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. bassiana and M. anisopliae. The experimental design applied completely randomized design consisting of 15 treatments and four replicates. There were five types of insecticides showing the highest vegetative growth on B. bassiana and M. anisopliae at 7 DAI was imidacloprid 0.5 x DF, while at 14 DAI was imidacloprid 0.5 x DF and chlorpyrifos 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. bassiana (Bl: 67.77) and M. anisopliae (Bl: 67.16). Key Words: B. bassiana, 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 andchlorpyrifos (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. bassiana and showed low inhibitory effect (< 5%) at the lowest field 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. bassiana and M. anisopliae. Thus, the purpose of this study was to determine the compatibility of five insecticides at particular doses to entomopathogenic fungus B. bassiana 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. bassiana was from Balai Besar Perbenihan dan Protelsi Tanaman Perkebunan (BBPPTP) Surabaya, and M. anisopliae was from Balai Besar Perbenihan dan Protelsi Tanaman Perkebunan (BBPPTP) Surabaya.
anisopliae was obtained from Balai Besar Penelitian Veterin (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, chlorpyrifos, imidacloprid, deltamethrin, 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.

Conidial suspension was taken and placed on a hemocytometer. Conidial germination was observed in 5 mm diameter of treatment with control, each with four replicates. The medium without insecticides 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 10⁶ conidia/ml) 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 determine 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 a 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 10⁶ conidia.ml⁻¹.

Calculation of Insecticidal Compatibility used the formula below:

\[
BI = \frac{10 \times (GR) + 47 \times (VG) + 43 \times (SPR)}{100}
\]

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.

M0 = The control (untreated check)
MPC0 = PDA + chlorpyrifos 0.5 x DF
MPC1 = PDA + chlorpyrifos 1 x DF
MPC2 = PDA + chlorpyrifos 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
MFT0 = PDA + thiodicarb 0.5 x DF
MFT1 = PDA + thiodicarb 1 x DF
MFT2 = PDA + thiodicarb 2 x DF
MPV0 = PDA + cypermethrin 0.5 x DF
MPV1 = PDA + cypermethrin 1 x DF
MPV2 = 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 chlorpyrifos 0.5 x DF (60.00%). The Moderate inhibitory insecticides included cypermethrin 0.5 x DF (51.44%), deltamethrin 1 x DF (46.36%), imidacloprid 1 x DF (42.49%), chlorpyrifos 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%), chlorpyrifos 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 dosage of imidacloprid causes highest percentage of conidial germination (> 90%) in the study.
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%), chlorpyrifos 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%), chlorpyrifos 2 x DL (38.24%), chlorpyrifos 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%), chlorpyrifos 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%), chlorpyrifos 1 x DL (52.87%), chlorpyrifos 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%), and deltamethrin 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 chlorpyrifos 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%), chlorpyrifos 1 x DL (74.38%), chlorpyrifos 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 chlorpyrifos 2 x DF (87.12%), followed by cypermethrin 2 x DF (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%), chlorpyrifos 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%), chlorpyrifos 1 x DL (44.94%), imidacloprid 1 x DL (43.56%), and thiodicarb 2 x DL (40.49%).
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%), chlorpyrifos 1 x DL (19.88%), chlorpyrifos 0.5 x DL (18.18%), imidacloprid 2 x DL (15.25%), chlorpyrifos 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 DL, 1 x DL, and 2 x DL) 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 DL (112.99%), cypermethrin 1 x DL (73.64%), chlorpyrifos 0.5 x DL (68.48%), cypermethrin 0.5 x DL (49.62%), imidacloprid 1 x DL (46.36%), thiocarb 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%), chlorpyrifos 1 x DL (25.95%), imidacloprid 2 x DL (24.77%), deltamethrin 2 x DL (14.28%), chlorpyrifos 2 x DL (6.55%), and thiodicarb 2 x DL (6.29%). Karnataka (2007) examined the effects of chlorpyrifos, thiocarb, 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 chlorpyrifos (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.](image1)

![Figure 8. Conidial production of M. anisopliae.](image2)

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Conidial Germination % Reduction (%)</th>
<th>Vegetative Growth * cm Reduction (%)</th>
<th>Conidial Production 1x10^6 Reduction (%)</th>
<th>Biological Index</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>MP0</td>
<td>97.50</td>
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<td>209.06</td>
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<td>38.00</td>
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Note: * Data taken from the average of colony diameter on 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. bassiana. 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. bassiana and M. anisopliae that were able to metabolize and liberate compounds as secondary nutrients.

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

<table>
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<th>Treatment</th>
<th>Conidial Germination % Reduction (%)</th>
<th>Vegetative Growth * cm Reduction (%)</th>
<th>Conidial Production 1 x 10⁶ Reduction (%)</th>
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Note: * Data taken from the average of colony diameter at the 14 DAI

**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.

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**REFERENCES**


