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# Efficacy of *Bacillus thuringiensis* and *Beauveria bassiana* in Controlling *Helicoverpa armigera*

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## ABSTRACT

*Helicoverpa armigera* (Lep.: Noctuidae) is one of the most important pests of crops, which imposes huge management costs on farmers every year, and also causes environmental harm to the agricultural ecosystem because of the utilization of different chemical pesticides. In this study, the effectiveness of three various formulations of the insect pathogenic fungus *Beauveria bassiana* and a biological insecticide based on the active ingredient *Bacillus thuringiensis* subsp. *kurstaki* and organic insecticide-based plants were investigated in laboratory conditions and two tomato fields. The results of laboratory bioassay of various formulations of *B. bassiana* fungus with a $1 \times 10^7$ conidia/ml concentration on third-instar larvae revealed that all three various formulations of the pathogenic fungus caused between 84-91% mortality on third-instar larvae. The results of different concentrations of biological compounds on third-instar *Helicoverpa armigera* larvae after eight days revealed that with increasing concentration of use, the mortality rate also increases significantly. The highest mortality rate was reported with 93.33% at a 1.5 ml/L concentration and the lowest mortality rate was reported with 48.89% at a 0.5 ml/L concentration. The findings of the studies in tomato fields revealed that the effectiveness of the treatments used enhances with time and the highest percentage of effectiveness in all experimental treatments was between about 50 and 60% on the seventh and tenth days after foliar spraying. In the tomato field, the percentage of efficacy of fungal formulations decreased on the tenth day of foliar spraying. Regarding the fruit perforation percentage in both studied fields, the fungal treatment and the control treatment containing distilled water were placed in the same experimental group and revealed the highest rate of fruit perforation.

**Keywords:** Biological pesticide, *Helicoverpa armigera,* Pathogenic fungus, Chemical pesticides.

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## INTRODUCTION

Tomato is an important vegetable of the family Solanaceae that is widely cultivated and produced in fields and greenhouses all over the world. Tomato is widely used in major agricultural industries, and in addition to fresh consumption, it is also used in various forms such as peeled fruit, fruit juice, pickles, dried fruit, chopped fruit, leaves, powder, paste, puree, various sauces, soups, and ketchup. *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae), which attacks tomato fruit, is one of the most important tomato pests in the world. Damage caused by this pest on tomatoes in an outbreak has been reported to be between 85 and 93% [1-3].

*Helicoverpa armigera* usually lays eggs singly on the terminal leaflets of leaves close to flowers or small fruits, and newly hatched larvae feed preferentially on immature fruits and bore holes in them, and the remaining larval excrement can be seen at the feeding site. Eventually, the fruits become unusable. Worm-infested fruits turn red earlier and the damage to the first fold is usually more severe. Ripe fruits are rarely attacked. The larvae make deep holes in the fruits. After the pest attack, the fruits often provide a way for bacteria and secondary fungi to penetrate, which causes complete rot and decay. However, sometimes the insect larvae also attack flower buds or stems [4, 5]. The activity of this pest begins in the spring of the first generation on peas and tomatoes, and its activity is observed in cotton fields from the second generation, its activity is more intense in the third generation, which coincides with the emergence of maximum buds and flowering and young cotton bolls. Tomato fruitworm is a polyphagous pest and the important crops attacked include peas, tomatoes, corn, cotton, tobacco, soybeans, and cotton weed [6-9].

To manage and control this pest, various non-chemical methods are recommended, including deep autumn and winter plowing, winter water freezing, observing crop rotation, installing light and pheromone traps, and releasing Trichogramma and Bracon bees. Every year, due to the outbreak of the pest, the use of various chemical pesticides is inevitable [8, 10-14].

Studies conducted on the management of pests in the fields show that the use of many pesticides has not been very successful in controlling the pests due to the pests remaining inside the fruit. Therefore, the use of biological control methods, including insect pathogenic fungi and the biological pesticide Bt, is worth considering, especially in tomato fields that are consumed fresh and the weather conditions are suitable for the use of biological control agents during the tomato growing season. In this study, the effectiveness of three various formulations of the insect pathogenic fungus *Beaveria bassiana* (Balsamo), the biological insecticide Biolep based on the active ingredient (BtK) *Bacillus thuringiensis* subsp. *kurstaki* and an organic insecticide on agro (Matrin) in controlling the tomato fruitworm pest in laboratory conditions and in two fields were investigated.

## MATERIALS AND METHODS

*Fungal isolate*

In this study, an isolate of the fungus *B. bassiana* was used, which in recent research had shown the best performance among several other fungal isolates on the cotton bollworm. Before use in the experiments, the desired fungal isolate was cultured in Potato Dextrose Agar (PDA) medium and kept in an incubator at 25 °C for 12-14 days in the dark. Mass production of this fungal isolate was carried out in a solid medium containing rice bran and 8% whey [15].

*Establishment of Helicoverpa armigera colony in the laboratory*

To establish a fruitworm population in the laboratory, this pest insect was collected from tomato fields as larvae and pupae, and all developmental stages of the pest were carried out in a breeding room at a temperature of 27 °C, relative humidity of 65%, and a light period of 16 hours and a dark period of 8 hours. To feed the larvae, an artificial diet containing 205 grams of cowpea soaked in water for 24 hours, 35 grams of baker's yeast, 30 grams of wheat germ powder, 1.1 grams of sorbic acid, 3.5 grams of ascorbic acid (vitamin C), 2.2 grams of methyl parahydroxybenzoate (nipagin), 1.4 grams of agar, 4.2 ml of sunflower oil (without antioxidant), 2.5 ml of 37% formaldehyde and 700 ml of sterile distilled water were used. 10% honey water solution was used to feed the adult insects.



*Laboratory studies*

In vitro, the bioassay of biological control agents including three different formulations of fungi (concentrated oil suspension, mixture of fungal conidia in oil, and mixture of fungal conidia in water) as well as three different concentrations of the biological pesticide Biolep (WP) containing BtK bacteria were investigated on the third instar larvae of *Helicoverpa armigera*. In this study, two types of oil formulations were used, based on the findings of Devi and Hari [16]. Thus, based on the findings of Devi and Hari [16] and Tupe *et al*. [17], prepared a 30% concentrated fungal suspension based on mineral oil (liquid paraffin) containing fungal conidia, liquid paraffin, and Tween 80, carboxymethyl cellulose and talc. In the method of Tupe *et al*. [17] fungal conidia were mixed in a mixture of sunflower oil and diesel in a ratio of 7:3. In the treatment of fungi with distilled water, fungal conidia were mixed in a ratio of 30% with sterile distilled water containing 0.05% Tween 80. Finally, fungal formulations were mixed in a ratio with sterile distilled water containing 0.05% Tween 80 to obtain a fungal suspension with a concentration of $10^7$ conidia per ml. This suspension was sprayed on third-instar fruitworm larvae using a hand sprayer. The experiments were conducted in triplicate, each with 20 larvae, along with a control treatment of sterile distilled water containing 0.05% Tween 80. After spraying, the larvae were placed on filter paper for five minutes to absorb excess moisture and were finally transferred to containers containing some artificial food and placed in an incubator with a temperature of 26 ± 5 °C, a relative humidity of 60 ± 5%, and 16 hours of light. Daily mortality was monitored and recorded for eight days. The experiments were conducted in a completely randomized design with three replications and a control treatment.

In the bioassay of bollworm larvae with Biolep (WP), three concentrations of the biological pesticide were prepared and sprayed on the bollworm artificial food.

*Field experiments*

This experiment was done in a randomized complete block design with 6 treatments and 3 replications in two regions. The experimental areas consisted of two tomato fields. The experimental treatments included three treatments containing fungal conidia, including 1) an oil formulation based on diesel and sunflower oil (BbOil), 2) a concentrated suspension formulation based on mineral oil (BbSuspension), 3) a fungal suspension dissolved in sterile distilled water with 0.05% Tween 80 (BbWater), 4) a biological product with Biolep at the recommended dose (one per thousand Bt), 5) an organic plant insecticide based on RuiAgro (Matrin) at the recommended dose (one per thousand), and 6) a control treatment (no foliar spray). In the treatments containing fungi, after preparing the fungal formulations according to the items mentioned in the laboratory studies section, in all fungal treatments, 250 mg of the above formulations were mixed in one liter of sterile distilled water using a laboratory homogenizer, so that the conidia content in this suspension was about $10^{10}$ conidia per ml. From this suspension, the amount required for field spraying was prepared. To investigate the effect of the mentioned treatments on larval losses, the number of pierced fruits and larvae was examined. The size of each plot was 5 by 10 meters and the distance between the plots was 4 meters. Foliar spraying was done using a motorized backpack sprayer and the time of spraying was at the peak of the emergence of second and third instar larvae according to previous studies. Foliar spraying was carried out early in the morning. Sampling was carried out one day before spraying and 3, 7, and 10 days after spraying. Larvae were collected by removing the marginal rows from the middle rows of the crop by randomly selecting 10 tomato plants from each plot. Larvae were collected from the plants and examined in the laboratory for fungal or bacterial contamination, and then the percentage of effectiveness of each treatment was obtained using the Henderson-Tilton formula. To calculate the number of fruits formed per plant and the percentage of pierced fruits, 20 plants were randomly selected from each plot one week before harvest and the number of healthy and pierced fruits was calculated [18].

Finally, after analyzing the variance of the results using SPSS software, the comparison of the treatment means was performed based on the findings of the variance analysis using Tukey's test.

*Statistical analysis*

Statistical analysis and comparison of means were performed using SPSS version 23 software. Laboratory biometry experiments were performed in a completely randomized design with three replications. In the biometry experiments, analysis of variance (ANOVA) was used, the mortality rate of bollworm larvae was corrected based on Abbott's formula, and the average percentage of pest losses was compared using Tukey's test at a probability level of five percent with SPSS version 23 software. Graphs were drawn using Excel software.

## RESULTS AND DISCUSSION

*Laboratory studies*

The results of laboratory bioassay of various formulations of *B. bassiana* fungus with a concentration of 1 × $10^7$ conidia/ml on third instar larvae of tomato fruitworm are shown in **Figure 1**. The findings of the variance analysis revealed that there was no significant difference between the experimental treatments including fungal suspension dissolved in sterile distilled water containing 0.05% Tween 80 (BbWater), oil formulation based on diesel and sunflower oil (BbOil), and concentrated suspension formulation based on mineral oil (BbSuspension) (P > 0.001, F = 1.807, df = 2).

BbSuspension with 90.96% mortality showed the highest and BbWater with 84% mortality showed the lowest mortality on third instar larvae of tomato fruitworm.

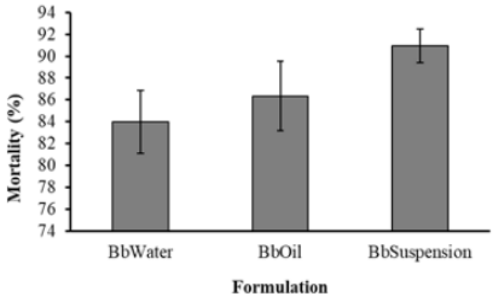


**Figure 1.** Laboratory bioassay of various formulations of Beaveria bassiana fungus (concentration 1 × $10^7$ conidia/ml) on third instar larvae of *Helicoverpa armigera*.

Similarly, the findings of the variance analysis of the bioassay of various concentrations of biocompound Bt on the third instar larvae of the fruitworm revealed that there was a significant difference between the experimental treatments including different concentrations of half per thousand, one per thousand, and one and a half per thousand (P < 0.05, df = 2, F = 152.045) **(Figure 2)**. Comparison of the means using the Tukey test grouped the treatments into three statistical levels (P < 0.05). In this experiment, it was observed that with increasing concentration of Bt consumption, the mortality rate also enhanced significantly. The highest mortality rate was 93.33% at a concentration of one and a half per thousand and the lowest mortality rate was 48.89% at a concentration of half per thousand.

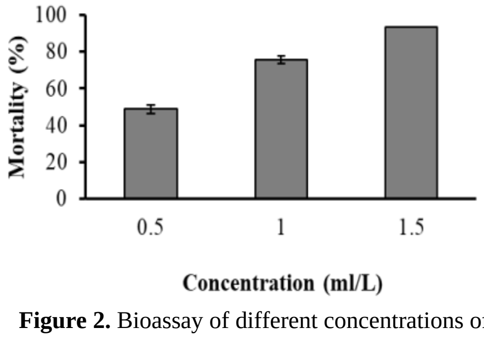


**Figure 2.** Bioassay of different concentrations of a commercial biological pesticide containing the bacterium *Bacillus thuringiensis* subsp. kurstaki (Biolep) (Bt) on the third instar larvae of *Helicoverpa armigera*.

*Field experiments*

*Field 1*

The results of the efficacy study of different biological control agents including three various formulations of insect pathogenic fungi (BbOil, BbSuspension, and BbWater) and a commercial biological pesticide containing the bacterium *Bacillus thuringiensis* subsp *kursaki* (Biolep) (Bt) as well as an organic plant-based pesticide RuiAgro are shown in **Figure 3**. Three days after foliar application, no significant difference was reported among the treatments, but the RuiAgro treatment showed the highest efficacy among the treatments with 38.77% (P > 0.05, F = 1.641, df = 4). In all treatments, the lowest efficacy was three days after foliar application, which gradually increased on the seventh day in all treatments. On the seventh day, the BOil and RuiAgro treatments showed the highest percentage of efficacy and the BbWater treatment showed the lowest efficacy. On the tenth day, BbWater, BSuspension, and Bt treatments increased the percentage of efficiency, while the percentage of efficiency in the RuiAgro treatment remained constant and decreased in the BbOil treatment. Overall, the highest percentage of efficiency was observed on the tenth day in the BbSuspension treatment with 61.57%.

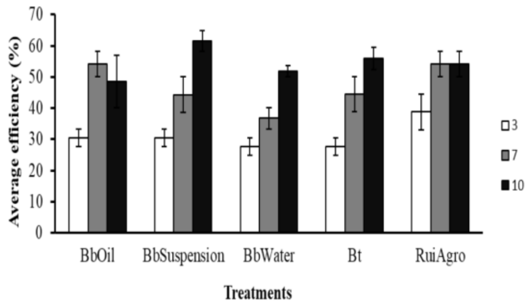


**Figure 3.** Efficacy of different biological control agents including three various formulations of insect pathogenic fungus *Beauveria bassiana* (BbOil, BbSuspension, and BbWater) and a commercial biological insecticide containing bacteria *Bacillus thuringiensis* subsp. kurstaki (Biolep) (Bt) and an organic insecticide based on Agro (Matrin) (RuiAgro) on the damage of *Helicoverpa armigera* at three, seven, and ten days after foliar spraying in Field 1.

*Field 2*

The results of the field experiments in Field 2 **(Figure 4)** showed that three days after foliar spraying, there was a significant difference between the experimental treatments (P < 0.05, df = 4, F = 3.365). The examination of the mean difference of the treatments showed that the BbWater treatment had the lowest efficiency at 30.53% and the Agro treatment had the highest efficiency at 55.55%. At seven days after foliar spraying, the efficiency increased in all experimental treatments, but the difference was not significant. There was no difference between treatments. Ten days after foliar application, the findings of the variance analysis revealed that there was a significant difference between the experimental treatments (P < 0.001, df = 4, F = 8.887). The percentage of efficacy decreased in treatments containing fungal conidia, while in the Bt treatment, an increase in efficacy was observed.

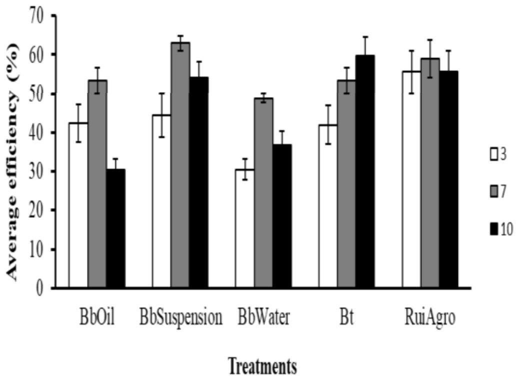


**Figure 4.** Efficacy of different biological control agents including three different formulations of insect pathogenic fungus *Beauveria bassiana* (BbOil, BbSuspension, and BbWater) and a commercial biological insecticide containing bacteria *Bacillus thuringiensis* subsp. kurstaki (Biolep) (Bt) and an organic insecticide based on Agro (Matrin) (RuiAgro) on the damage of *Helicoverpa armigera* at three, seven, and ten days after foliar spraying in field 2.

*Percentage of pierced fruits*

One week before fruit harvest, the percentage of fruits pierced by *Helicoverpa armigera* compared to healthy fruits in both studied plots was examined. The results **(Figure 5)** showed that in plot 1 there was a significant difference between the experimental treatments (P < 0.001, df = 4, F = 22.062). The difference in means showed that the control and BbWater treatments showed the highest percentage of pierced fruits with 23.33% and 18% of pierced tomato fruits, respectively, and the RuiAgro, BbSuspension, BbOil, and Bt treatments had the lowest percentage of pierced fruits, respectively.

In Plot 2, the trend of the percentage of pierced fruits in the experimental treatments was similar to Plot 1. There was a significant difference between the experimental treatments (P < 0.001, df = 4, F = 9.481). The control and BbWater treatments were placed in an experimental group and revealed the highest fruit perforation rate with 35.79% and 22.38% of tomato fruit perforation, respectively. The RuiAgro, BbSuspension, BbOil, and Bt treatments were also placed in an experimental group and had the lowest percentage of fruit perforation, respectively.

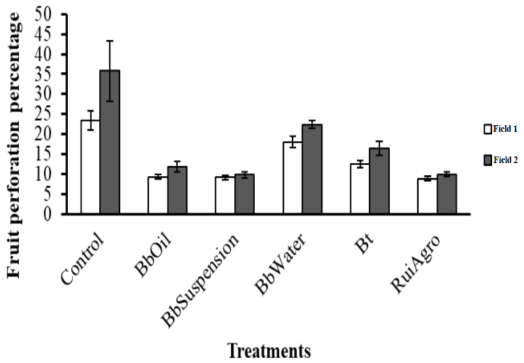


**Figure 5.** Percentage of fruits perforated by *Helicoverpa armigera* in both studied plots.

The results of laboratory bioassay of various formulations of *B. bassiana* fungus at a concentration of 1 × $10^7$ conidia/ml on third-instar larvae of *Helicoverpa armigera* showed that all three various formulations of the pathogenic fungus caused between 84-91% mortality on third-instar larvae. In the study of the sensitivity of third-instar larvae of bollworm to seven fungal isolates of different genera in vitro, it was observed that between 68 and 100% mortality was caused by these isolates. In this study, an isolate of *B. bassiana* fungus at a concentration of 1 × $10^7$ conidia/ml caused 100% mortality on third-instar larvae. These isolates also caused a 74.4 to 100% reduction in pupal emergence [19]. In another study, among three fungi evaluated in vitro on cotton bollworm larvae, an isolate of *N. rileyi* showed the highest mortality rate in the spot inoculation method. In this experiment, an isolate of *B. bassiana* showed about 58% mortality on bollworm larvae and pupae [20]. In Pakistan, 5 of 22 fungal isolates belonging to the *B. bassiana* and *M. anisopliae*, which showed more than 75% mortality on second-instar cotton bollworm larvae, caused 72.92-100% and 65.24-93.96% mortality on third-instar and fourth-instar larvae, respectively. In this study, increasing fungal concentrations increased the pathogenicity of these fungi on bollworm larvae [21]. Apart from the direct mortality of insect-pathogenic fungi, these factors also affected the larval period and the rate of pupal and adult emergence.

The results of laboratory bioassays of different concentrations of Bt biocompound on third-instar fruitworm larvae after eight days showed that with increasing Bt concentration, mortality also increased significantly. The highest mortality rate was 93.33% at a concentration of 1.5 parts per thousand and the lowest mortality rate was 48.89% at a concentration of 0.5 parts per thousand. Similar studies also confirm the appropriate effect of Bt on bollworm larvae in laboratory conditions. According to research by Bouslama *et al*. [22], an isolate of *B. thuringiensis* bacteria called Hrl, in vitro and at a concentration of $1.8 \times 10^9$ cfu/ml, resulted in 93% mortality on first-instar cotton bollworm larvae within 96 hours of use. Also, in the initial screening of concentrated spore-crystal suspensions (1000 µg/ml) of four bacterial isolates, it was observed that 96 to 100% of bollworm larvae were killed within seven days [23].

Comparison of the effectiveness of different fungal formulations with the commercial product Bt and the insecticide RuiAgro under field conditions in two regions shows that in Plot 1, the effectiveness of the treatments used generally increases over time, and the highest effectiveness in all experimental treatments was between about 50 and 60% on the seventh and tenth days. Data from station 2 show that on the tenth day of spraying, the effectiveness of the fungal formulations decreased, while the treatments on RuiAgro and Bt did not change much. It seems that given that the experiments at station 2 were conducted a little later and the air temperature had increased on the days of the experiment, and because insect pathogenic fungi are very sensitive to environmental temperature and humidity, their effectiveness decreased with increasing temperature. A comparison of the effects of 14 fungal isolates belonging to *B. bassiana* and *M. anisopliae* and a commercial Bt powder (BIO-T-PLUSTM 5 WP) on bollworm larvae under laboratory and field conditions in pea fields showed that in laboratory conditions, an isolate of *B. bassiana* had the highest mortality on third-instar larvae. Field studies showed that this isolate was effective in reducing the number of larvae, reducing damage to the pods, and increasing pea yield. Bt powder caused the highest mortality on second-instar larvae (69%) compared to third-instar larvae (56.5%) at a concentration of 0.2 g/200 ml eight days after treatment [18].

In Turkey, treatment of bollworm pests in tomato fields with Bt compared to common insecticides such as malathion, deltamethrin, and lambda-C-halothrin did not significantly differ in terms of damage to fruit [24]. In a study by Younas *et al*. [25], using *B. bassiana* fungus, jasmonic acid, and the insecticide chlorantraniliprole alone or in combination to control cotton bollworm in chickpea fields showed that the lowest number of larvae per plant and pod infection was in the *B. bassiana* treatment with a concentration of $3.21 \times 10^6$ conidia/mL along with the use of the insecticide chlorantraniliprole over two consecutive years. In this treatment, crop yield also increased with a decrease in larval population.

## CONCLUSION

In this study, the effectiveness of three various formulations of the insect pathogenic fungus *Beauveria bassiana* and a biological insecticide based on the active ingredient *Bacillus thuringiensis* subsp. *kurstaki* and organic insecticide-based plants were investigated in laboratory conditions and two tomato fields. The results of laboratory bioassay of various formulations of *B. bassiana* fungus with a $1 \times 10^7$ conidia/ml concentration on third-instar larvae revealed that all three various formulations of the pathogenic fungus caused between 84-91% mortality on third-instar larvae. The results of different concentrations of biological compounds on third-instar *Helicoverpa armigera* larvae after eight days revealed that with increasing concentration of use, the mortality rate also increases significantly. The highest mortality rate was reported with 93.33% at a 1.5 ml/L concentration and the lowest mortality rate was reported with 48.89% at a 0.5 ml/L concentration. The findings of the studies in tomato fields revealed that the effectiveness of the treatments used enhances with time and the highest percentage of effectiveness in all experimental treatments was between about 50 and 60% on the seventh and tenth days after foliar spraying. In the tomato field, the percentage of efficacy of fungal formulations decreased on the tenth day of foliar spraying. Regarding the fruit perforation percentage in both studied fields, the fungal treatment and the control treatment containing distilled water were placed in the same experimental group and revealed the highest rate of fruit perforation. In general, considering the decrease in the effectiveness of fungal formulations on the tenth day of foliar spraying, it can be stated that repeated foliar spraying of biological control agents is essential for better pest control, or combining these methods with organic or low-risk pesticides should be considered.

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