Compatibility studies of entomopathogenic fungi and botanical pesticide for controlling *Spodoptera exigua*

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Abstract. *Spodoptera exigua* is one of main pest for horticulture crops. In sustainability agriculture, entomopathogenic like *Beauveria bassiana* and *Metharizium anisopliae* are solution to control this pest. This study aimed to examine the compatibility between the entomopathogenic fungus Beauveria bassiana and the vegetable insecticide neem leaf extract against the larvae of *Spodoptera exigua*. Value LC 50 single testing B. bassiana and neem leaf extract respectively is 0.08% (y=1,65x + 0.16) and 0.627% (y=1,59x-1.02). While the value of LC 50 treatment. combinations B. bassiana with neem leaf extract is 0.079% (y = 1,22x + 1.45). Based on the value of LC 50, it is known that the LC 50 value of the combination treatment is smaller than the LC 50 value of the single treatment, this indicates that the combination treatment has a higher toxicity than the combination treatment in killing S. exigua larvae. The LT 50 values from the LC 50 obtained from the single test of B. bassiana and neem leaf extract were 7.96 days and 6.1 days, respectively. While the value of LT 50 from LC 50 obtained from testing the combination of B. bassiana with neem leaf extract was 5.79 days. Based on the LT 50 value, it can be seen that the LT 50 value of the combination treatment is smaller than the LT 50 value of the single treatment.

1 Introduction

The larvae of Spodoptera exigua (Hubner) (Lepidoptera: Noctuidae) are the main pests that attack shallot plants starting from the vegetative phase to harvest with the most severe attack impact, namely losing 100% of crop yields if not controlled [1,2]. The control of S. exigua larvae by farmers until now generally only relies on chemical pesticides. The unwise use of chemical pesticides will cause negative impacts including pest resistance, pest resurgence, the emergence of pesticide residues causing environmental pollution, and kill useful insects such as natural enemies and pollinating insects [3–5]. One alternative that can be done is to develop environmentally friendly pest control technology so that it can reduce the impact negative impact on the environment and can reduce the cost of controlling pests using the principle of control biological agents [6,7].

The entomopathogenic fungus Beauveria bassiana is an alternative control that can be used because it is easy to breed, environmentally friendly, and not harmful to humans and

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plants. B. bassiana is an aggressive parasite for various types of insects and attacks both the larval and adult stages of insects [8,9]. In addition to biological control, pest control that can be used as an alternative and environmentally friendly is the use of vegetable pesticides. Some types and parts of plants that can be used as vegetable pesticides, one of which is neem leaves. Neem leaves contain compounds azadirachtin, meliantriol, salanin and nimbin which have a negative effect on pests [10]. One of the weaknesses of bioinsecticides and vegetable insecticides is that they work slowly and their efficacy is often below that of chemical insecticides when applied singly so that they still cause high damage due to pest attacks. This can be circumvented by combining two types of insecticides.

Applications of compatible insecticide combinations have benefits including requiring lower doses than single insecticide applications, application of combination insecticides with lower doses can save application costs and reduce the risk of poisoning to non-target organisms and the environment, increase insecticidal activity and can slow the occurrence of resistance. pests to insecticides [11]. The combination of B. bassiana with the vegetable insecticide neem has been suggested in several studies. Based on the results of the study, it was found that the combination treatment of neem at a concentration of 1% with B. bassiana at a concentration of 10^8 conidia/mL on eggplant was synergistic and increased the mortality percentage of the pest Bemisia tabaci [12]. The purpose of this study was to examine the compatibility and effectiveness of the application of the combination of the entomopathogenic fungus B. bassiana with the vegetable insecticide neem leaf extract against S. exigua larvae.

2 Material and method

2.1 Study area

This research was conducted at the Laboratory of Pests, Faculty of Agriculture, University of Jember as a place to borrow laboratory equipment, Greenhouse Pests and Plant Diseases, Faculty of Agriculture, University of Jember. The study was conducted from March to September 2021.

2.2 Rearing test larvae

Larvae of S. exigua were obtained from shallot farmers' land in Kraksaan District, Probolinggo, East Java. Then the S. exigua larvae were propagated by rearing which aimed to obtain insects of uniform age. Larvae were kept in plastic containers covered with gauze. During rearing, the larvae were fed daily with pesticide-free fresh shallots. S. exigua larvae were reared until the larvae entered the pupa stage and the pupae were separated into another container. Pupae that have become moths are transferred to moth jars and fed with a 10% honey solution which is absorbed on cotton media tied to the ceiling of the jars and the walls of the jars are covered with plastic. After the imago appears and performs copulation, the imago will place the eggs in plastic in a jar. The cultured insects are reared until they are ready to become suitable insects for the test material.

2.3 Botanical pesticide preparation

The old neem leaves (dark green) are separated from the stalks. Then the neem leaves are washed, then air-dried for about 7 days until the leaves dry. The dried leaves are then mashed using a blender to form a powder. The fine powder was weighed with a powder weight of 500 g, then the powder was put into a maceration container and then macerated in 2500 mL of methanol solution for 2-3 days, then filtered using filter paper. The final filtered filtrate was then evaporated using a rotary evaporator at a temperature of 45ºC and a pressure of 337 mBar until a pure extract of neem leaves was produced. The extraction results are stored in a refrigerator (temperature of approximately 4º C) until the extract will be tested.

2.4 Material of B. bassiana

B. bassiana used is commercial B. bassiana Inoculum in the form of flour with a density of 4.5x 10^{10} spores/g (trade name Natural BVR) produced by PT. Natural Nusantara Yogyakarta and has been traded in shops for agricultural facilities. The recommended concentration stated on the package is 2 gL-1 or 0.2%.

2.5 Experimental design

Study consisted of two tests, the first single test of B. bassiana and neem leaf extract. The second is the combination test of B. bassiana with neem leaf extract. This study used a completely randomized design (CRD) consisting of 6 treatments with 4 replications, 1 replication used 12 test insects so that in 1 treatment 48 test insects were needed (table 1).

Table 1. Combination concentration of B. bassiana with neem leaf extract.

Code Treatment	Combination Treatment
AB1	LC50 BB + LC50 EDM
AB2	LC50 BB + 1/2 LC50 EDM
AB3	LC50 BB + 1/4 LC50 EDM
AB4	LC50 BB + 1/8 LC50 EDM
AB5	LC50 BB + 1/16 LC50 EDM
AB0	*Control*

3 Result and discussion

The test results showed that the mortality of S. exigua increased from the beginning to the end of the observation, namely from observations 1-7 days after treatment. The amount of concentration of an insecticide can affect the mortality rate of insects [13,14]. The higher the application of insecticide concentration, the higher the percentage of insect mortality. Based on Figure 1, it is known that the different concentrations of B. bassiana have an effect on the mortality of S. exigua larvae. The concentration of B. bassiana which caused the highest mortality of S. exigua at 7 days after treatment was found at a concentration of 0.2%, which caused larval mortality of 76.60%.

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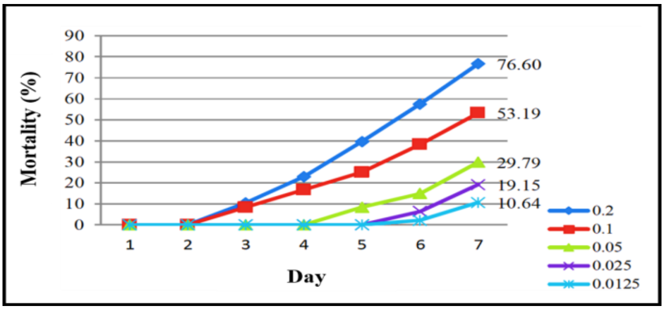

Fig. 1. Effect of B. bassiana application on S. exigua Larvae mortality.

The ability of B. bassiana as a bio-insecticide has been investigated by Rosmiati et al., [15], that administration of a suspension of B. bassiana with a concentration of 1010 spores/mL can kill 82.50% of S. litura larvae within 7 days. According to Maze et al., [16], the higher the concentration of B. bassiana applied, the more conidia attached to the larval body, so the faster the infection process and the higher the percentage of mortality in S. exigua larvae (figure 2).

Fig. 2. S. exigua larvae infected with B. bassiana (a) Healthy S. exigua larvae (b).

Symptoms caused by the application of B. bassiana to S. exigua larvae are first the symptoms of larvae being less active, decreased appetite, body color becomes pale, the body is difficult to move so that in some time it will die. The cuticle of the dead larvae becomes stiff and turns black in color, after a few days the larva's body secretes a white fungal mycelium. Based on research by Masyitah et al. [17], infection caused by B. bassiana occurs through the integument and then damages the immune system of the larvae. Conidia in contact with the integument will germinate and form hyphae. Germinated hyphae will release beauvericin toxin and absorb nutrients in the larva's body so that it can destroy the structure in the larva's body and result in the death of the larvae. The main parts of the larval body that are most easily penetrated by the mycelium of the fungus are the parts of the body and mouth so that in the end it covers the entire body of the larvae.

The test results showed that the vegetable insecticide neem leaf extract is toxic to insect pests so that it can have an effect on mortality in S. exigua larvae. The high mortality of S. exigua larvae was directly proportional to the level of neem leaf extract concentration. Based on Figure 3, it can be seen that the application of several concentrations of neem leaf extract had an effect on the mortality of S. exigua larvae. The concentration of neem leaf extract that caused the highest mortality of S. exigua at 7 days after observation was at a concentration of 5%, which caused a mortality of 91.67% (Figure 3).

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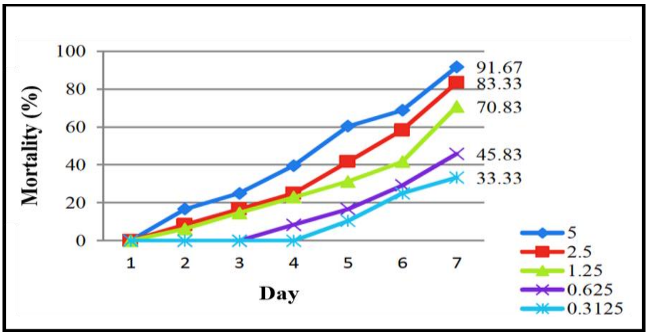

Fig. 3. Effect of Neem Leaf extract application on S. exigua Mortality.

The toxicity value of Lethal Concentration 50 (LC50) is a concentration that can result in 50% death in the test insects within a certain time of observation. The LC50 toxicity value of B. bassiana and neem leaf extract can be determined from the probit regression equation (Figure 4;5). The function of the probit regression equation is to find the effective concentration by determining the mortality of the test insects. Based on Figure 4, it is known that the regression equation for observations 7 days after treatment in B. bassiana treatment (y=1.65x+0.16) with an LC50 value of 0.08%. The pattern of the relationship between the concentration of B. bassiana and mortality of S. exigua larvae is very strong because it has a coefficient of determination (R2) of 1.

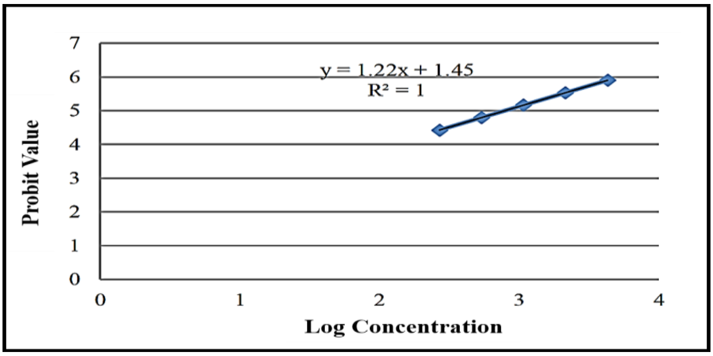

Fig. 4. Analysis of Probit and LC50 in B. bassiana.

The test results showed that the LC50 value in B. bassiana was smaller than the LC50 value in neem leaf extract, so it could be seen that B. bassiana had higher toxicity than neem leaf extract in killing S. exigua larvae. Insecticides with active ingredients of entomopathogenic fungi in controlling S. exigua have higher toxicity than vegetable insecticides. Based on Sofiana's research [18], the LC50 value of B. bassiana in killing C. pavonana larvae was 5.8 grams/liter. Meanwhile, in the study of Su'ud et al. [19], the LC50 value of neem leaf extract in killing S. exigua larvae was 107.363 grams/liter. The results of this study proved that the application of B. bassiana was more effective than the vegetable insecticide neem leaf extract. Based on Figure 4, it is known that the regression equation for observations 7 days after treatment in the combination treatment of B. bassiana with neem leaf extract (y=1.22x+1.45) with an LC50 value of 0.079%. The pattern of the relationship between the concentration of the combination of B. bassiana with neem leaf extract and mortality of S. exigua larvae is very strong because it has a coefficient of determination (R2) of 1.

The LC50 value for testing B. bassiana, neem leaf extract, and their combination was 0.08%; 0.627%; 0.079%. The results of the combination test of B. bassiana with neem leaf extract obtained a lower LC50 value than the single test. This shows that the combination of B. bassiana with neem leaf extract has a higher toxicity than the test alone. Combination insecticide applications are more effective than single applications because B. bassiana and neem leaf extract have different active ingredients, entry routes, target points, and different effects in the process of killing insect pests [20]. This is also confirmed by the research of Sain et al. [21], that the combination application of entomopathogenic fungi with vegetable insecticides is more effective than single application, because the application of combination insecticides has different active ingredients so that they can complement each other and combination insecticides can delay the emergence of pest resistance to pests. insecticides used.

4 Conclusion

The concentration of the combination insecticide that caused the highest mortality of S. exigua at 7 days after observation was in treatment AB1 (BB 0.08% + EDM 0.627) which caused a mortality of 82.98%. The LC50 values for testing B. bassiana and neem leaf extract alone and in combination were 0.08%; 0.627%; 0.079%. The LT50 value of testing B. bassiana and neem leaf extract alone and in combination was 7.96 days; 6.1 days; 5.79 days.

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