Suitable Combination Between Beauveria bassiana (Balsamo) Vuillemin and Four Plant Leaf Extracts to Control Spodoptera litura (Fabricius)

Aminudin Afandhi*, Vivi Renna Pratiwi, Mochammad Syamsul Hadi, Yogo Setiawan and Retno Dyah Puspitarini

Department of Plant Pest and Disease, Faculty of Agriculture, Universitas Brawijaya, East Java, Indonesia

ARTICLE INFO

Keywords:
- Beauveria bassiana
- Compatibility
- Conidial density and viability
- Larval mortality
- Plant extracts

Article History:
Received: April 27, 2020
Accepted: June 8, 2020

* Corresponding author:
E-mail: aaf_fp@ub.ac.id

ABSTRACT

Lepidopteran pests such as S. litura might cause yield losses in many crops especially during pest outbreak. The combinations of microbial and botanical insecticides were expected to increase the effectiveness in controlling the respected insect pest. This research aimed to evaluate the combination of plant extracts and B. bassiana to control S. litura. The compatibility of B. bassiana with different plant extracts such as Neem, Chinaberry, Mexican Sunflower and Lantana leaves was studied in the laboratory. The compatibility was evaluated based on B. bassiana colony growth, conidia density, conidia viability, and mortality of S. litura larvae. After 12 days after application (DAA), colony growth of B. bassiana was reduced by all plant extract treatments. For the mortality of S. litura, the combined mixture of B. bassiana and 0.25% Chinaberry extract resulted in the maximum mortality rate (44%). After 12 and 15 DAA, the 0.25% Chinaberry showed the highest colony growth (3.93 and 4.37 cm). The most suitable treatment was B. bassiana with 0.25% Chinaberry that had the conidial density at 1.77 x 10⁸ conidia/ml and conidial viability of 75.63% and can enhanced the mortality of S. litura larvae.

INTRODUCTION

As the major pest on crops, Spodoptera litura Fabricius is also has a strong ability to be a migratory pest. These insect pests have been distributed from tropical to temperate regions (Fu, Zhao, Xie, Ali, & Wu, 2015). Spodoptera litura larvae can attack over 112 plants in farmland (Houguo, Zhengxiang, Shuijin, Jian, & Renhua, 2004). Spodoptera litura management is mostly use pesticides and leading to many negative effects to environment and human health (Ahmad & Mehmood, 2015). The application of various chemical insecticides has made Spodoptera litura developed resistance to several type insecticides (Tong, Su, Zhou, & Bai, 2013). Recently, the concern of the use of more environmentally friendly insect pest control has been increase worldwide (Dhar, Jindal, Jariyal, & Gupta, 2019). Pest control with environmentally friendly strategies is need to solve the above-mentioned problems (Revathi, Chandrasekaran, Thanigaivel, Kirubakaran, & Senthil-Nathan, 2014).

Entomopathogenic fungi have been used to control pest because they efficiently kill insects and relatively safe for the human health and environment (Garrido-Jurado, Ruano, Campos, & Quesada-Moraga, 2011). Beauveria bassiana has a broad host range and over 700 species of vertebrate (Glare et al., 2012). Like other entomopathogenic fungi, conidia of B. bassiana penetrate through the cuticle insects pest (Ortiz-Urquiza, Luo, & Keyhani, 2015). Beauveria bassiana is considered to have minimal effects on non-target organism, including animals and humans (Gouli, Gouli, & Kim, 2014). B. bassiana has ability to produce infectious conidia rapidly and high effectiveness against different insect pests (Taylor, Edginton, Luke, & Moore, 2013).

Several factors have been observed to be the limitation of the entomopathogenic fungi

ISSN: 0126-0537 Accredited First Grade by Ministry of Research, Technology and Higher Education of The Republic of Indonesia, Decree No: 30/E/KPT/2018

Cite this as: Afandhi, A., Pratiwi, V. R., Hadi, M. S., Setiawan, Y., & Puspitarini, R. D. (2020). Suitable combination between Beauveria bassiana (Balsamo) vuillemin and four plant leaf extracts to control Spodoptera litura (Fabricius). AGRIVITA Journal of Agricultural Science, 42(2), 341–349. https://doi.org/10.17503/agrivita.v42i2.2678
as biocontrol agents. These factors include environmental conditions, speed of action, shelf life and their compatibility with chemical fungicides (Jaber, Araj, & Qasem, 2018). Botanical insecticides were more effective when applied in combination with microbial insecticides, than single application (Islam, 2006). The compatibility of Beauveria bassiana (Balsamo) with neem (Azadirachta indica) extracts caused higher Bemisia tabaci mortality than individual treatments of B. bassiana (Islam, Castle, & Ren, 2010). Bemisia argentifolii mortality was significantly higher in Azadirachta indica combined with Paecilomyces fumosoroseus fungi than only one of agents was used (James, 2003). The compatibility of B. bassiana with different plant extracts such as Neem (Azadirachta indica), Chinaberry (Melia azedarach), Mexican Sunflower (Tithonia diversifolia) and Lantana (Lantana camara) leave was tested in this study. This research aimed to investigate the most effective plant extract formulation in combination with B. bassiana as an alternative pest control for S. litura.

**MATERIALS AND METHODS**

This research was conducted at the Laboratory of Biological Control, Department Plant Pest and Disease, Faculty of Agriculture, Universitas Brawijaya from April to June 2016.

**Preparation of Beauveria bassiana Isolates**

Beauveria bassiana isolates were obtained from the Department of Plant Pest and Disease, Faculty of Agriculture, Universitas Brawijaya. The isolates were cultured on Potato Dextrose Agar medium (PDA), composed by Potato extract solution (250 g potatoes and 1200 ml sterile water), 20 g dextrose, 10 g peptone, and 1 ml of chloramphenicol. The water solution was poured into a medium bottle, and the bottles were sterilized using autoclave for 30 minutes at 121°C and 1 atm pressure. After the isolates inoculated in the media, the cultures were shaken for 48 hours at a speed of 120 rpm. After that, the isolates were incubated for 6 days. The conidia density was counted under the microscope using a hemocytometer from 1 ml suspension. Conidia density was calculated using the method of Singleton & Sainsbury (2006).

**Preparation of Plant Leaf Extracts**

The materials used in this study were Neem (Azadirachta indica), Chinaberry (Melia azedarach), Mexican Sunflower (Tithonia diversifolia) and Lantana (Lantana camara) leaves. The leaves were washed with distilled water and air-dried at room temperature. The plant leaf was weighted at 1000 g each. The leaves were put on 1 l sterile water and blended by hand mixer and then kept for 24 hours. The leaves were then filtered to get the extract. The chosen concentrations for each plant extracts were 0.25%, 0.5%, and 1%.

**Colony Growth of B. bassiana on Plant Leaf Extracts**

The compatibility of B. bassiana isolates on plant extracts was studied under laboratory conditions. The research design for all treatments was a non factorial completely randomized experiment. For each treatment, 10 ml Sabouraud Dextrose Agar with yeast extract (SDAY) medium (10 g dextrose, 2.5 g yeast extract, 2.5 – 20 g peptone, 0.5 g chloramphenicol and 1 l sterile water) were prepared and put on petri dishes (Samuels, Coracini, Martins dos Santos, & Gava, 2002). The plant extracts were added in the SDAY medium (2 ml) for each concentration (Table 1). Next, filter paper with 5 mm diameter was put on B. bassiana suspension. The filter paper contained B. bassiana was the put into petri dishes with the SDAY medium. All process was conducted in Laminar Air Flow Cabinet (LAFC) to protect from contamination, after inoculation B. bassiana and plant extracts were incubated at 26-29°C.

The compatibility was calculated based on three variables, namely colony growth, conidial viability, and conidial density. The colony growth of B. bassiana isolate was studied based on the growth of mycelia as well as the production of aerial conidia on the treated medium. The mean diameter of the colonies was obtained from 6 to 15 days after application (Depieri, Martinez, & Menezes Jr., 2005). The colony growth of B. bassiana isolate was calculated based on the diameter of growth of the colony by Vincent (1947).

\[
I = \left( \frac{(C - T)}{C} \right) \times 100
\]

Where: I, C, and T are inhibition of colony (%), growth of B. bassiana colony in control (cm), and growth of B. bassiana colony in treatment (cm), respectively.
The conidial viability of B. bassiana on the plant extracts was studied by adding 10 ml sterile water to Sabouraud Dextrose Agar with yeast extract (SDAY) with plant extracts. For about 1 ml suspensions were taken and put on microscopic glass slides with agar water layer. There were three replications per treatment. The material was incubated about 48 hours at 24°C in the growth chamber (petri dish with moist tissue paper). The viability of conidia was recorded on day 6 after incubation. The viability of conidia as described by the germinated conidia was calculated by using a hemocytometer under a compound microscope at 400× magnification.

After 15 days incubation, 5 ml sterile water was put on SDAY with plant extract. The conidia on each medium was taken by using fine brushes. A suspension of each treatment was put into hemocytometer and the conidial density (conidia/ml) was counted. Conidial density was calculated using the method of Singleton & Sainsbury (2006). The compatibility of B. bassiana was calculated by the formula of Sain et al. (2019):

\[
T = \frac{[20 \times VG + 80 \times SP]}{100} \quad \cdots \quad \text{2)}
\]

Where: VG and SP are vegetative growth, and sporulation values (conidiogenesis) to the control (%), respectively. The compatibility of B. bassiana (T) is categorized as very toxic (from 0 to 30), toxic (from 31 to 45), moderately toxic (from 46 to 60), and compatible (above 60).

### Insect Rearing

The larvae of S. litura for this study were obtained from Indonesian Sweetener and Fiber Crops Research Institute (ISFCRI). Spodoptera litura was reared by using a natural diet with castor leaves (Ricinus communis). The larvae of S. litura were placed in the container, each container was placed one larva until it became a pupa. The pupa was transferred to a new plastic cylinder container until it became a moth. Moths were held for mating and oviposition in cages with 15-20% honey solution as food. Castor leaf was provided as an ovipositional substrate. After the eggs hatch into larvae (F1 progeny), the larvae were transferred to a new container and reared on castor leaves. The 2nd instar larvae were selected and used in this research.

### Effect of B. bassiana and Plant Extracts combinations to S. Litura

Three different plant extracts, Neem (Azadirachta indica), Chinaberry (Melia azedarach), Mexican Sunflower (Tithonia diversifolia) and Lantana (Lantana camara) with the concentrations (0.1%, 0.3%, and 0.5%) and 10 ml B. bassiana isolate (1 x 10⁸ conidial/ml) were mixed and sprayed on 20 of the 2nd instar larvae of S. litura for each treatment. Each treatment setup was repeated

#### Table 1. The treatment combination of B. bassiana conidial density and plant leaf extracts

| No. | Treatment (Plant extracts and Conidial density of B. bassiana) |
|-----|----------------------------------------------------------------|
| 1.  | Control (B. bassiana x 10⁸/ml)                                |
| 2.  | 0.25% of Neem + B. bassiana x 10⁸/ml                         |
| 3.  | 0.50% of Neem + B. bassiana x 10⁸/ml                         |
| 4.  | 1.00% of Neem + B. bassiana x 10⁸/ml                         |
| 5.  | 0.25% of Chinaberry + B. bassiana x 10⁸/ml                  |
| 6.  | 0.50% of Chinaberry + B. bassiana x 10⁸/ml                  |
| 7.  | 1.00% of Chinaberry + B. bassiana x 10⁸/ml                  |
| 8.  | 0.25% of Mexican Sunflower + B. bassiana x 10⁸/ml           |
| 9.  | 0.50% of Mexican Sunflower + B. bassiana x 10⁸/ml           |
| 10. | 1.00% of Mexican Sunflower + B. bassiana x 10⁸/ml           |
| 11. | 0.25% of Lantana + B. bassiana x 10⁸/ml                     |
| 12. | 0.50% of Lantana + B. bassiana x 10⁸/ml                     |
| 13. | 1.00% of Lantana + B. bassiana x 10⁸/ml                     |
three times. Larval mortality was recorded daily on day 7. Fungal infection of larvae was distinguished by the growth of mycelia on larval bodies. The percentage of mortality was calculated using the following formula (Abbott, 1925):

\[
\text{% mortality} = \left( \frac{\text{Number of died insect}}{\text{Number of insects tested}} \right) \times 100
\]

Data Analysis

Data were analyzed by the two ways - Analysis of Variance (ANOVA) at \(\alpha = 5\%\) using Microsoft Excel. If there were significant differences between treatments, the Duncan’s Multiple Range Test is adopted to perform the mean comparison among the treatments.

RESULTS AND DISCUSSION

Compatibility of \(B.\ bassiana\) and Plant Extracts on Colony Growth, Density, and Viability of Conidia

The results showed that the addition of plant extracts to the growth medium can reduce the growth of \(B.\ bassiana\) colonies. All treatments had lower colony growth compared to control (Table 2). The growth rate of \(B.\ bassiana\) colonies in Chinaberry extract was higher than Neem, Mexican Sunflower, and Lantana leaf extract.

At 12 DAA, the colony growth showed that in the control, 0.25% Chinaberry, and 0.5% Chinaberry were not significantly different (4.28, 3.93 and 3.88 cm, respectively). At 15 DAA, medium was added 0.25% Chinaberry leaf extract had the highest colony growth rate (4.37 cm) compared to other leaf extract treatments (Fig. 1). The percentage of inhibition in the growth of \(B.\ bassiana\) colonies was low in each treatment. The highest percentage of inhibition was in 1% Lantana leaf (0.22%) and the lowest percentage inhibition was in 0.25% Mexican sunflower (0.15%) (Table 2).

Depieri, Martinez, & Menezes Jr. (2005) stated that emulsiable neem oil, in different concentrations can reduce vegetative growth of colonies and the conidiogenesis of \(B.\ bassiana\) significantly, as compared with the control. Nana et al. (2012) stated that the extract of Calpurnea aurea leaves with various concentrations did not significantly affect the growth of \(M.\ anisopliae\) colony at 3 and 6 DAA. Plants extracts contain toxic substances/compounds with inhibitory effects on colony growth of \(B.\ bassiana\) (Tuan, 2014).

Table 2. Growth of \(B.\ bassiana\) colony at 3, 6, 9, 12, and 15 days after application (DAA) with plant extracts

| Treatment                      | 3 DAA | 6 DAA | 9 DAA | 12 DAA | 15 DAA | % Inhibition (6 DAA) |
|--------------------------------|-------|-------|-------|--------|--------|---------------------|
| Control (\(B.\ bassiana\) (10^8 conidia/ml)) | 1.54 b | 2.22 b | 3.49 c | 4.28 b | 4.83 c | -                   |
| 0.25% of Neem                   | 1.04 a | 1.82 a | 2.56 ab | 3.42 a | 3.84 a | 18                  |
| 0.50% of Neem                   | 1.04 a | 1.81 a | 2.49 ab | 3.27 a | 3.79 a | 18                  |
| 1.00% of Neem                   | 1.00 a | 1.80 a | 2.37 a  | 3.19 a | 3.64 a | 19                  |
| 0.25% of Chinaberry             | 1.07 a | 1.87 a | 2.89 b  | 3.93 b | 4.73 c | 16                  |
| 0.50% of Chinaberry             | 1.00 a | 1.87 a | 2.78 ab | 3.88 b | 4.34 b | 16                  |
| 1.00% of Chinaberry             | 1.00 a | 1.81 a | 2.43 a  | 3.48 a | 3.82 a | 18                  |
| 0.25% of Mexican Sunflower      | 0.92 a | 1.88 a | 2.65 ab | 3.45 a | 3.87 a | 15                  |
| 0.50% of Mexican Sunflower      | 0.92 a | 1.87 a | 2.61 ab | 3.24 a | 3.66 a | 16                  |
| 1.00% of Mexican Sunflower      | 0.90 a | 1.81 a | 2.60 ab | 3.21 a | 3.62 a | 18                  |
| 0.25% of Lantana                | 1.00 a | 1.79 a | 2.58 ab | 3.26 a | 3.66 a | 19                  |
| 0.50% of Lantana                | 1.00 a | 1.75 a | 2.58 ab | 3.23 a | 3.66 a | 21                  |
| 1.00% of Lantana                | 0.93 a | 1.74 a | 2.49 ab | 3.22 a | 3.55 a | 22                  |

Remarks: Numbers followed by different letters in the same column are significantly different based on DMRT (\(\alpha = 0.05\))
The highest value of conidia density was recorded at 1.77 x 10^8 conidia/ml on 0.25% Chinaberry while, the lowest was in 1% Neem (1.19 x 10^8 conidia/ml). Conidial density decreased along with the increase of plant extracts concentration. The highest conidial viability was detected on 0.025% Lantana leaf yet the values of all treatments were not significant (Table 3). These condition inferred that the extract of Neem, Chinaberry, Mexican sunflower and Lantana leaves did not inhibit the germination of B. bassiana. In contrast, Seyed-Talebi, Kheradmand, Talaei-Hassanloui, & Talebi-Jahromi (2012) stated that Ginkgo biloba extracts can inhibit the germination of B. bassiana conidia fungi. The inability of B. bassiana conidia to germinate in some of the plant extracts is due to toxic compounds present in these extracts (Taskeen-Un-Nisa, Wani, Bhat, Pala, & Mir, 2011).

The whole plant leaf extract in all concentrations were compatible with the B. bassiana (Table 4). The T value decreases with increasing concentrations of each plant extract. The results show that the highest T value (82.10) was observed on 0.25% Chinaberry while the lowest (60.09) was in 1% Neem. Decreasing the concentration of plant extract in this study affected to the increase of T values. Sousa et al. (2011) reported that the B. bassiana fungus combined with Melia azedarach is compatible and capable of controlling Rhipicephalus microplus. Deb, Rajesh, Majumdar, & Tombisana (2017) stated that B. bassiana was also found compatible with aqueous garlic extract at all three concentrations. Usha, Babu, & Padmaja (2014) also studied the compatibility of entomopathogenic fungus B. bassiana with pesticides, fungicides, and botanicals different concentrations.

**Compatibility of B. bassiana with Plant Extracts on the Mortality of S. litura Larva**

The study showed that the control of B. bassiana (10^8 conidia/ml) in control treatment had higher mortality on S. litura larvae (Table 5). Whereas, the lowest value of mortality was in 1% Mexican sunflower. However, the mortality of S. litura larvae treatment was not significantly different with all concentrations in Neem and Lantana leave extract, and 0.25 and 1% Mexican sunflower. Without combination, Beauveria bassiana produces significantly higher mortality on whitefly compared to plant extracts (Jaber, Araj, & Qasem, 2018). Islam & Omar (2012) showed that the combination treatment of neem with B. bassiana (10^8 conidia/ml) is compatible and increased the percentage of Bemisia tabaci mortality in eggplant plants. Sousa et al. (2011) also reported that B. bassiana combined with chinaberry is compatible and capable of controlling R. microplus. Fernández-Grandon, Harte, Ewany, Bray, & Stevenson (2020) showed that a combination of the entomopathogenic fungi, Metarhizium anisoplae, and pyrethrum produces a higher rate of mortality in Aphis fabae than the pesticide without combination. There are successful synergies and compatibilities between entomopathogen fungi and different plant-based pesticides to improve the pest control. Ali et al. (2018) indicated that entomopathogen fungi and botanical extracts (neem or eucalyptus) caused a significant reduction in survival and fecundity of Aphid.
### Table 3. Conidial viability and density of *Beauveria bassiana*

| Treatment (Plant extract concentrations + *(B. bassiana* *(10⁸ conidia/ml)*)) | Conidial viability (%) | Reductions (%) | Conidial density *(x 10⁴ conidia/ml)* | Reductions (%) |
|---|---|---|---|---|
| Control *(B. bassiana* *(10⁸ conidia/ml))* | 85.51 a | - | 2.17 a | - |
| 0.25% of Neem | 75.95 a | 11 | 1.68 a | 20 |
| 0.50% of Neem | 74.60 a | 13 | 1.33 a | 17 |
| 1.00% of Neem | 73.05 a | 15 | 1.19 a | 10 |
| 0.25% of Chinaberry | 75.63 a | 12 | 1.77 a | 8 |
| 0.50% of Chinaberry | 68.65 a | 20 | 1.32 a | 17 |
| 1.00% of Chinaberry | 63.29 a | 26 | 1.29 a | 18 |
| 0.25% of Mexican Sunflower | 77.88 a | 9 | 1.58 a | 12 |
| 0.50% of Mexican Sunflower | 75.89 a | 11 | 1.39 a | 15 |
| 1.00% of Mexican Sunflower | 74.17 a | 2 | 1.31 a | 18 |
| 0.25% of Lantana | 80.19 a | 6 | 1.65 a | 10 |
| 0.50% of Lantana | 78.93 a | 8 | 1.41 a | 15 |
| 1.00% of Lantana | 71.38 a | 17 | 1.38 a | 17 |

Remarks: Numbers followed by different letters in the same column are significantly different based on DMRT *(α = 0.05)*; * = significantly different

### Table 4. Plant extracts and compatibility classification with *B. bassiana* *(T value)*

| Treatments | T value (%) | Classification |
|---|---|---|
| Neem | | |
| 0.25% | 78.33 | Compatible |
| 0.50% | 65.34 | Compatible |
| 1.00% | 60.09 | Compatible |
| Chinaberry | | |
| 0.25% | 82.10 | Compatible |
| 0.50% | 65.51 | Compatible |
| 1.00% | 63.87 | Compatible |
| Mexican sunflowers | | |
| 0.25% | 75.18 | Compatible |
| 0.50% | 68.09 | Compatible |
| 1.00% | 64.60 | Compatible |
| Lantana | | |
| 0.25% | 66.18 | Compatible |
| 0.50% | 67.75 | Compatible |
| 1.00% | 66.18 | Compatible |
CONCLUSION

At 12 Days After Application (DAA), the plant extract of Neem, Chinaberry, Mexican sunflower, and Lantana leaves reduce colony growth of *B. bassiana*. For the mortality of *S. litura*, the combined mixture of *B. bassiana* and 0.25% Chinaberry extract had the maximum mortality rate (44%). At 12 and 15 DAA, the Chinaberry (0.25%) showed the highest colony growth (3.93 and 4.37 cm). While the combination of *B. bassiana* (1 x 10^8 conidia/ml) with 0.25% Chinaberry extract is considered to be the most inhibit treatment with the conidial density of 1.77 x 10^8 conidia/ml and conidial viability of 75.63%. Therefore, combination of *B. bassiana* (1 x 10^8 conidia/ml) with 0.25% Chinaberry extract might be used as promising natural alternatives to mycoinsecticides against *S. litura*.

ACKNOWLEDGEMENT

The authors would like to thanks all those who helped and worked during the conduct of the research and thank to Hagus Tarno for manuscript advices.

REFERENCES

Abbott, W. S. (1925). A method for computing the effectiveness of an insecticide. *Journal of Economic Entomology, 18*(2), 265–267. https://doi.org/10.1093/jee/18.2.265a

Ahmad, M., & Mehmood, R. (2015). Monitoring of resistance to new chemistry insecticides in *Spodoptera litura* (Lepidoptera: Noctuidae) in Pakistan. *Journal of Economic Entomology, 108*(3), 1279–1288. https://doi.org/10.1093/jee/tov085

Ali, S., Farooqi, M. A., Sajjad, A., Ullah, M. I., Qureshi, A. K., Siddique, B., … Asghar, A. (2018). Compatibility of entomopathogenic fungi and botanical extracts against the wheat aphid, *Sitobion avenae* (Fab.) (Hemiptera: Aphididae). *Egyptian Journal of Biological Pest Control, 28*, 97. https://doi.org/10.1186/s41938-018-0101-9

Deb, L., Rajesh, T., Majumdar, D., & Tombisana, R. K. (2017). Evaluation of biological compatibility of *Beauveria bassiana* with fungicides and botanicals. *Journal of Pharmacognosy and Phytochemistry, SP1*, 1120–1124. Retrieved from https://pdfs.semanticscholar.org/28fd/60a5580fa6b0334c6177270b7f030ccbb5f18.pdf

Depieri, R. A., Martinez, S. S., & Menezes Jr., A. O. (2005). Compatibility of the fungus *Beauveria bassiana* (Bals.) Vuill. (Deuteromycetes) with extracts of neem seeds and leaves and the emulsible oil. *Neotropical Entomology, 34*(4), 601–606. https://doi.org/10.1590/s1519-566x2005000400010

Table 5. The mortality of *S. litura* treated by plant extracts and *B. bassiana*

| Treatment | *S. litura* Larvae Mortality (%) |
|-----------|---------------------------------|
| Control (*B. bassiana* (10^8 conidia/ml)) | 42.10 c |
| 0.25% of Neem | 35.00 abc |
| 0.50% of Neem | 34.10 abc |
| 1.00% of Neem | 31.10 abc |
| 0.25% of Chinaberry | 44.00 c |
| 0.50% of Chinaberry | 30.80 ab |
| 1.00% of Chinaberry | 29.90 ab |
| 0.25% of Mexican Sunflower | 38.10 abc |
| 0.50% of Mexican Sunflower | 36.10 abc |
| 1.00% of Mexican Sunflower | 26.80 a |
| 0.25% of Lantana | 39.10 abc |
| 0.50% of Lantana | 34.90 abc |
| 1.00% of Lantana | 35.00 bc |

Remarks: Numbers followed by different letters in the same column are significantly different based on DMRT (α = 0.05); * = significantly different, ns = not significantly different.
Dhar, S., Jindal, V., Jariyal, M., & Gupta, V. K. (2019). Molecular characterization of new isolates of the entomopathogenic fungus *Beauveria bassiana* and their efficacy against the tobacco caterpillar, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). *Egyptian Journal of Biological Pest Control*, 29(8). https://doi.org/10.1186/s41938-019-0110-3

Fernández-Grandon, G. M., Harte, S. J., Ewany, J., Bray, D., & Stevenson, P. C. (2020). Additive effect of botanical insecticide and entomopathogenic fungi on pest mortality and the behavioral response of its natural enemy. *Plants*, 9(2), 173. https://doi.org/10.3390/plants9020173

Fu, X., Zhao, X., Xie, B., Ali, A., & Wu, K. (2015). Seasonal pattern of *Spodoptera litura* (Lepidoptera: Noctuidae) migration across the Bohai Strait in Northern China. *Journal of Economic Entomology*, 108(2), 525–538. https://doi.org/10.1093/jee/tov019

Garrido-Jurado, I., Ruano, F., Campos, M., & Quesada-Moraga, E. (2011). Effects of soil treatments with entomopathogenic fungi on soil dwelling non-target arthropods at a commercial olive orchard. *Biological Control*, 59(2), 239–244. https://doi.org/10.1016/j.biocontrol.2011.07.001

Glare, T., Caradus, J., Gelernter, W., Jackson, T., Keyhani, N., Köhl, J., … Stewart, A. (2012). Have biopesticides come of age? *Trends in Biotechnology*, 30(5), 250–258. https://doi.org/10.1016/j.tibtech.2012.01.003

Gouli, V., Gouli, S., & Kim, J. S. (2014). Production of *Beauveria bassiana* air conidia by means of optimization of biphasic system technology. *Brazilian Archives of Biology and Technology*, 57(4), 571–577. https://doi.org/10.1590/S1516-8913201401745

Houguo, Q., Zhengxiang, Y., Shujin, H., Jian, D., & Renhua, L. (2004). The correlation of the different host plants with preference level, life duration and survival rate of *Spodoptera litura* Fabricius. *Chinese Journal of Eco-Agriculture*, 12(2), 40–42. Retrieved from https://europemc.org/article/cba/403189

Islam, M. T., & Omar, D. B. (2012). Combined effect of *Beauveria bassiana* with neem on virulence of insect in case of two application approaches. *The Journal of Animal and Plant Sciences*, 22(1), 77–82. Retrieved from http://www.thejaps.org.pk/docs/v-22-1/29.pdf

Islam, Md T., Castle, S. J., & Ren, S. (2010). Compatibility of the insect pathogenic fungus *Beauveria bassiana* with neem against sweetpotato whitefly, *Bemisia tabaci*, on eggplant. *Entomologia Experimentalis et Applicata*, 134(1), 26–34. https://doi.org/10.1111/j.1570-7458.2009.00933.x

Islam, M. B. (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entomology*, 51, 45–66. https://doi.org/10.1146/annurev.ento.51.110104.151146

Jaber, L. R., Araj, S.-E., & Qasem, J. R. (2018). Compatibility of endophytic fungal entomopathogens with plant extracts for the management of sweetpotato whitefly *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae). *Biological Control*, 117, 164–171. https://doi.org/10.1016/j.biocontrol.2017.11.009

James, R. R. (2003). Combining azadirachtin and *Paecilomyces fumosoroseus* (Deuteromycotina: Hypomyces) to control *Bemisia argentifolii* (Homoptera: Aleyrodidae). *Journal of Economic Entomology*, 96(1), 25–30. https://doi.org/10.1603/0022-0493-96.1.25

Nana, P., Maniania, N. K., Maranga, R. O., Boga, H. I., Kutima, H. L., & Elloff, J. N. (2012). Compatibility between *Calpurnia aurea* leaf extract, attraction aggregation, and attachment pheromone and entomopathogenic fungus *Metarhizium anisopliae* on viability, growth, and virulence of the pathogen. *Journal of Pest Science*, 85, 109–115. https://doi.org/10.1007/s10340-011-0399-5

Ortiz-Urquiza, A., Luo, Z., & Keyhani, N. O. (2015). Improving mycoinsecticides for insect biological control. *Applied Microbiology and Biotechnology*, 99, 1057–1068. https://doi.org/10.1007/s00253-014-6270-x

Revathi, K., Chandrasekaran, R., Thanigaivel, A., Kirubakaran, S. A., & Senthil-Nathan, S. (2014). Biocontrol efficacy of protoplast fusants between *Bacillus thuringiensis* and *Bacillus subtilis* against *Spodoptera litura* Fabr. *Archives of Phytopathology and Plant Protection*, 47(11), 1365–1375. https://doi.org/10.1080/03235408.2013.840999

Sain, S. K., Monga, D., Kumar, R., Nagrale, D. T., Hiremani, N. S., & Kranthi, S. (2019). Compatibility of entomopathogenic fungi with insecticides and their efficacy for IPM of *Bemisia tabaci* in cotton. *Journal of Pesticide Science*, 44(2), 97–105. https://doi.org/10.1584/jpestics.D18-067

Samuels, R. I., Coracini, D. L. A., Martins dos Santos, C. A., & Gava, C. A. T. (2002). Infection of *Blissus antillus* (Hemiptera: Lygaeidae) eggs by the entomopathogenic fungi *Metarhizium*...
Aminudin Afandhi et al.: Beauveria bassiana with Leaf Extract

*anisopliae* and *Beauveria bassiana*. Biological Control, 23(3), 269–273. https://doi.org/10.1006/bcon.2001.1009

Seyed-Talebi, F. S., Kheradmand, K., Talaei-Hassanloui, R., & Talebi-Jahromi, K. (2012). Sublethal effects of *Beauveria bassiana* on life table parameters of two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae). Biocontrol Science and Technology, 22(3), 293–303. https://doi.org/10.1080/09683157.2012.655709

Singleton, P., & Sainsbury, D. (2006). *Dictionary of microbiology and molecular biology* (3rd ed.). West Sussex, England: John Wiley & Sons, Ltd. Retrieved from https://enggbiochem.files.wordpress.com/2014/08/dictionary-of-microbiology-molecular-biology.pdf

Sousa, L. A. D., Pires Júnior, H. B., Soares, S. F., Ferri, P. H., Ribas, P., Lima, E. M., … Borges, L. M. F. (2011). Potential synergistic effect of *Melia azedarach* fruit extract and *Beauveria bassiana* in the control of *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) in cattle infestations. Veterinary Parasitology, 175(3–4), 320–324. https://doi.org/10.1016/j.vetpar.2010.10.012

Taskeen-Un-Nisa, Wani, A. H., Bhat, M. Y., Pala, S. A., & Mir, R. A. (2011). *In vitro* inhibitory effect of fungicides and botanicals on mycelial growth and spore germination of *Fusarium oxysporum*. Journal of Biopesticides, 4(1), 53–56. Retrieved from http://www.jbiopest.com/users/lw8/efiles/vol_4_1_238.pdf

Taylor, B., Edington, S., Luke, B., & Moore, D. (2013). Yield and germination of the entomopathogenic fungus *Beauveria bassiana* when grown on different rice preparations. Journal of Stored Products Research, 53, 23–26. https://doi.org/10.1016/j.jspr.2013.02.004

Tong, H., Su, Q., Zhou, X., & Bai, L. (2013). Field resistance of *Spodoptera litura* (Lepidoptera: Noctuidae) to organophosphates, pyrethroids, carbamates and four newer chemistry insecticides in Hunan, China. Journal of Pest Science, 86, 599–609. https://doi.org/10.1007/s10340-013-0505-y

Tuan, P. P. (2014). Dermal toxicity of white muscardine fungus, *Beauveria bassiana* (Bals.) Vuill. on vertebrates. International Journal of Innovative Science, Engineering & Technology, 1(10), 122–128. Retrieved from http://www.ijiset.com/v1s10/IJISET_V1_I10_17.pdf

Usha, J., Babu, M. N., & Padmaja, V. (2014). Detection of compatibility of entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. with pesticides, fungicides and botanicals. International Journal of Plant, Animal and Environmental Sciences, 4(2), 613–624. Retrieved from https://www.cabdirect.org/cabdirect/abstract/20143220338

Vincent, J. M. (1947). Distortion of fungal hyphae in the presence of certain inhibitors. Nature, 159, 850. https://doi.org/10.1038/159850b0