Entomology and Applied Science Letters
Volume 11, Issue 1, Page No: 48-55
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Available Online at: **www.easletters.com**

ISSN No: 2349-2864

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

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ABSTRACT

The overuse and unjustified use of chemical pesticides pose a great threat not only to the environment and natural enemies in the agro-ecosystem but also to human health, such as cancer, skin, and respiratory disease. To avoid such an incidence, the use of alternative chemical pesticides is dire. In this situation, using the green control method by using biopesticides is a safe and climate-smart method for insect control. In the current experiment, we observed the pathogenicity of different entomopathogenic fungal strains. We found that fungal strains have potential and could be used against the tested insect "fall armyworm (FAW) *Spodoptera frugiperda* " early larval instar to manage pest populations below the economic injury level. For this *Beauveria bassiana* strain (Bb885 & 86), *Cordyceps cicadae*, *Metarhizium anisopliae* strain (73&42) and *Paecilomyces fumosoroseus* were observed. We found that the *B. bassiana* strain (Bb885), *C. cicadae, and M. anisopliae* 73 were more lethal to larvae of FAW, with maximum spore concentration against early second instar larvae. However, further experiments are required to observe the fungal pathogenicity potential on different life parameters that would be more helpful in the management of FAW control in agro-forestry ecosystems.

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

HOW TO CITE THIS ARTICLE: Bugti GA, Chen H, Bin W, Rehman A, Ali F. Pathogenic Response of Entomopathogenic Fungal Strains on Larvae of Fall Armyworm (*Spodoptera frugiperda*). Entomol Appl Sci Lett. 2024;11(1):48-55. https://doi.org/10.51847/5hfCqbNbSd

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Received: 29/10/2023
Accepted:14/01/2024

INTRODUCTION

Fall armyworm, technically known as *Spodoptera rugiperda)* (Lepidoptera: Noctuidae) a serious migratory lepidopteron insect pest [1]. It is polyphagous in nature and feeds on about 80 recorded plant species in 26 plant families, including the most economically important crops sorghum, maize, rice, millet, sugarcane, and sugarcane [2-4]. Previous studies indicate that in 2018, the first fall armyworm was reported in China in Yunnan province [5]. Because of the wide range of host plants and high reproduction rate, the pest spread out within a few years and became a serious pest of the maize crop in the northeast and northwest of China [6]. This region is famous for corn production, with an area of 13 million hectares. It is considered that the FAW is more serious than *Helicoverpa armigera* (Hübner) [7] and causes more losses to the maize crop. It was observed that, due to rapid control, most of the farmer community depends on chemical pesticides to control FAW [8]. It has been observed that the time of the chemical application greatly affects its efficiency, and FAW caterpillars are typically located in corn whorls

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where they are mainly shielded from pesticide treatments [9]. Due to the extensive application of chemical pesticides, FAW has developed resistance to commonly used chemical pesticides [10, 11]. It is estimated that FAW has developed resistance to more than 30 active ingredients of chemical pesticides from well-known classes. (https://www.pesticideresistance.org/). So new green control approaches are needed to minimize pest infestation and the adverse effects of pesticides on the environment [12]. Among chemical and biocontrol agents for pest control, entomopathogenic fungi are the most efficient and easy to use; about 1000 insect-pathogenic fungal strains are known globally [13]. Whereas, over 100 microbial insecticides are available on the market to control insect pests [14, 15]. The entomopathogenic fungus *Isaria fumosorosea*, *Metarhizium anisopliae,* and *Beauveria bassiana* are widely used against different insects [16]. Previous literature also confirmed the potential of entomopathogenic fungi against such insect pests [17, 18]. These fungi have special characteristics to infect their host insect through their cuticle [19]. After penetration into the cuticle, the host dies within 3-6 days In most cases, the fungal efficiency depends on environmental factors; among other environmental factors, humidity is known as a key factor for successful infection [20]. The host insect and its life stages are also important for successful infection [16, 20]. Therefore, in this study, we investigated different entomopathogenic fungi to evaluate the potential entomopathogenic fungal strains against (FAW) under laboratory conditions. We hope the results will be helpful for researchers who are working on microbial control of insect pests in their IPM program.

MATERIALS AND METHODS

Entomopathogenic fungal strain selection

The multiple strains of entomopathogenic fungi, e.g., *Beauveria bassiana* strain (Bb885 & 86), *Cordyceps cicadae, Metarhizium anisopliae* strain (73&42) and *Paecilomyces fumosoroseus,* were obtained from the fungal bank of the Research Center on Entomogenous Fungi (RCEF) at Anhui Agricultural University, Hefei, China (Latitude 31°N and Longitude 117°E) for the bioassay test**.** These strains were preserved at -70°C prior to use.

Preparation of fungal spore suspension

Two hundred microliters of conidial suspension tube were obtained from the fungal bank and inoculated with sabouraud dextrose agar in 9 cm-diameter Petri dishes, containing 20 g of agar, 10 g of peptone, and 40 g of dextrose. The mixture was then maintained at 24 ± 1°C in an incubation chamber for 12 days. Penicillin/streptomycin (2.5 ml/l), potassium (0.5 mg), and cycloheximide (40 g) were added to the medium to support bacterial growth before plating. Completely developed conidia were obtained by scraping the upper layer of the culture and then diluted using 100 milliliters of 0.05% Tween®80 in a 200-milliliter conical flask. For five minutes, the flask containing the conidia was homogenized in a vortex. The diluted conidia were transferred into a sterile beaker using cotton and a sterile 30 ml syringe. Using a hemocytometer and a microscope, suspensions were brought to predetermined concentrations. The standardization of conidia suspensions was done at 1 x 10^5, 1 x 10^6, 1 x 107, and 1 × 10^8 conidia /ml [21].

Insect collection and bioassay procedures

Newly hatched larvae of the fall armyworm (early 2nd and 3rd instar) were collected from an already established insect culture in the laboratory of the Institute of Plant Protection, Agro-Products Safety, Anhui Academy of Agricultural Sciences, and Hefei China. The collected FAW larvae were brought to the Anhui Provincial Key Laboratory of Microbial Control, Anhui Agricultural University, Hefei, China, for the fungal bioassay test. Two different bioassay methods, i.e., direct larvae dipping and the plant leaf inoculation method, were used for the bioassay test. Each treatment was repeated four times, along with the control treatment. We used four different concentrations with four replications, with 6 larvae/ replicate; a total of 24 larvae for each concentration and 120 for each treatment, along with a control treatment. We haven't found significant results against the 3rd instar at the initial test, so data is not mentioned in this manuscript.

Direct dipping method

The newly hatched 2nd instar larvae of FAW were dipped for 3 seconds into different fungal spore suspensions, such as 1 x 10^5, 1 x 10^6, 1 x $10^{7,}$ and 1 × 10^8). After inoculation, the larvae were placed

on tissue paper to remove excessive liquid spores and placed into plastic boxes (with six holes to avoid the action of cannibalism). A small piece of green Chinese cabbage leaf was cut about 1 square inch and placed in each whole as food with a controlled relative humidity of 70 ± 5% in a rearing chamber at 24 ± 1°C. Old leaves were replaced after 24 hours with fresh leaves. Boxes were covered with lids to avoid the larvae escaping. Two cotton swabs were placed in each box to maintain humidity and promote fungal infection. For 10 days, daily observations were noted. The dead insects were taken out of the boxes and put onto Petri dishes with moist filter paper so that the fungal infection could be confirmed by mycelial growth.

Plant leaf inoculation method

In the plant leaf inoculation method, the Chinese cabbage leaves were cut into small pieces of about 1 inch and dipped into different fungal spore suspensions, such as 1 x 10^5, 1 x 10^6, 1 x 10^7, and 1 × 10^8). After dipping leaves, pieces were placed on tissue paper to remove excessive water and offered to newly hatched 2nd instar larvae of FAW. The larvae were placed into plastic boxes with six holes to avoid cannibalism. The inoculated small piece of Chinese cabbage leaves (1 inch) was offered to the larvae as food, and the larvae were kept at a relative humidity of 70 ± 5% in a rearing chamber at 24 ± 1°C. Boxes were covered with lids to avoid the larvae escaping. Two cotton swabs were placed in each box to maintain humidity and promote fungal infection. Old leaves inoculated with fungal spores were replaced after 24 hours with fresh leaves. For 10 days, daily observations were noted. The dead insects were taken out of the boxes and put onto Petri dishes with moist filter paper so that the fungal infection could be confirmed by mycelial growth.

Data analysis

The data was statistically analyzed using one-way analysis of variance (ANOVA) using Minitab statistical software, while the effectiveness of the fungi was compared by Fisher's least significant difference (LSD) test to determine differences among fungal concentrations at a significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

We noticed that the leaf dipping method was more effective than the larval dipping method after conducting an initial bioassay screening of fungal strains and testing on FAW larvae. Therefore, we carried out additional experiments using the leaf dipping method with three virulent strains, namely *B. bassiana* (Bb885), *C. cicadae*, and *M. anisopliae* strains (73), which were found to be more lethal to FAW larvae **(Figure 7)**.

According to our findings, the larval population was significantly infected when Chinese cabbage leaves were dipped in *B. bassiana* (Bb885) fungal spores.

Our results showed that the highest mortality rate, 66.67%, was observed in the conidial rate of 1 × 108 spores/ml. Subsequently, mortality rates of 20.83%, 29.17%, and 45.83% were noticed at conidial concentrations of 1 x 105, 1 x 106, and 1 x 107 spores/ml, respectively **(Figure 1)**. Whereas, 9.38 % was observed in the control treatment which was significantly less than other treatments observed by the LSD test (Df=4, F=3.45, P=0.009) **Figure 2**.

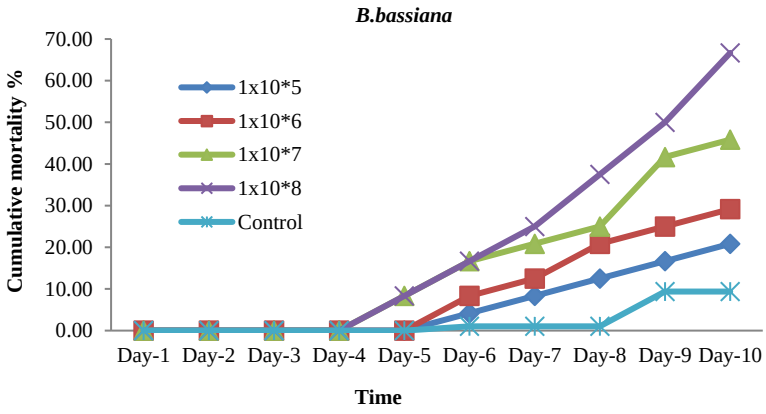


Figure 1. Showing cumulative larval mortality of FAW treated with different spore concentrations of B.bassiana strain-885.

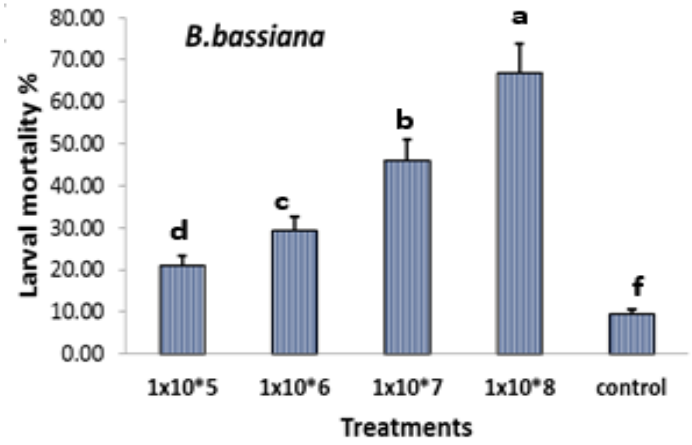


Figure 2. Showing overall larval mortality of FAW treated with different spore concentrations of B.bassiana strain-885.
Note: In the Figure means values that do not share a letter are significantly different at P<0.05 level by using the Fisher LSD method.

Whereas, maximum cumulative mortality of larvae 54.17 %, 41.67%, 37.50%, and 16.67 was observed on 1 x 10^5/ ml, 1 x 10^6/ ml, 1 x 10^7 / ml, and 1 × 10^8 spores/ml respectively on *cordyceps cicadae* treatment **(Figure 3)**. We found a significant difference among all concentrations. However, all concentrations were also significantly different from the control treatment when compared with LSD test (Df=4, F=2.85, P=0.009) **Figure 4.**

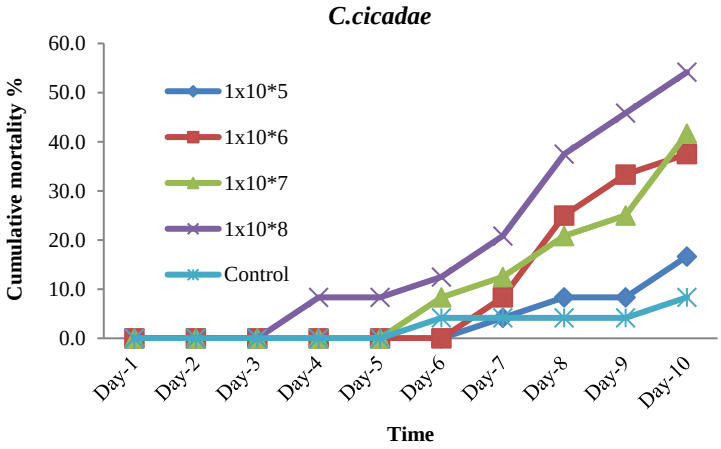


Figure 3. Showing cumulative larval mortality of FAW treated with different spore concentrations of C.cicadae.

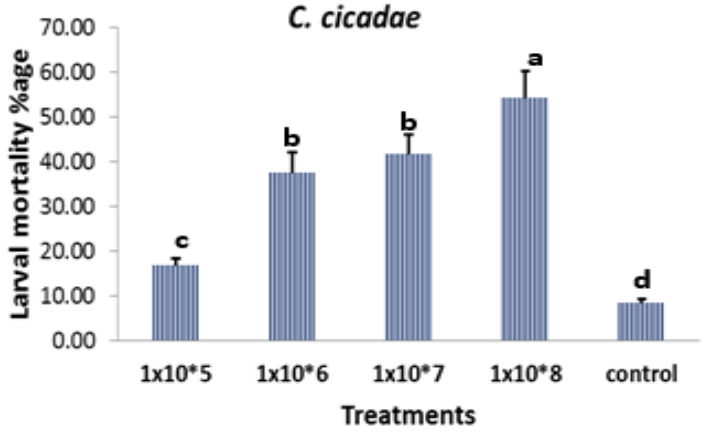


Figure 4. Showing overall larval mortality of FAW treated with different spore concentrations of C.cicadae.
Note: In the Figure means values that do not share a letter are significantly different at P<0.05 level by using the Fisher LSD method

The *M. anisopliae* strains also performed a good pathogenicity and observed maximum mortally rates of 45.83%, 37.50%, 20.83%, and 12.50% was observed on conidial concentrations of 1 x 10^5, 1 x 10^6, 1 x 10^7, and 1 × 10^8/ ml respectively **(Figure 5)**. We observed a great difference in pathogenicity between the lowest and highest concentrations. However all the concentrations were statistically different from the control treatment LSD showed values of (Df=4, F=2.23, P=0.067) **Figure 6.**

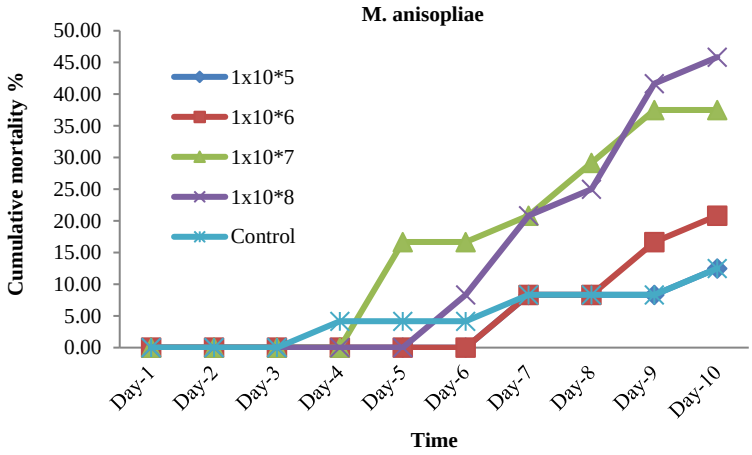


Figure 5. Showing cumulative larval mortality FAW treated with different spore concentrations M. anisopliae 73 strain.

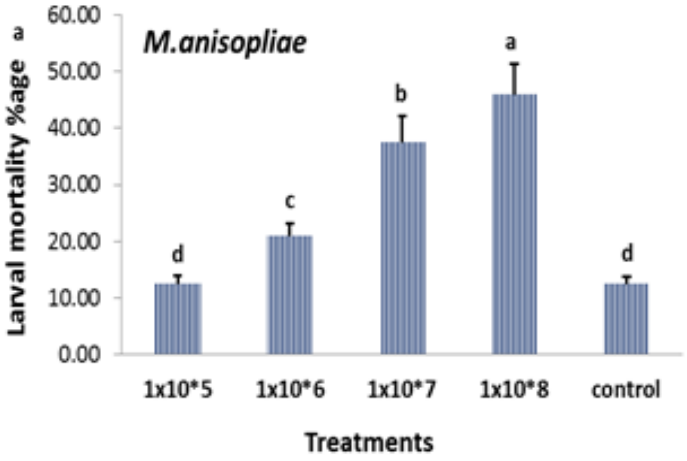
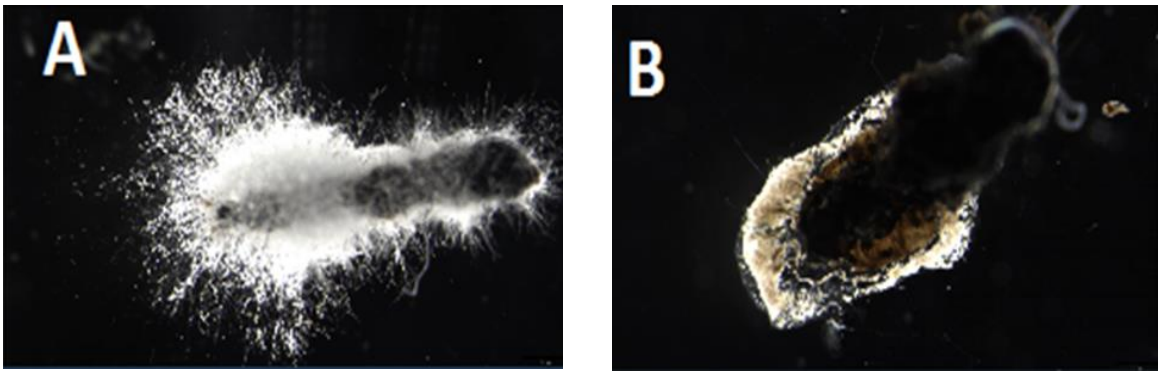


Figure 6. Showing over all larval mortality FAW treated with different spore concentrations M. anisopliae 73 strain.
Note: In the Figure means values that do not share a letter are significantly different at P<0.05 level by using the Fisher LSD method



a) b)

c)

Figure 7. Showing infection caused by different entomopathogenic fungi.
Note: The alphabetic letter shown on the figures may read as: a) B.bassiana-885 strain, b) C.cicadae, c) M. anisopliae -73 strain.

Alternative approaches to insect management might be beneficial to prevent an over-reliance on pesticides. Previous research confirmed the control potential of insect pests using entomopathogenic fungi. In the present study, we observed that the application of the fungal spore suspension has a great influence on causing infection to targeted insect larvae. While applying *B. bassiana* strains (Bb885), *Cordyceps cicadae*, *and Metarhizium anisopliae* strains (73) have different levels of control potential to early-stage larvae of FAW. We found that fungal strain *B. bassiana* (Bb885) post-good infection caused maximum mortality after 10 days of incubation period and observed 66.67% mortality. While 54.17% mortality was observed in *Cordyceps cicadae.* Whereas, *M. anisopliae* showed 45.83% mortality.

In our results, we observed some degree of host-specificity of entomopathogenic fungi in the targeted larvae. Similar results were observed by [22] reported that a different insect pathogenic fungi *Isaria sp*. showed 98.6% control of the immature population of *B. tabaci* while *B. bassiana* showed 84.1% response and *M. anisopliae* caused 23.2% mortality. Kavallieratos, Athanassiou [23] noticed the pathogenic response of various fungal strains against *Sitophilus myzae* and found great variation in the pathogenicity of *B. bassiana*, *M. anisopliae,* and *I. fumosorosea.* Similarly, [24] compared the pathogenicity of commercially available fungal strains likewise *B. bassiana* GHA, *M. brunneum* F52, and *I. fumosorosea* Apopka 97 on chili *Scirtothrips dorsalis* and observed 84–93%, 81–94%, and 62–66%, control respectively.

We observed dosage-dependency response among fungal species, both on causing infection and on mortality percentage. The lower spore concentration showed the lowest infection on tested larvae while the higher spore concentration showed the highest infection on tested larvae. As it is recorded *B.bassiana* lower concentration $1x10^5$ posts about 20%.83 corrected mortality while $1x10^8$ post 66.67% corrected mortality. Similar results were also found in C. cicada and *M. Anisopliae* **(Figure 1)** [25] observed killing time, infection process, and mortality of *Locuslana pardalina* by applying different conidial concentrations of *M.anisopliae* and noticed a maximum mortality rate of 100 in 3–4 days in $1x10^8$ conidia/ml, while $1x10^7$,$1x10^6$ and $1x10^5$ conidia/ml showed killing time 5–6, 6–10 and 12–14 days respectively. This showed the highest number of conidia causes fast and rapid infection to the targeted insect pest.

Usually, a higher density of conidia resulted in faster insect control. So, for the control of higher pest populations, the higher concentration dosage would be more effective than the lower conidial dosage to limit the pest population below the economic threshold level.

CONCLUSION

It was concluded from the current experiment that all pathogenic fungal strains have the ability to cause infection in fall armyworms in early larval instars. For older instars, we need higher conidial concentration to obtain a better control.

ACKNOWLEDGMENTS: I would like to express my special thanks of gratitude to Dr. Haoliang Chen, Director Department of Agricultural Entomology, Institute of Plant Protection at Anhui Academy of Agricultural Sciences, as well as head of the Ahui Academy of Agricultural Sciences, Hefei, China for invitation to avail this

opportunity and conduct research studies on the fall armyworm at Hefei China.
I would also like to extend my thanks to Professor Dr. Wang Bin who allowed his laboratory to conduct a partial experimental trial at Anhui Agricultural University, Hefei China. I also appreciate my project colleagues, and lab mates who helped me from time to time to finalize this project within the limited time.

CONFLICT OF INTEREST: None

FINANCIAL SUPPORT: This research project was financially supported by the Anhui Provincial Key Research and Development Project (2022h11020001) and "The Belt and Road" Innovative Talents Exchange Project (DL2021019001L)

ETHICS STATEMENT: None

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