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Research Article

In Vitro Effectiveness of *Beauveria bassiana* as a Control Agent against Invasive Fall Armyworm (*Spodoptera frugiperda*) Larvae

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ABSTRACT

Fall armyworm or *Spodoptera frugiperda* is one of the main pests of corn. It can caused up to 28.08% yield loss. Pest control efforts with broad-spectrum insecticides can cause negative impacts that disrupt ecosystems, non-target biotic factors and the surrounding environment. *Beauveria bassiana* is an alternative biological control agent that is target-specific. This study was conducted to determine toxic and antifeedant activities of *B. bassiana* application on third to fifth instar *S. frugiperda* larvae. *B. bassiana* isolates were propagated using corn flakes media and resulted in colonies with conidia density of 1.1×10^8 conidia.g^{-1}. This study used a completely randomized design with 5 treatments and 5 replications. Treatments consisted of P1 namely *B. bassiana* propagation with a concentration of 60 g.L^{-1}, P2 = 70 g.L^{-1}, and P3 = 80 g.L^{-1}. P0 was a negative control (untreated with *B. bassiana*) and P4 was a positive control (diazinon with concentrations of 2 ml.L^{-1}). The variables observed were mortality and weight of the leftover feed. The results showed that the formulation of *B. bassiana* with concentrations of 80 g.L^{-1} was able to infect armyworms with a mortality percentage of 53.5% which was statistically similar as the effect of chemical insecticides. In addition, inhibition of feeding activity due to treatment could prevent leaf damage up to 68.21%.

Keywords: antifeedants; biological agents; entomopathogenic fungi; larvae; pests

INTRODUCTION

Corn is a staple food in Indonesia. It provides carbohydrates and protein (Nurmaisah & Purwati, 2021). Harvest residues, known as stover, have roles as animal feed sources (Ishak *et al.*, 2013). Efforts to increase productivity still experience many obstacles causing Indonesia's corn production not to be able to meet national demands (Kasryno *et al.*, 2015). One of the limiting factors for maize production is the invasive fall armyworm (*Spodoptera frugiperda*). This species is known to be more invasive than *S. litura* (Herlinda *et al.*, 2021). Crop loss caused by this pest both in sweet and glutinous corn can reach 28.08 and 25.04 % respectively (Sunari *et al.*, 2022). Usually, pests start attacking corn plants (*Zea mays*) at 10 days after planting (DAT). The most detrimental period of fall armyworms damage occurs during the larval stage, especially instars 3 to 5 (Subiono, 2019). This period lasts for approximately 14 days, during where larvae consume leaves the most as an energy reserve for preparation to metamorphose to the pupa stage (Navik *et al.*, 2021).

Farmers mostly use chemical pesticides to control fall armyworm in corn plants. If it is done continuously, it harms the environment and the ecosystem (Lubis *et al.*, 2020). Farmers must be continuously introduced to the potential of biological control agents. One of the biological control agents is entomopathogenic fungi including *Metarhizium* sp and *Beauveria bassiana* (Grijalba *et al.*, 2018). *B. bassiana* is cosmopolitan and able to survive in endophytic land (Meyling & Eilenberg, 2007). The potential ability of *B. bassiana* to control fall armyworms needs to be compared to effectiveness of chemical insecticides. This research aims to test *B. bassiana* toxic and antifeedant activity on *S. frugiperda*.

Results can be used as an argument to support changes in farmers' pest control methods to more environmentally friendly ones.

The propagation of *B. bassiana* isolates is easy and has been widely practiced by farmers (Sopialena, 2018). Also, the propagated *B. bassiana* is less sensitive to nutritional and environmental condition compared to other entomopathogenic fungi. It may support the stability of the conidia virulence (Grijalba *et al*., 2018). The efficacy of propagated *B. bassiana* needs to be tested. This study aims to compare the effectiveness of controlling *S. frugiperda* between chemical insecticides and biological agents (*B. bassiana* produced by propagation) based on mortality and antifeedant rate.

MATERIALS AND METHODS

The research was carried out at the Plant Protection Laboratory of Polytechnic Agricultural Development Malang from March to May 2022. The study used a completely randomized design with 5 treatments and 5 replications. The treatments consisted of negative control, P1 concentration of 60 $g.L^{-1}$, P2 concentration of 70 $g.L^{-1}$, P3 concentration of 80 $g.L^{-1}$, and P4 as positive control, using the insecticide diazinon 2 $mL.L^{-1}$. Each treatment was repeated 5 times. Each replication consisted of 10 larvae and was considered one experimental unit. Parameters observed were the percentage of dead larvae in each experimental unit and the weight of remaining diet. Tested larvae were 3rd instar of fall armyworm larvae. Every larvae was placed individually in a plastic jar due to their cannibal characteristic (Navik *et al*., 2021). The larvae were the second generation (F2) from a reared colony of the Plant Protection Laboratory of Polytechnic Agricultural Development Malang. Observations were done for 14 days or until the larvae became pupae (Navik *et al*., 2021). Leaf diets were changed every day. Data were analyzed using ANOVA and an HSD (Tukey) post-hoc test was performed.

Propagation of *B. bassiana* Isolates

The isolates were obtained from the Pests Control Labs of The Technical Implementing Unit for Food Crop Protection, Department of Agriculture, Food Security, East Java Province. *B. bassiana* were propagated on corn flakes media. Corn media were soaked for 24 hours and steamed until 25% done. At room temperature, 100 g of media were placed in a plastic bag, steamed again for 30 minutes at a temperature of 110°C. *B. bassiana* isolates were diluted with distilled water and stirred using a sterile iron stirrer. As much as 2 mL of *B. bassiana* solution was inoculated into a plastic bag containing sterilized media. *B. bassiana* was incubated for 2 weeks and propagated *B. bassiana* was ready to use (Mascarin *et al*., 2015).

Calculation of Conidia Density

Conidia density of propagated *B. bassiana* was calculated from 1 mg of media dissolved in 9 mL of distilled water. The solution was homogenized at 200 rpm. One milliliter suspension was taken and diluted with 100 mL of distilled water. This step was repeated to obtain dilution with countable conidia's density. As much as 1 ml of final dilution was dropped into the hemocytometer, then the conidia were counted using a binocular microscope with 400 times magnification. Conidia's density calculated by conidia's densities formula in Herlinda *et al.* (2006):

$$C = \frac{t}{(n \times 0.25)} \times 10^5$$

C = conidia density per mL of solution; t = total number of conidia in the observed sample box; n = number of sample boxes (5 large boxes × 16 small boxes); 0.25 = correction factor. The propagated *B. bassiana* has 1.1×10^8 conidia unit in each gram media based on calculation in conidia's densities formula.

In Vitro Mortality and Antifeedant Testing

Suspension was made by mixing the propagated *B. bassiana* with distilled water according to designated treatments: P1 60 $g.L^{-1}$, P2 70 $g.L^{-1}$, and P3 80 $g.L^{-1}$, and for comparison, Diazinon was used (2 $mL.L^{-1}$) as P4 and distilled water was used as a control (P0). As much as 5 mL of solution treatments were sprayed to 5 g of corn leaves used for diets the tested larvae. The calculation of conidia unit quantity used the following formula;

$$P1 = \frac{60 \times 1.1 \times 10^8}{1000 \text{ ml}} \times 5 \text{ ml} = 3.3 \times 10^7$$

Thus, P2 contains 3.85×10^7 unit conidia. P3 contains 4.4×10^7 unit conidia.

RESULTS AND DISCUSSION

Larval Mortality

Results of the pathogenicity test showed *B. bassiana* formulation was able to infect army-worms and cause mortality from the sixth day. Dead larvae were stiff and covered by mycelium (Figure 1). Research by Suharto (2004) showed similar symptoms on *Plutella xylostella* larvae infected with *B. bassiana*. Infection was indicated by the wrinkled body and color changes. Observation on day 6 showed that the color of the larval body changed from dark green to dull black. Black larval bodies indicate melanization, a body defense mechanism in response to pathogen infection. Other infection symptoms on the 4th day were decreased movement, decreased appetite, and larvae forming C shapes. The entomo-pathogenic fungi will release a toxin that causes paralysis on larvae. Paralysis causes a loss of body coordination resulting in irregular and slow movement that will cause larval mortality. Another infection symptom was the white hyphae covering the larval body. The white hyphae initially appeared as small spots and eventually covered the whole larval body over time. At this point, the fungi has been reproduced insidelarvae's body. Dead larvae were stiff on 11th day after treatment (Figure 1D). The white powdery layer on larvae surfacewere *B. bassiana* hyphae. Indriyanti *et al.* (2021) also stated larvae stiff bodies was caused due to larvae fluid being absorbed by the fungi inside the larvae cells.

Efficacy results showed the highest mortality occurred in the P3 concentration treatment (80 $g.L^{-1}$) (Figure 2). *B. bassiana* fungal infection cumulatively from the 6th day to the 14th day caused the death rate in armyworms reached to 53.5±6.71% (Table 1). The results of Tukey tests showed the efficacy of *B. bassiana* with these concentrations matched the mortality rate due to the treatment of the insecticide diazinon (P4). The concentration of 80 $g.L^{-1}$ with a spray volume of 5 mL per leaf of feed, containing conidia with a density of 4.4×10^7. This conidia density is sufficient to cause death for larvae of *S. litura* instars 3 to 5 (Indriyanti *et al.*, 2017). A mortality percentage of 50% was achieved on day 14.

Two *B. bassiana* inoculation treatments, namely P2 (70 $g.L^{-1}$) and P1 (60 $g.L^{-1}$ concentration) have not been able to cause a 50 % mortality percentage. P2 with a conidia density of 3.85×10^7 was only able to reach a mortality rate of 32.5±13.00%. P1 with a conidia density of 3.3×10^7 only killed 30.5±22.4% of the test larvae population until the 14th day of observation. The application of diazinon insecticide was able to cause the death of the test larvae by 74.5±8.94 % under laboratory conditions.

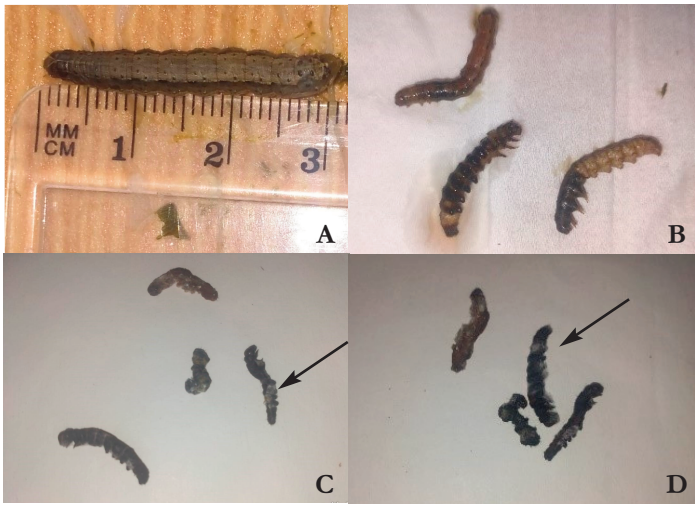

Figure 1. Differences in the appearance of *Spodoptera frugiperda* larvae treated with *Beauveria bassiana* at concentrations of 80 $g.L^{-1}$; (A) healthy larvae; (B) dead larvae on day 6, (C) dead larvae on day 11, and (D) dead larvae on day 14. The white fibers (arrow) that emerge from cadavers are fungal mycelia

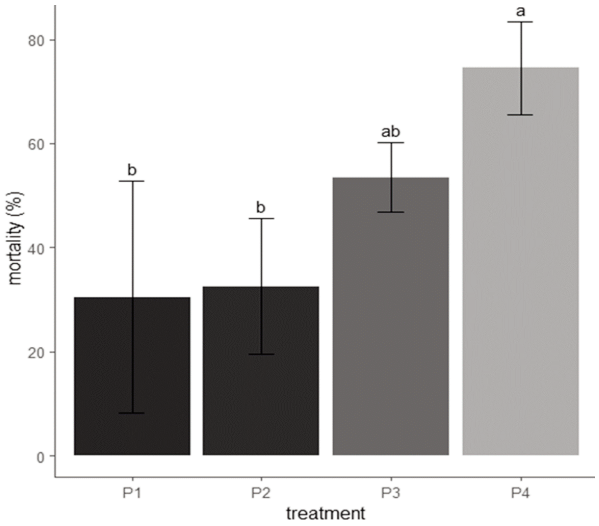

Figure 2. Mortality rate of *Spodoptera frugiperda* larvae due to treatments; P1 (inoculated with *Beauveria bassiana* 60 g.L^{-1}); P2 (inoculated with *B. bassiana* 70 g.L^{-1}); P3 (inoculated with *B. bassiana* 80 g.L^{-1}) and P4 diazinon 2 ml.L^{-1}). Different lowercase letters indicate significant differences between treatments based on Tukey test [n = 5, p < 0.05]; data are presented as average value ± standard deviation [n = 5]); n = replication

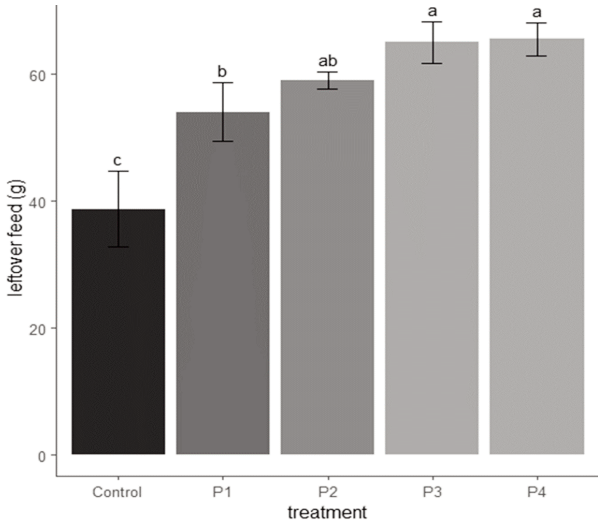

Figure 3. Weights of remaining diet after treatments; P1 (inoculated with *Beauveria bassiana* 60 g.L^{-1}); P2 (inoculated with *B. bassiana* 70 g.L^{-1}); P3 (inoculated with *B. bassiana* 80 g.L^{-1}) and P4 (as positive control, using the insecticide diazinon 2 ml.L^{-1}). Different lowercase letters indicate significant differences between treatments based on Tukey test [n = 5, p < 0.05]; data are presented as average value ± standard deviation [n = 5]); n=replication

Table 1. Average mortality rate of *Spodoptera frugiperda* due treatment

Treatment	Average mortality rate (%)	SD (n = 5)
P1	30.5 b	22.4
P2	32.5 b	13.0
P3	53.5 ab	6.71
P4	74.5 a	8.94

Notes: P1= inoculated with *Beauveria bassiana* 60 g.L^{-1}; P2= inoculated with *B. bassiana* 70 g.L^{-1}; P3= inoculated with *B. bassiana* 80 g.L^{-1}; P4= as positive control, using the insecticide diazinon 2 ml.L^{-1}; n=replication

Table 2. Weights of remaining diet due treatments

Treatment	Average leftover feed (g)	SD (n = 5)
Control	38.7 c	5.97
P1	54.1 b	4.65
P2	59.0 ab	1.38
P3	65.1 a	3.26
P4	65.5 a	2.68

Notes: P1= inoculated with *Beauveria bassiana* 60 g.L^{-1}; P2= inoculated with *B. bassiana* 70 g.L^{-1}; P3= inoculated with *B. bassiana* 80 g.L^{-1}; P4= as positive control, using the insecticide diazinon 2 ml.L^{-1}; n=replication

The difference in mortality rates was due to an increase in the number of *B. bassiana* conidia contained in the treatment suspension. The higher the concentration of the *B. bassiana* formulation, the higher the *B. bassiana* conidia content and the higher the chance of infection (González-Mas *et al.*, 2019). The mortality rate achieved was not optimal because the application of *B. bassiana* was only on the leaves of the early feed. Applications of biological agents need to be repeated at short intervals so that they can immediately kill the larvae (Grijalba *et al.*, 2018)

Antifeedant Effect

Leaf diets were from 5 g young corn leaves and changed daily. Antifeedant rate was calculated by accumulating the weight of remaining diet (Fateha *et al.*, 2020). Results of this study showed *B. bassiana* infection in treatments P1, P2, and P3 showed significantly differences of antifeedant activity compared to controls (Figure 3). However, only P2 and P3 that showed feeding inhibition levels similar to insecticide application. Remaining diet increased from 38.7 g to 59.0 g in P2 and 65.1 g in P3 (Table 2). Thus, feeding activity decreased due to insecticide

treatment as much as P3 treatment. Decreased feeding activity has the potential to reduce corn leaf damage. Damaged corn leaves intensity, especially on the shoots or young leaves, that can be reduce may restore productivity, considering that the young leaves are the site of photosynthesis (Muhadjir, 2018).

CONCLUSION

Propagated *B. bassiana* 80 $g.L^{-1}$ with conidia density of 4.4×10^7 unit has the potential to be used as a biological control agent with a mortality rate of 53.5±6.71% which was statistically similar with the efficacy of diazinon treatment. *B. bassiana* infection also reduced leaf damage. The total weight of remaining diet increased from 38.7 to 65.1g or 68.21% as a result of the antifeedant mechanism on 3rd to 5th instar larvae of *S. frugiperda*.

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