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Pathogenic Effects of Entomopathogenic Fungal Strains on Fall Armyworm (*Spodoptera frugiperda*) Larvae

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ABSTRACT

The excessive and inappropriate use of chemical pesticides presents significant risks to the environment, natural predators in agroecosystems, and human health, leading to diseases such as skin disorders, cancer, and respiratory problems. To mitigate these dangers, it is essential to adopt alternative pest control strategies. In this context, the use of biopesticides offers a safer and climate-friendly approach to insect management. This research focused on evaluating various entomopathogenic fungi for their effectiveness against the fall armyworm (FAW) under controlled laboratory conditions. The experiment evaluated the pathogenicity of various fungal strains and showed that some fungi showed promise in controlling the early larval stages of FAW and keeping the pest population below economic thresholds. Among the fungi tested, *Beauveria bassiana* (Bb885 and 86), *Cordyceps cicadae*, *Metarhizium anisopliae* (73 and 42), and *Paecilomyces fumosoroseus* demonstrated potential, with *B. bassiana* (Bb885), *C. cicadae*, and *M. anisopliae* (73) proving particularly lethal to FAW larvae, especially at high spore concentrations against the early second instar stage. However, further studies are necessary to assess the full range of fungal pathogenicity effects on different life stages, which will provide valuable insights for FAW management in agroforestry settings.

Keywords: *Spodoptera frugiperda*, Entomopathogenic fungi, Pathogenicity, Microbial control

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Introduction

The fall armyworm (FAW), scientifically referred to as *Spodoptera frugiperda* (Lepidoptera: Noctuidae), is a significant migratory pest that threatens agriculture. It is polyphagous, feeding on about 80 plant species across 26 plant families, including vital crops such as sorghum, rice, maize, millet, and sugarcane [1-4]. FAW was first reported in China in 2018, in Yunnan province [5], and has since spread rapidly due to its wide range of host plants and high reproduction rate. Within a few years, it became a major pest in China's maize-producing regions in the northeast and northwest, areas covering 13 million hectares. FAW has proven to be a more serious threat than *Helicoverpa armigera* (Hübner) [6, 7], causing greater damage to maize crops. Due to the rapid spread of the pest, many farmers have resorted to chemical pesticides for control [8]. However, the effectiveness of these

pesticides is significantly impacted by the timing of application, as FAW larvae are typically concealed in corn whorls, making pesticide treatments less effective [9]. The widespread application of chemical pesticides has led to FAW's development of resistance to more than thirty pesticide active ingredients [10, 11], with further information available at https://www.pesticideresistance.org/. This underscores the urgent need for alternative pest management approaches to reduce pest infestations and minimize the harmful environmental effects of pesticides [12]. Entomopathogenic fungi have become a promising biocontrol agent, offering efficiency and ease of use in pest control. Around one thousand insect-pathogenic fungal strains are recognized worldwide [13], and more than one hundred microbial insecticides are commercially available to combat insect pests [14, 15]. Fungal species like *Metarhizium anisopliae*, *Isaria fumosorosea*, and *Beauveria bassiana* are frequently utilized for pest management [16]. These fungi infect their hosts through the insect cuticle, leading to death within 3-6 days after penetration. The efficiency of these fungi is influenced by environmental factors, particularly humidity, and the life stage of the host insect, both of which are key to successful infection [16-20]. This study aims to assess the potential of various entomopathogenic fungal strains against FAW under laboratory conditions. The results are expected to aid researchers focused on microbial pest control within integrated pest management (IPM) programs.

Materials and Methods

Selecting entomopathogenic fungal strain

The various strains of entomopathogenic fungi, including *Beauveria bassiana* (Bb885 and 86), *Cordyceps cicadae*, *Metarhizium anisopliae* (73 and 42), and *Paecilomyces fumosoroseus*, were sourced from the fungal collection at the Research Center on Entomogenous Fungi located at Anhui Agricultural University, Hefei, China (Latitude 31 degrees North, Longitude 117 degrees East) for bioassay testing. These fungal strains were stored at -70 °C before their application in the experiment.

Preparing fungal spore suspension

200 microliters of conidial suspension were retrieved from the fungal collection and inoculated onto Sabouraud dextrose agar in 9 cm-diameter Petri dishes containing 20 grams of agar, 10 grams of peptone, and 40 grams of dextrose. The mixture was incubated at 24 ± 1 °C for 12 days. To facilitate bacterial growth, the medium was supplemented with 2.5 ml/l of penicillin/streptomycin, 0.5 mg of potassium, and 40 grams of cycloheximide. Fully developed conidia were harvested by scraping the top layer of the culture and then diluted with 100 mL of 0.05% Tween®80 in a 200 mL conical flask. The conidia suspension was vortexed for five minutes to ensure proper homogenization. The diluted conidia were then transferred into a sterile beaker using a cotton filter and a sterile 30-milliliter syringe. The concentration of the conidia suspension was adjusted to 1×10^5, 1×10^6, 1×10^7, and 1×10^8 conidia/mL, determined using a microscope and a hemocytometer [21].

Collecting insect and bioassay procedures

Early second and third-instar larvae of the FAW were sourced from a pre-established insect culture maintained in the laboratory of the Institute of Plant Protection, Agro-Products Safety, Anhui Academy of Agricultural Sciences, Hefei, China. The collected larvae were then transported to the Anhui Provincial Key Laboratory of Microbial Control, Anhui Agricultural University, Hefei, China, for fungal bioassay testing. Two bioassay techniques were employed: direct larvae dipping and plant leaf inoculation. Each treatment was conducted with four replicates, including a control group. Four different concentrations were tested, with each replicate containing six larvae, leading to a total of 24 larvae per concentration and 120 larvae per treatment, including the control. No significant results were observed for the 3rd instar larvae in the preliminary tests, so these data are not included in this manuscript.

Direct dipping method

The freshly hatched second instar larvae of FAW were submerged for 3 seconds in fungal spore suspensions with concentrations of 1×10^5, 1×10^6, 1×10^7, and 1×10^8. Following inoculation, the larvae were placed on tissue paper to remove any excess spores and then transferred into plastic containers with 6 holes to prevent cannibalism. A small 1-inch square piece of green Chinese cabbage leaf was provided as food in each compartment. The containers were maintained in a rearing chamber at a controlled temperature of 24 ± 1 °C and relative humidity of 70 ± 5%. After 24 hours, the old leaves were replaced with fresh ones. The containers were covered with lids

to prevent the larvae from escaping, and 2 cotton swabs were added to each container to keep humidity and encourage fungal infection. The larvae were monitored daily for 10 days. Dead larvae were removed and placed on Petri dishes containing moist filter paper, where the fungal infection was confirmed through mycelial growth.

Plant leaf inoculation method

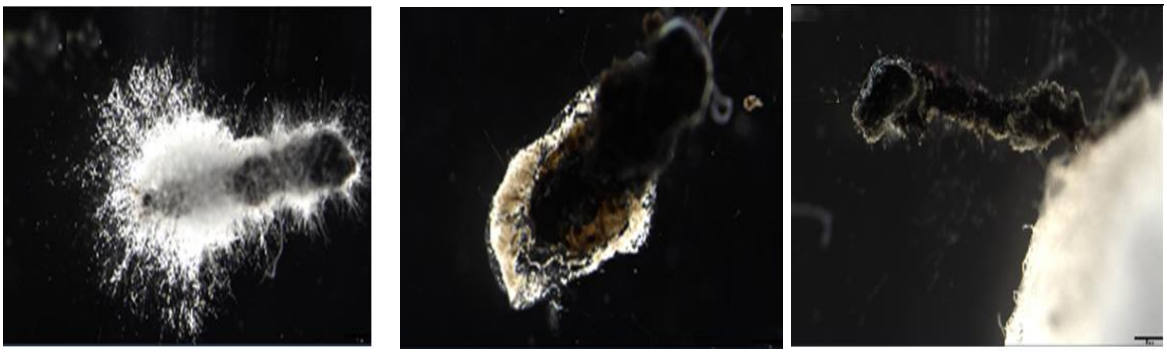
For the plant leaf inoculation method, small pieces of Chinese cabbage leaves, approximately 1 inch in size, were immersed in fungal spore suspensions with concentrations of 1 x 10^5, 1 x 10^6, 1 x 10^7, and 1 x 10^8. After immersion, the leaf pieces were placed on tissue paper to remove excess moisture and then offered to newly hatched second-instar larvae of FAW. The larvae were housed in plastic containers with 6 holes to prevent cannibalism. The inoculated cabbage leaf pieces were provided as food, and the larvae were kept in a rearing chamber at 24 ± 1 °C with 70 ± 5% relative humidity. The containers were sealed with lids to prevent the larvae from escaping, and 2 cotton swabs were placed in each container to keep humidity and encourage fungal infection. After 24 hours, the old, inoculated leaves were replaced with fresh ones. The larvae were observed daily for 10 days. Dead larvae were removed and placed on Petri dishes with moist filter paper to confirm fungal infection through the growth of mycelium.

Analyzing data

The data were subjected to statistical analysis using one-way analysis of variance (ANOVA) through Minitab statistical software. To assess the efficacy of the fungi, Fisher's least significant difference (LSD) test was applied to compare the fungal concentrations, with statistical significance set at $P \leq 0.05$.

Results and Discussion

After performing an initial bioassay screening of fungal strains on FAW larvae, we saw that the leaf dipping method was more efficient than the larval dipping method. As a result, we proceeded with further experiments using the leaf dipping technique, focusing on three highly virulent strains: *B. bassiana* (Bb885), *C. cicadae*, and *M. anisopliae* strain (73) that were particularly lethal to FAW larvae **(Figure 1)**.



a) b) c)

Figure 1. Infection induced by various entomopathogenic fungal strains.

Note: The letters presented in the figures correspond to: a) *B. bassiana* strain 885, b) *C. cicadae*, and c) *M. anisopliae* strain 73.

Our findings showed an important infection of the larval population when Chinese cabbage leaves were treated with *B. bassiana* (Bb885) fungal spores. The highest mortality rate of 66.67% was recorded at a conidial concentration of 1 × 108 spores per milliliter. Mortality rates of 20.83%, 29.17%, and 45.83% were observed at conidial concentrations of 1 × 105, 1 × 106, and 1 × 107 spores per milliliter, respectively **(Figure 2)**. In contrast, the control treatment resulted in a mortality rate of 9.38%, which was significantly lower than the other treatments, as indicated by the LSD test (Df = 4, F = 3.45, P = 0.009) **(Figure 3)**.

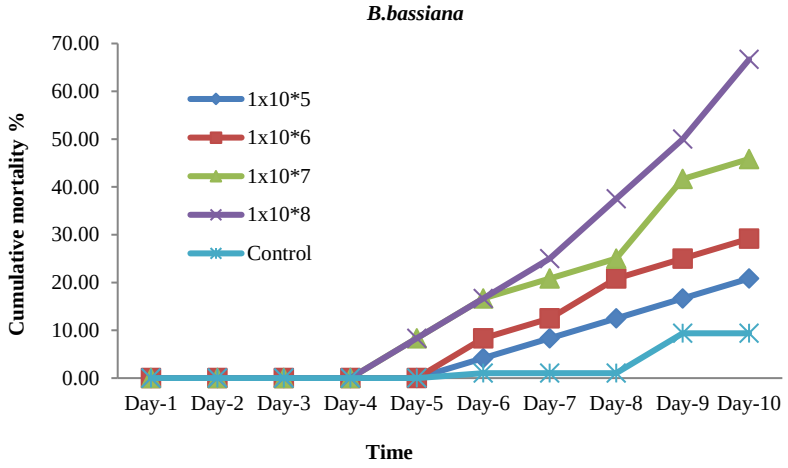


Figure 2. Cumulative larval mortality of FAW treated with different spore concentrations of *B. bassiana* strain-885.

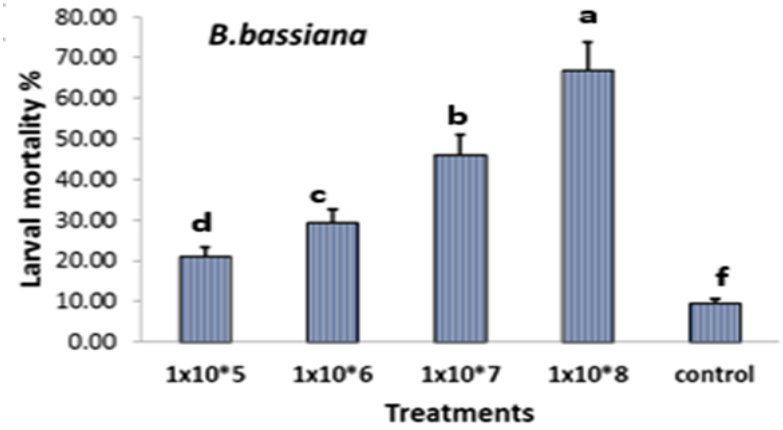


Figure 3. Overall larval mortality of FAW treated with different spore concentrations of *B. bassiana* strain-885.
Note: In the figure, values marked with different letters are significantly distinct at the P < 0.05 level according to the Fisher LSD method.

The highest cumulative larval mortality of 54.17%, 41.67%, 37.50%, and 16.67% was observed at concentrations of 1 x 105spores per milliliter, 1 x 106 spores per milliliter, 1 x 107 spores per milliliter, and 1 x 108 spores per milliliter, respectively, in the *Cordyceps cicadae* treatment **(Figure 4)**. A significant difference was detected among all concentrations. Additionally, when compared using the LSD test (Df = 4, F = 2.85, P = 0.009), all concentrations were significantly different from the control treatment **(Figure 5)**.

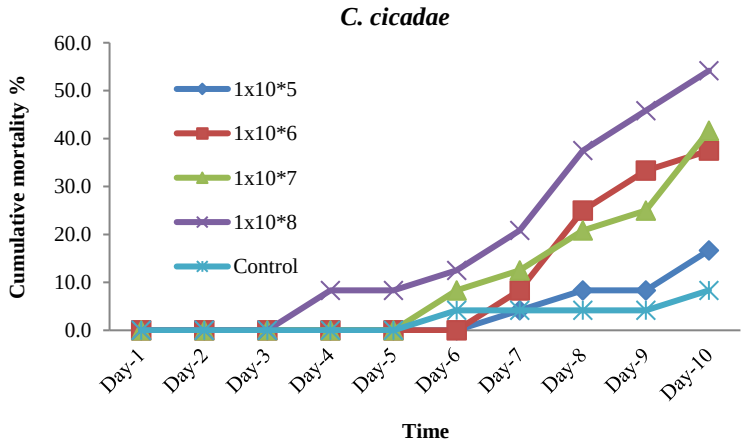


Figure 4. Cumulative larval mortality of FAW treated with various spore concentrations of *C. cicadae*.

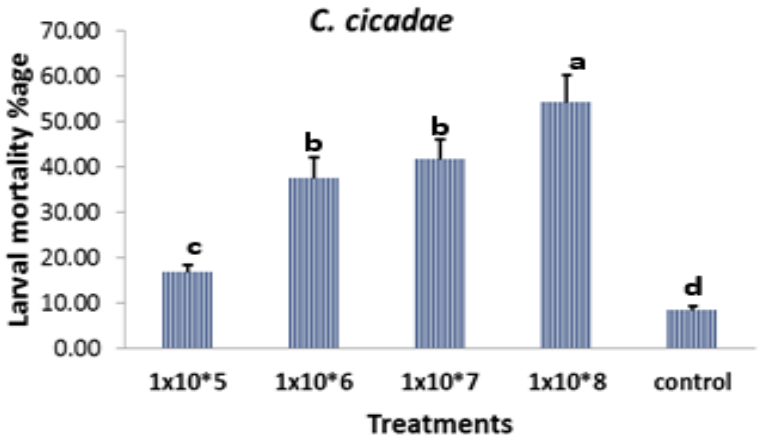


Figure 5. Overall larval mortality of FAW treated with various spore concentrations of *C.cicadae*.
Note: In the figure, values without a common letter indicate a significant difference at the P < 0.05 level, as determined by the Fisher LSD method.

The M. anisopliae strains exhibited strong pathogenicity, with the highest mortality rates of 45.83%, 37.50%, 20.83%, and 12.50% recorded at conidial concentrations of 1 x 105, 1 x 106, 1 x 107, and 1 x 108 spores/ml, respectively **(Figure 6)**. A notable difference in pathogenicity was observed between the highest and lowest concentrations. Additionally, all concentrations showed significant variation compared to the control treatment (Df = 4, F = 2.23, P = 0.067) **(Figure 7)**.

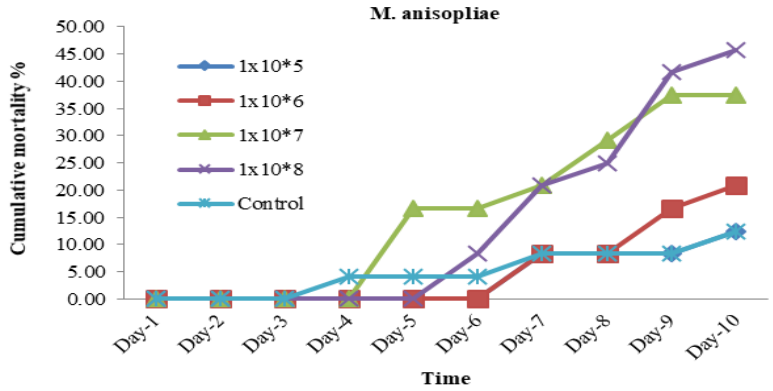


Figure 6. Cumulative larval mortality FAW treated with various spore concentrations *M. anisopliae* 73 strain.

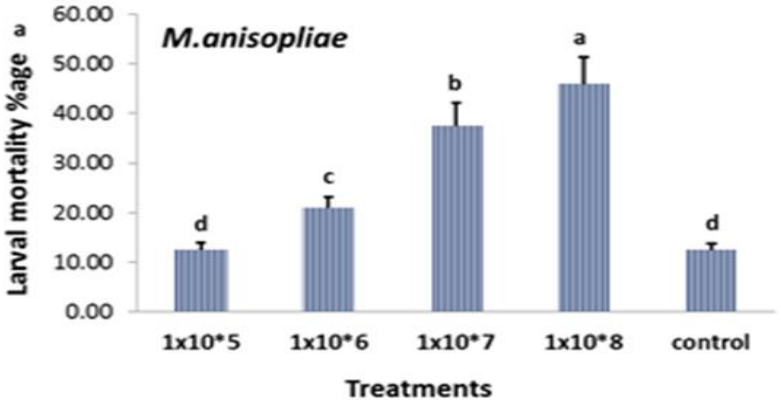


Figure 7. Overall larval mortality FAW treated with various spore concentrations *M. anisopliae* 73 strain.
Note: In the figure, values marked with different letters are considered significantly distinct at the P < 0.05 level based on the Fisher LSD test.

Alternative strategies for insect management could help reduce dependency on chemical pesticides. Previous studies have demonstrated the effectiveness of entomopathogenic fungi in controlling insect pests. In our research, we found that the application of fungal spore suspensions significantly impacted the infection rates in target insect larvae. Different fungal strains, including *B. bassiana* (Bb885), *Cordyceps cicadae*, and *M. anisopliae* (73), exhibited varying levels of efficacy against early instar FAW larvae. Among these, *B. bassiana* (Bb885) resulted in the highest mortality rate of 66.67% after a 10-day incubation period, followed by 54.17% mortality with *Cordyceps cicadae*, and 45.83% mortality with *M. anisopliae*.

Our findings indicated a degree of host specificity in the response of entomopathogenic fungi to the targeted larvae. A similar observation was made by Potrich *et al*. [22], who reported that the insect pathogenic fungus *Isaria* sp. achieved 98.6% control of *B. tabaci* immatures, whereas *B. bassiana* caused an 84.1% response, and *M. anisopliae* resulted in 23.2% mortality. In another study, Kavallieratos *et al*. [23] examined the pathogenic effects of different fungal strains on *Sitophilus myzae* and noted significant differences in the pathogenicity of *B. bassiana*, *M. anisopliae*, and *I. fumosorosea*. Likewise, [24] evaluated the effectiveness of commercially available fungal strains, including *B. bassiana* GHA, *M. brunneum* F52, and *I. fumosorosea* Apopka 97, against *Scirtothrips dorsalis* on chili plants and found control rates ranging from 84–93%, 81–94%, and 62–66%, respectively.

Our observations revealed a dose-dependent relationship between fungal species and both infection rates and mortality percentages. Lower spore concentrations resulted in reduced infection rates in the larvae, while higher concentrations led to increased infection. For instance, *B. bassiana* at a concentration of 1x105 spores/ml produced about 20.83% corrected mortality, while the concentration of 1x108 spores/ml resulted in 66.67% corrected mortality. Similar patterns were noted with *C. cicadae* and *M. anisopliae*. In a related study, Muller [25] investigated the impact of various conidial concentrations of *M. anisopliae* on *Locuslana pardalina*, finding that a 1x108 conidia/ml concentration caused 100% mortality within three to four days, while lower concentrations (1x107, 1x106, and 1x105 conidia/ml) led to mortality over 5 to 6, 6 to 10, and 12 to 14 days, respectively. This highlights that a higher number of conidia leads to a faster and more efficient infection process. Typically, increased conidial density accelerates insect control, suggesting that for managing larger pest populations, higher concentrations would be more effective in keeping pest numbers below the economic threshold.

Conclusion

The results of the present study indicate that all the pathogenic fungal strains were capable of infecting FAW during the early larval stages. However, for effective control of older instars, higher conidial concentrations are required.

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Conflict of Interest: None

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Ethics Statement: None

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