative growth of *M. anisopliae* at 14 DAI. Insecticide that revealed the highest vegetative growth of the fungus was chlorphyrifos 2 x DF (87.12%), followed by cypermethrin 2 x DL (80.98%), imidacloprid 0.5 x DL (78.22%), cypermethrin 0.5 x DL (67.33%), cypermethrin 1 x DL (64.11%), thiodicarb 1 x DL (54.29%), imidacloprid 2 x DL (53.83%), chlorphyrifos 0.5 x DL (53.22%), thiodicarb 0.5 x DL (52.91%), deltamethrin 1 x DL (51.69%), dan deltamethrin 0.5 x DL (49.85%). deltamethrin 2 x DL (46.27%), chlorphyrifos 1 x DL (44.94%), imidacloprid 1 x DL (43.56%), and thiodicarb 2 x DL (40.49%).

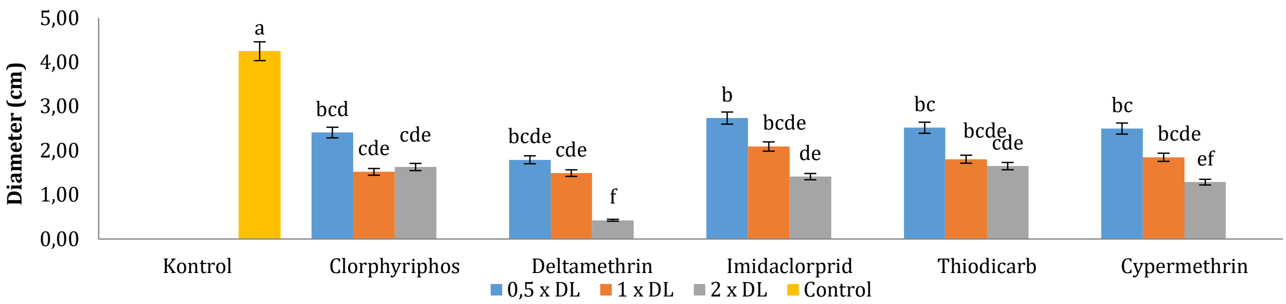

Figure 3. Colony diameter of *B. basiana* at 7 DAI.
Mean followed by different letters indicate significant differences at DMRT p <0.05 (Mean±SD)

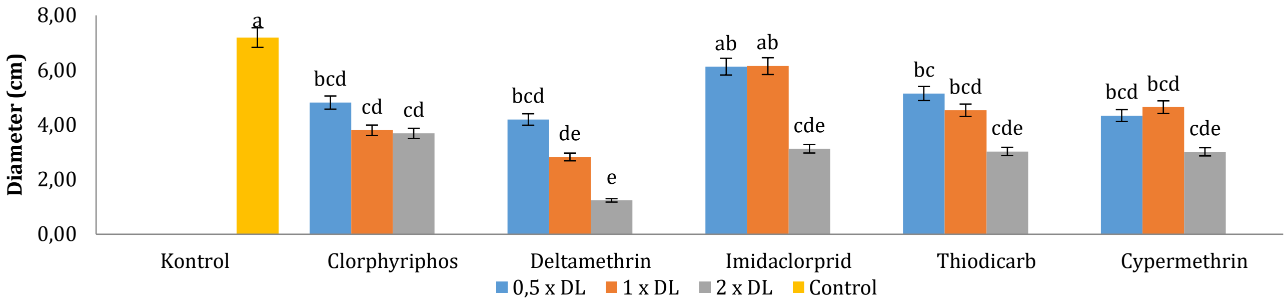

Figure 4. Colony diameter of *B. basiana* at 14 DAI.
Mean followed by different letters indicate significant differences at DMRT p <0.05 (Mean±SD)

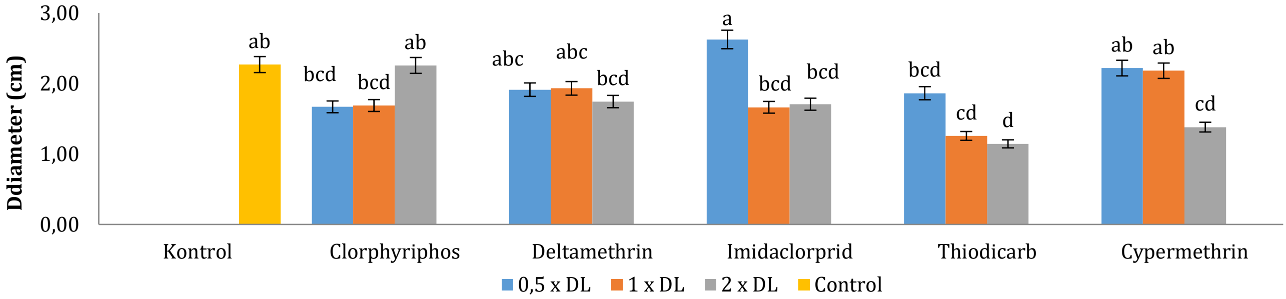

Figure 5. Colony diameter of *M. anisopliae* at 7 DAI.
Mean followed by different letters indicate significant differences at DMRT p <0.05 (Mean±SD)

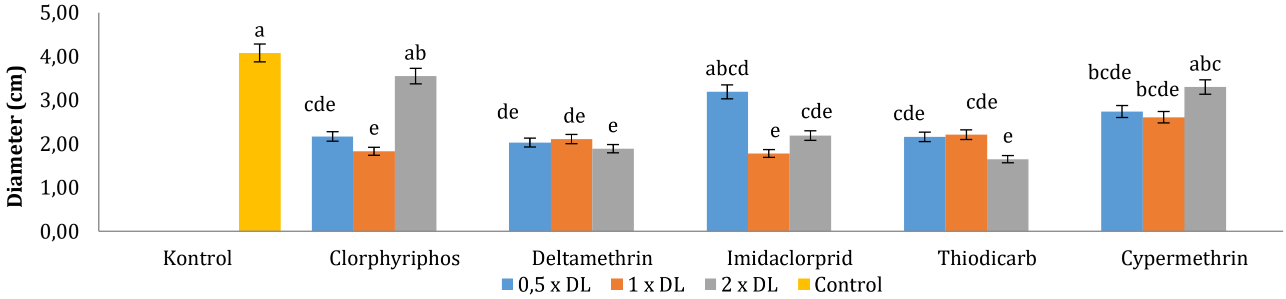

Figure 6. Colony diameter of *M. anisopliae* at 14 DAI.
Mean followed by different letters indicate significant differences at DMRT p <0.05 (Mean±SD)

Shumacher & Poehling (2012) stated that imidacloprid at lower concentration showed the smallest growth inhibition for *M. anisopliae*. Karnataka (2007) examined the effects chlorphyrifos, cypermethrin, deltamethrin, imidacloprid and thiodicarb to vegetative growth of *M. anisopliae*, and demonstrated that the lowest inhibition was by imidacloprid (11.1%).

Based on the comparison with the control, insecticide that indicated the highest conidial production for *B. basiana* were imidacloprid 0.5 x DF (46.04%), cypermethrin 0.5 x DL (39.43%), imidacloprid 1 x DL (37.67%), thiodicarb 0.5 x DL (32.71%), thiodicarb 2 x DL (25.38%), thiodicarb 1 x DL (25.17%), cypermethrin 1 x DL (22.48%), cypermethrin 2 x DL (22.18%), deltamethrin 0.5 x DL (21.17%), deltamethrin 1 x DL (20.06%), chlorphyrifos 1 x DL (19.88%), chlorphyrifos 0.5 x DL (18.18%), imidacloprid 2 x DL (15.25%), chlorphyrifos 2 x DL (12.44%) and deltamethrin 2 x DL (20.06%). James & Elzen (2001) stated that imidacloprid had no effects on conidial germination, vegetative growth and conidia production in *B. basiana*.

Alizadeh *et al.* (2007) investigated the effect of three doses of imidacloprid (0.5 x DF, 1 x DF, and 2 x DF) to conidial production of *B. basiana* and reported that all three doses of imidacloprid had the lowest reduction value (3–46%).

Comparison to the control showed that the highest conidia production of *M. anisopliae* were on imidacloprid 0.5 x DF (112.99%), cypermethrin 1 x DL (73.64%), chlorphyrifos 0.5 x DL (68.48%), cypermethrin 0.5 x DL (49.62%), imidacloprid 1 x DL (46.36%), thiodicarb 1 x DL (43.60%), deltamethrin 0.5 x DL (40.42%) thiodicarb 0.5 x DL (29.77%), cypermethrin 2 x DL (29.02%), chlorphyrifos 1 x DL (25.95%), imidacloprid 2 x DL (24.77%), deltamethrin 2 x DL (14.28%), chlorphyrifos 2 x DL (6.55%), andthiodicarb 2 x DL (6.29%). Karnataka (2007) examined the effects of chlorphyrifos, thiodicarb, cypermethrin, deltamethrin, and imidacloprid on conidial production of *M. anisopliae*. The results showed that the highest conidial production were those with imidacloprid (293 x 10^6 conidia.ml^{-1}), followed by deltamethrin (193 x 10^6 conidia.ml^{-1}), cypermethrin (150 x 10^6 conidia.ml^{-1}), thiodicarb (71 x 10^6 conidia.ml^{-1}) and chlorphyrifos (17 x 10^6 conidia.ml^{-1}). This was supported by Akbar *et al.* (2012) who showed conidial production of *M. anisopliae* were higher in imidacloprid than cypermethrin.

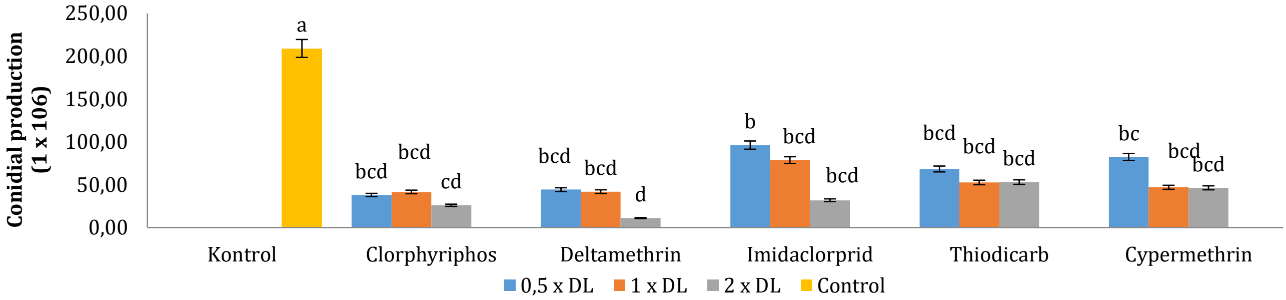

Figure 7. Conidial production of *B. basiana.*
Mean followed by different letters indicate significant differences at DMRT p <0.05 (Mean±SD)

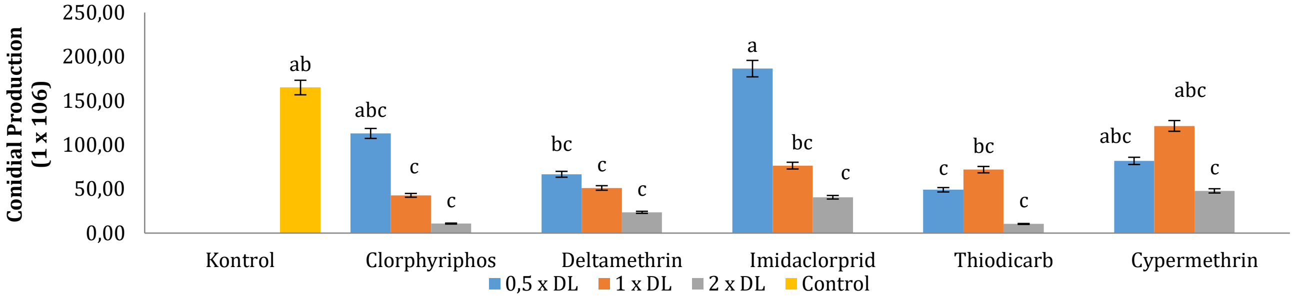

Figure 8. Conidial production of *M. anisopliae.*
Mean followed by different letters indicate significant differences at DMRT p <0.05 (Mean±SD)

Table 1. Compatibility of Insecticides and *B. basiana* based on equation of Alves *et al.* (2007)

Treatment	Conidial Germination		Vegetative Growth *		Conidial Production		Biological Index	Classification
	%	Reduction (%)	cm	Reduction (%)	1x10^6	Reduction (%)		
MPO	97,50	-	7.19	-	209.06	-	-	-
MPC0	67.71	30.56	4.81	33.04	38.00	81.82	46.23	Moderately Toxic
MPC1	57.75	40.77	3.80	47.13	41.56	80.12	39.32	Toxic
MPC2	1.00	98.97	3.69	48.70	26.00	87.56	29.56	Toxic
MPD0	87.50	10.26	4.19	41.65	44.25	78.83	45.50	Moderately Toxic
MPD1	43.75	55.13	2.83	60.70	41.94	79.94	31.59	Toxic
MPD2	2.00	97.95	1.24	82.78	11.06	94.71	10.57	Toxic
MPI0	77.22	20.80	6.13	14.78	96.25	53.96	67.77	Compatible
MPI1	55.83	42,74	6,14	14.52	78.75	62.33	62,10	Compatible
MPI2	6.25	93.59	3.13	56.52	31.88	84.75	27.63	Toxic
MPT0	61.35	37.08	5.14	28.43	68.38	67.29	53.99	Moderately Toxic
MPT1	29.08	70.17	4.53	36.96	52.63	74.83	43.44	Moderately Toxic
MPT2	1.25	98.72	3.03	57.91	53.06	74.62	30.82	Toxic
MPY0	59.89	38.57	4.34	39.65	82.44	60.57	51.46	Moderately Toxic
MPY1	46.33	52.48	4.64	35.39	47.00	77.52	44.79	Moderately Toxic
MPY2	0.75	99.23	3.01	58.09	46.38	77.82	29.31	Toxic

Note: * Data taken from the average of colony diameter on the 14 DAI

Table 2. Compatibility of Insecticides and M. Anisopliae based on Alves *et al.* (2007) equation

Treatment	Conidial Germination		Vegetative Growth *		Conidial Production		Biological Index	Classification
	%	Reduction (%)	cm	Reduction (%)	1 x 10^6	Reduction (%)		
MPO	94.72	-	4.08	-	165.00	-	-	-
MPC0	60.00	36.66	2.17	46.78	113.00	31.52	43.58	Moderately Toxic
MPC1	35.25	62.79	1.83	55.06	42.81	74.05	24.40	Toxic
MPC2	0,00	100.00	3.55	12.88	10.81	93.45	25.44	Toxic
MPD0	76.02	19.74	2.03	50.15	66.69	59.58	34.80	Toxic
MPD1	46.36	51.06	2.11	48.31	51.19	68.98	29.06	Toxic
MPD2	1.25	98.68	1.89	53.73	23.56	85.72	17.30	Toxic
MPI0	77.72	17.95	3.19	21.78	186.44	12.99	67.16	Compatible
MPI1	42.49	55.14	1.78	56.44	76.50	53.64	31.70	Toxic
MPI2	1.25	98.68	2.19	46.17	40.63	75.38	22.83	Toxic
MPT0	73.12	22.81	2.16	47.09	49.13	70.23	31.70	Toxic
MPT1	31.81	66.41	2.21	45.71	71.94	56.40	32.53	Toxic
MPT2	0.50	99.47	1.65	59.51	10.38	93.71	12.97	Toxic
MPY0	51.44	45.69	2.74	32.67	81.88	50.38	40.06	Toxic
MPY1	20.16	78.72	2.61	35.89	121.50	26.36	44.14	Moderately Toxic
MPY2	0.25	99.74	3.58	12.12	47.88	70.98	33.29	Toxic

Note: * Data taken from the average of colony diameter at the 14 DAI

Alves *et al.* (2007) proposed the Biological Index (BI) equation as a reference to classify the toxicological effects of chemical compounds on entomopathogenic fungi under in vitro condition. The equation was based on conidial germination, vegetative growth, and conidia production data for the compatibility parameter, which differed from T factor formula previously published by Alves *et al.* (1998). The later equation had not included conidia germination as one of the compatibility parameters. Earlier, Hassan (1989) also proposed an equation called the Hasan classification scheme, but it was based on vegetative growth data only as the compatibility parameter. Accordingly, the Biological Index of Alves *et al.* (2007) was the most relevant equation to apply to this study.

Based on the equation of Alves *et al.* (2007), the compatible insecticides to *B. basiana* (Table 1) were imidacloprid 0.5 x DF (BI value 67.77) and imidacloprid 1 x DF (62,10). These results were consistent with some reference. Alizadeh (2007) and Singh *et al.* (2014) suggested that the insecticide imidacloprid (0.5 x DF) was compatible with the *B. basiana*. There were similar compatibility insecticides in *M. anisopliae* (Table 2). Based on the equation of Alves *et al.* (2007), the imidacloprid 0.5 x DF was compatible with *M. anisopliae* (BI value 67.16). This finding was consistent with Shumacher & Poehling (2012) who claimed that imidacloprid at low concentration tended to increase the germination of conidia of the *M. anisopliae*. Vijila *et al.* (2011), Akbar *et al.*, (2012), and Singh *et al.* (2014) revealed that the lowest dose of imidacloprid was compatible with *M. anisopliae*. Imidacloprid was neurotoxic to insects but had no adverse effect on *B. basiana* and *M. anisopliae* that were able to metabolize and liberate compounds as secondary nutrients.

CONCLUSION

It was concluded that insecticides showing the highest conidial germination percentage on *B. basiana* and *M. anisopliae* were deltamethrin 0.5 x DF and imidacloprid 0.5 x DF, respectively. Those demonstrating the most significant vegetative growth on *B. basiana* and *M. anisopliae* at 7 DAI were imidacloprid 0.5 x DF, while at 14 DAI were imidacloprid 0.5 x DF and chlorpyrifos 2 x DF. Insecticides showing the highest conidial production on *B. basiana* and *M. anisopliae* were imidacloprid 0.5 x DF. The compatible insecticide with *B. basiana* and *M. anisopliae* was imidacloprid 0,5 x DF with BI values of 67.77 and 67.16.

ACKNOWLEDGMENTS

We thank to the BBPPTP Surabaya and BBalitvet Bogor for entomopathogenic fungi isolates, and Mutiara Widawati for preserving the chemical substances.

REFERENCES

Akbar S, Freed S, Hameed A, Gul HT, Akmal M, Malik MN, Naeem M, Khan MB. 2012. Compatibility of *Metarhizium anisopliae* with different insecticides and fungicides. African Journal of Microbiology Research. 6(17):3956-3962.

Alizadeh A, Samih MA, Khezri M, Riseh RS. 2007. Compatibility of *Beauveria bassiana* (Bals.) Vuill. Eith several insecticides. International Journal of Agriculture & Biology. 9(1):31-34.

Alves SB, Monino A. Jr, Almeida JEM. 1998. Produtosfitossanitários eentomopatogênicos. Sâo Paulo: Fealq.

Alves SB, Haddad ML, Faion M, de Baptista GC, Rossi-Zalaf LS. 2007. Novo ındice biologico para classificacao da toxicidade de agrotoxicos para fungos entomopatogenicos. Brasılia: Anais do X Siconbiol.

Ambethgar V. 2009. Potential of entomopathogenic fungi in insecticide resistance management (IRM): A review. Journal of Biopesticides. 2(2):177-193.

Asi MR, Bashir MH, Afzal M, Ashfaq M, Sahi ST. 2010. Compatibility Of Entomopathogenic Fungi, *Metarhizium anisopliae* and *Paecilomyces fumosoroseus* with Selective Insecticides. Pak. J. Bot. 42(6):4207-4214.

de Oliveira CN, Neves PMOJ, Kawazoe LS. 2003. Compatibility Between The Entomopathogenic Fungus *Beauveria bassiana* and Insecticides Used In Coffee Plantations. Scientia Agricola. 60(4):663-667.

Golshan H, Saber M, Majidi-Shilsar F, Bagheri M, Mahdavi V. 2013. Effects of Common Pesticides Used in Rice Fields on the Conidial Germination of Several Isolates of Entomopathogenic Fungus, *Beauveria bassiana* (Balsamo) Vuillemin. J. Entomol. Res. Soc. 15(1):17-22.

Hassan SA. 1989. Testing methodology and the concept of the IOBC/WPRS working group. P.C. Jepson, editor. Pesticides and Non-Target Invertebrates. Wimborne: Dorset.

Herdatiarni F, Himawan T, Rachmawati R. 2014. Eksplorasi Cendawan Entomopatogen *Beauveria* sp. menggunakan Serangga Umpan pada Komoditas Jagung, Tomat dan Wortel Organik Di Batu, Malang. Jurnal HPT. 1(3):1-11.

James R, Elzen GW. 2001. Antagonism between *Beauveria bassiana* and Imidacloprid when combined for *Bemisia argentifolii* (Homoptera: Aleyrodidae) control. J. Econ. Entomol. 94(2):57-61.

Karnataka. 2007. Effect of Agrochemicals on Growth and Sporulation of *Metarhizium anisopliae* (Metschnikoff) Sorokin. J. Agric. Sci. 20(2):410-413.

Moorhouse ER, Charnley AK, Gillespie AT. 1992. Review of the biology and control of the vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae). Ann. Appl. Biol. 121(2):431-454.

Schumacher V, Poehling H. 2012. In vitro effect of pesticides on the germination, vegetative growth, and conidial production of two strains of *Metarhizium anisopliae.* Fungal Biologi. 116:121-132.

Singh RK, Vats S, Singh B. 2014. Compatibility Analysis of Entomopathogenic Fungi *Beauveria bassiana* (NCIM No-1300) With Several Pesticides. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 5(1):837-844.

Vijila SM, Das SSM, Sekar RRJ. 2011. A Novel Chemical-Microbial Hybrid Pesticides in the Management of *Sitohpilus oryzae* L. International Journal of Pharma and Bio Sciences. 2(4):1-6.