Online ISSN: 2588-3526
Journal of Wildlife and Biodiversity
*Volume 9(1): 183-199 (2025) (*http://www.wildlife-biodiversity.com/*)*
Research Article

Virulence of different entomopathogenic fungal strains against different life stages of fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

Muhammad Usman Haider*, Shahbaz Ahmad
Department of Entomology, Faculty of Agricultural Sciences, University of the Punjab, Quaid-I Azam Campus, Lahore 54590, Pakistan
*Email: musmanhaider32@gmail.com
Received: 19 November 2024 / Revised: 29 December 2024 / Accepted: 31 December 2024/ Published online: 06 January 2025.
How to cite: Haider, M.U, Ahmad, S.H. (2025). Virulence of different entomopathogenic fungal strains against different life stages of fall armyworm, Spodoptera frugiperda (Lepidoptera: Noctuidae) , Journal of Wildlife and Biodiversity, 9(1), 183-199 **DOI**: https://doi.org/10.5281/zenodo.14634911

Abstract
Maize holds great significance in Pakistan's agricultural landscape as it is an essential crop that plays a vital role in the country's food security and agricultural economy. However, the recent invasion of *Spodoptera frugiperda*, commonly known as fall armyworm (FAW), poses a significant threat to the sustainability of maize production in Pakistan. Therefore, it is imperative to address and manage this challenge to safeguard the nation's agricultural stability and ensure the continued well-being of its communities. The evaluation of entomopathogenic fungi (EPF), including *Beauveria bassiana, Metarhizium anisopliae, Trichoderma viride, Isaria fumosorosea, Lecanicillium lecanii,* and *Aspergillus Niger*, is a valuable initiative to understand their effectiveness in inducing mortality across various biological parameters against fall armyworm. The findings are noteworthy, indicating the varying efficacy of different entomopathogenic fungi in causing mortality across different life stages of fall armyworm. *B. bassiana, M. anisopliae*, and *A. Niger* stand out for causing the highest egg mortality at 34.09%, 25.44%, and 23.60%, respectively. Moreover, the impact on larval mortality is significant, with *B. bassiana* and *M. anisopliae* demonstrating their effectiveness across multiple instars. Notably, *B. bassiana* led in causing the highest mortality in the 1st, 4th, and 6th instars, while *M. anisopliae* had the highest impact in the 2nd, 3rd, and 5th instars. Additionally, *B. bassiana* and *M. anisopliae* caused the highest larval mortality of 31.43% and 22.77%, 23.43% and 18.77%, 22.76% and 13.44%, 29.02% and 20.36%, 19.92% and 15.43%, 21.64% and 12.32% against 1st instar, 2nd instar, 3rd instar, 4th instar, 5th instar and 6th instar respectively. The consistent pattern of mortality caused by *B. bassiana* and *M. anisopliae* extends to pupae of Fall Armyworm (FAW), with respective rates of 18.76% and 12.77%. The reliable impact on different life stages shows potential utility of *B. bassiana* and *M. anisopliae* in comprehensive pest management strategies.
Keywords: Virulence, Fall Armyworm, Fungal strains, Entomopathogenic fungi, FAW mortality.

Introduction

Zea mays L., commonly known as maize, holds the third position among the major cereal crops cultivated in Pakistan, following Wheat (*Triticum aestevum* L.) and Rice (*Oryzea sativa* L.) (Ahmad et al., 2020). The reduction in maize yield can be attributed to various abiotic and biotic factors, with the Fall Armyworm (FAW) being a significant constraint. The invasion of FAW has notably impacted maize production in Asia, leading to substantial economic losses estimated at 13 billion and an annual loss of up to U$S 4000 million (IPPC, 2018). This pest is a critical factor limiting maize production across Afro-Asia, posing a threat to food security in regions heavily reliant on maize (Day et al., 2017). The most concerning and distinctive aspect of Fall Armyworm (FAW) infestation, as opposed to stem borers, is its impact on all stages of plant development, ranging from seedling to maturity (Goergen et al., 2016). Over the past few decades, FAW, scientifically known as *Spodoptera frugiperda*, has gained notoriety as a highly polyphagous crop pest, affecting a wide spectrum of more than 350 crops. Originally native to the tropics and sub-tropics of the Americas, FAW is no longer confined to this region, having dispersed globally (Erasmus, 2019). The invasive nature of this pest was first documented in the African continent in 2016 (Goergen et al., 2016). Despite initial attempts to contain its spread, FAW was reported in Asia for the first time in 2018, starting with India (Kalleshwaraswamy et al., 2018) and subsequently in Pakistan in 2019 (Naeem-Ullah et al., 2019; Gilal et al., 2020).

When addressing the management of this destructive pest, chemical control was initially regarded as the sole dependable method (Valicente & Cruz, 1991). However, the indiscriminate use of pesticides against Fall Armyworm (FAW) has resulted in its resistance to most of these chemicals (Gutiérrez-Moreno et al., 2019). It has been observed that a singular formulation is insufficient, given the widespread presence of FAW on all parts of the plant, including the whorl, stem, and cobs. Not only are chemicals environmentally unfriendly, but they are also economically impractical (Jeyanthi and Kombairaju (2005); Akutse et al., 2020a). When considering living factors, biological control emerges as the primary approach. Biological control agents act as living barriers, serving as a natural and potential means of reducing pest populations. Conservative biological control, integral to Integrated Pest Management (IPM), represents an economically viable approach for long-term pest control. Effective biological control initiatives contribute to the sustainability of pest management and decrease reliance on non-renewable inputs (Quimby et al., 2002). Using microorganisms stands out as a sustainable approach to pest control efforts (West & Gwinn, 1993).

Microorganisms have significantly impacted borers, particularly Fall Armyworm (FAW) larvae (Ríos-Velasco et al., 2010). The evolution of biopesticides and plant extracts has revolutionized pest management, contributing to innovation, and increased agricultural yield (Qadir *et al.*, 2021; Ahmed *et al.*, 2022; Liu *et al.*, 2022; Idrees *et al.*, 2022b). The utilization of certain fungal strains against stem borers has yielded fruitful results (Idrees *et al.*, 2021; Idrees *et al.*, 2022a). The larvae of FAW exhibit high susceptibility to various microbes, including fungi, viruses, and bacteria, owing to their delicate skin that allows microbes to enter and ultimately lead to the death of the insect (Molina-Ochoa *et al*., 2003; Ríos-Velasco et al., 2010). The mode of action for pathogenic fungi involves their spores coming into contact with the dehydrated skin of the insect's immature stages (Dara, 2017; Altinok et al., 2019; Ebani & Mancianti, 2021). Insects infected by fungal spores cease feeding and egg laying and experience developmental blockages, ultimately leading to their demise (Akutse et al., 2019). These infections are contagious and weaken the host's immune system by penetrating its system through the soft cuticle (Donzelli and Krasnoff, 2016; Mondal et al., 2016).

Biological control, with its expansive and targeted impact on pest control, sustainability maintenance, and the elimination of resistance factors, has revolutionized the field of pest management. Beauveria *bassiana* stands out as one of the most extensively employed entomopathogenic fungi (EPF) against lepidopteron insects (Kumar et al., 1999; Wraight et al., 2000; Dannon et al., 2020; Wu et al., 2022). The effectiveness of EPF is notable, with reported pest control rates as high as 90% (Lovett & St. Leger, 2018). EPF has established itself as a viable alternative to synthetic chemicals that pose hazards to the environment and human health (Roberts & Hajek, 1992). Globally, over 1500 EPF species have been identified (St Leger & Wang, 2010; Araújo & Hughes, 2016). Furthermore, approximately 150 biopesticides derived from EPF have been commercially developed and are utilized across a broad spectrum of insects, including beetles, weevils, and larvae (Clifton et al., 2020). A research initiative was devised considering all relevant factors and prioritizing sustainability in addressing pest attacks. This involved the application of entomopathogenic fungi (EPF) strains against Fall Armyworm (FAW) in the laboratory. The primary objective of this research was to formulate an alternative pest management strategy to chemical interventions. The aim was to develop a goal-oriented tactic capable of effectively addressing and mitigating the impact of FAW infestations without relying solely on chemical approaches.

Material and methods

Insect rearing

FAW eggs sourced from a well-established lab colony were housed in a ventilated rectangular plastic box. Newly hatched larvae were given fresh, pesticide-free maize leaves. The first to third instar larvae were kept together in a rectangular plastic box, while fourth to sixth instar larvae were placed individually in six-well plates to prevent them from feeding on each other until they reached the pupation stage. Newly emerged adult moths were placed in cylindrical glasses covered with a paper towel. Honey-soaked (10%) sterile cotton balls were provided in a plastic bottle lid inside the glasses. The larvae were maintained at a temperature of 25°C ± 2°C, with a 12:12 (dark: light) photoperiod and 65% ± 5% relative humidity. The study involved fifty generations of laboratory-reared larvae for experimentation and analysis.

Entomopathogenic fungal isolates

The fungal isolates were cultured by inoculating a small amount onto Sabouraud dextrose agar media in 90 mm diameter Petri dishes. These dishes were subsequently incubated in darkness for a period of 2-3 weeks. Following the incubation period, fungal conidia were harvested from sporulated cultures over the specified duration. In universal bottles, the conidia were suspended in 10 mL of distilled water, supplemented with 0.05% Tween-80. Each bottle contained glass beads, ranging from six to nine beads with a diameter of 3 mm. To ensure homogeneity, the fungal conidial suspensions underwent vortexing for 5 minutes at approximately 700 rpm, effectively breaking up conidial clumps. Before commencing fungal bioassay four different conidial concentrations (1×10^6, 1×10^7, 1×10^8, and 1×10^9 conidia/mL) were planned and made by using a hemocytometer. Before starting bioassay, viability tests were carried out on fungal isolates to ensure their effectiveness, as Opisa et al. (2018) outlined. The results indicated that all eight fungal isolates exhibited germination rates of ≥90%.

Fungal Isolates assessment on eggs and larvae of FAW

In the experimental setup, Fall Armyworm (FAW) eggs, aged one to two days, were extracted from the adult chamber. Under the light microscope, fifty (50) eggs were separated gently by using a camel brush. Subsequently, 10 mL concentration from each treatment was showered on separated eggs using a manual atomized hand spray (20 mL). A sterile paper was placed at the bottom of the rectangular box to manage excess spore suspension. As a control, distilled water with 0.10% Tween-80 was used. After exposed eggs were then air-dried for an hour under laminar flow hood to avoid any contamination. The treated eggs were then placed into Petra plates and incubated at room temperature (25°C ± 2°C) with 65% ± 5% relative humidity (RH). Mortality was assessed seven days post-treatment by observing hatched and unhatched eggs. Unhatched with hyphae were considered dead. The neonate larvae from the treated eggs were housed in a separate plastic box having tiny holes. Fresh maize leaves were fed to the neonate larvae daily, and their data was recorded for up to 14 days post-treatment. All the bioassays were repeated twice, employing a completely randomized design (CRD) with three replications for each treatment.

The daily observation of neonate larvae mortality continued for 7 days. The cumulative mortality of both neonates and larvae of FAW was determined by counting the deceased eggs and neonate larvae of FAW divided by the total number of eggs at 14 days post-treatment. To assess mycosis presence, the cadavers were examined using the approach outlined by Akutse *et al.*, (2019). The deceased cadavers were sterilized externally using 70% alcohol and then washed three times with distilled water. These surface-sterilized cadavers were then placed in Petri dishes containing sterile filter paper, and mortality due to the target entomopathogenic fungi was determined by observing hyphae and conidia on the body of dead cadavers.

Efficacy of fungal Isolates on the feeding performance of different Instar larvae of FAW

Using a hand spray bottle (20 mL), 15 second-instar larvae of Fall Armyworm (FAW) were exposed to 10 mL of an Entomopathogenic Fungus (EPF) at four different concentrations described earlier, following the method outlined by Fargues and Maniania in 1992. 15-20 g of fresh maize leaves were added to a square plastic container. The control group was treated with sterilized distilled water containing 0.10% Tween-80. The second-instar larvae were weighed before and after being provided with a diet of fresh maize leaves.

For this study, the feeding performance of the larvae was calculated in terms of feeding percentage. This was determined by dividing the total weight of the fresh maize leaf surface in grams consumed by the larvae by the total weight of leaves not consumed by the larvae, then multiplying the result by 100. The feeding performance was observed at 24- and 48-hour post-treatment. The experimental layout followed a Completely Randomized Design (CRD) with three replications.

EPF Efficacy on pupae of FAW

Employing manual spray bottles (20 mL), 10 mL of each concentration of fungal isolates were investigated against pupae of Fall Armyworm (FAW). In each treatment, 15 pupae were placed within a rectangular plastic box measuring 28 cm × 17 cm × 18 cm. The control group was treated with sterilized distilled water containing 0.10% Tween-80. Pupal mortality was observed over 15 days, following the protocol outlined by Liu et al. (2022). Pupae were considered dead if they did not exhibit a blackened appearance, emergence, or any movement upon touch. The experimental design adhered to a Completely Randomized Design (CRD) with three replications.

Statistical analysis

To determine the percentage mortality of larval stages, Abbott's formula (Abbott, 1925) was employed. The obtained data were visually represented graphically and subjected to statistical analysis using Statistix® Version 8.1 (Analytical Software, Tallahassee, FL). Factorial ANOVA was employed to examine the interaction of various factors, encompassing fungal conidial concentrations, treatments, and immature stages. Subsequently, Tukey's Highly Significant Difference (HSD) post hoc test was applied for further analysis. The statistical analysis was conducted using SPSS (version 22.0).

Results

Efficacy of different concentration of entomopathogenic fungus strains on eggs of Fall Armyworm (FAW)

According to the results, the entomopathogenic fungal strains caused 49.57% egg mortality at the highest concentration of 1×10^9 spores/ml. The concentrations of 1×10^8 spores/ml and 1×10^7 spores/ml also caused significant egg mortality, with 35.68% and 27.35% respectively at 10 days after treatment ($F_{4, 89} = 16306.10$; $p = 0.0000$). However, there were no significant differences in egg mortality at a concentration of 1×10^6 spores/ml and in the control treatment, which registered at 4.02% and 3.46% respectively (Table 1).

Efficacy of different concentration of entomopathogenic fungus strains on different larval instars of Fall Armyworm (FAW)

The entomopathogenic fungal strains induced 46.24% motility in 1st instar larvae of *S. frugiperda* when used at a concentration of 1×10^9 spores/ml. When the concentration was reduced to 1×10^8 spores/ml and 1×10^7 spores/ml, the motility percentages decreased to 27.91% and 15.13% respectively, in 1st instar larvae of *S. frugiperda* after 10 days of treatment. The statistical analysis showed significant results ($F_{4, 89} = 13078.37$; $p = 0.0000$). However, no significant differences were observed in the percent mortality of 1st instar larvae of *S. frugiperda* when treated with a concentration of 1×10^6 spores/ml and in the control treatment. The mortality rates recorded were 4.02% and 3.46%, respectively (Table 1).

The entomopathogenic fungal strains exhibited a mortality rate of 42.91% in 2nd instar larvae of *S. frugiperda* when applied at a concentration of 1×10^9 spores/ml. Decreasing the concentration to 1×10^8 spores/ml and 1×10^7 spores/ml resulted in motility percentages of 19.02% and 9.57%, respectively, in 2nd instar larvae of *S. frugiperda* after a 10-day treatment period. The statistical analysis indicated these results were significant ($F_{4, 89} = 10535.39$; $p = 0.0000$). However, no significant differences were observed in the percent mortality of 1st instar larvae of *S. frugiperda* when treated with a concentration of 1×10^6 spores/ml and in the control treatment. The recorded mortality rates were 4.57% and 4.02%, respectively (Table 1).

During a 10-day treatment period, the application of entomopathogenic fungal strains at different concentrations was tested on 3rd instar larvae of *S. frugiperda*. The results showed that a concentration of 1×10^9 spores/ml resulted in a motility rate of 34.02%. When the concentration was reduced to 1×10^8 spores/ml and 1×10^7 spores/ml, the motility percentages decreased to 22.35% and 11.24%, respectively. These results were statistically significant ($F_{4, 89} = 6198.67$; $p = 0.0000$). However, there were no significant differences in the percent mortality of 1st instar larvae of *S. frugiperda* treated with a concentration of 1×10^6 spores/ml compared to the control treatment. The recorded mortality rates were 4.02% and 3.46%, respectively (Table 1).

At a 1×10^9 spores/ml concentration, entomopathogenic fungal strains induced a motility rate of 43.83% in 4th instar larvae of *S. frugiperda*. The reduction in concentration to 1×10^8 spores/ml

and 1×10^7 spores/ml resulted in decreased motility percentages of 25.50% and 12.72%, respectively, in 1st instar larvae of *S. frugiperda* after a 10-day treatment period. Statistical analysis underscored the significance of these findings ($F_{4, 89} = 13078.37$; $p = 0.0000$). Conversely, no significant differences were noted in the percent mortality of 1st instar larvae of *S. frugiperda* treated with a concentration of 1×10^6 spores/ml compared to the control treatment. The recorded mortality rates were 1.61% and 1.05%, respectively (Table 1).

Based on the results, entomopathogenic fungal strains induced a mortality rate of 39.40% in 5th instar larvae of *S. frugiperda* at the highest concentration of 1×10^9 spores/ml. Notably, concentrations of 1×10^8 spores/ml and 1×10^7 spores/ml also resulted in significant mortality in 5th instar larvae of *S. frugiperda*, recording 15.51% and 6.06%, respectively, after a 10-day treatment period ($F_{4, 89} = 13722.05$; $p = 0.0000$). However, there were no significant differences in mortality at a concentration of 1×10^6 spores/ml compared to the control treatment, with recorded rates of 1.51% and 1.19%, respectively (Table 1).

According to the results, entomopathogenic fungal strains caused a mortality rate of 32.90% in 6th instar larvae of *S. frugiperda* when the highest concentration of 1×10^9 spores/ml was used. It is worth noting that concentrations of 1×10^8 spores/ml and 1×10^7 spores/ml resulted in non-significant mortality in 6th instar larvae of *S. frugiperda*, recording 11.23% and 10.12%, respectively, after a 10-day treatment period ($F_{4, 89} = 6198.67$; $p = 0.0000$). However, there were no significant differences in mortality at a concentration of 1×10^6 spores/ml compared to the control treatment, with recorded rates of 2.90% and 2.34%, respectively (Table 1).

Efficacy of different concentration of entomopathogenic fungus strains on pupae of Fall Armyworm (FAW)

Based on the research findings, entomopathogenic fungal strains caused a mortality rate of 25.68% in pupae of *S. frugiperda* when the concentration was at its highest level of 1×10^9 spores/ml. Notably, concentrations of 1×10^8 spores/ml and 1×10^7 spores/ml also resulted in significant pupal mortality, with rates of 16.79% and 10.13%, respectively, after a 10-day treatment period ($F_{4, 89} = 3520.48$; $p = 0.0000$). However, there were no significant differences in pupal mortality of *S. frugiperda* at a concentration of 1×10^6 spores/ml compared to the control treatment, with recorded rates of 4.02% and 3.46%, respectively (Table 1).

Efficacy of different fungal isolates on eggs of Fall Armyworm (FAW)

The findings indicate that *B. bassiana* resulted in the highest egg mortality at 34.09%, followed by *M. anisopliae*, *A. Niger*, *Trichoderma viride*, *I. fumosorosea*, and *L. lacanii* with percent mortality rates of 25.44%, 23.60%, 22.80%, 19.99%, and 18.18%, respectively, when subjected to various concentrations over a 10-day treatment period ($F_{4, 89} = 1041.52$; $p = 0.0000$) (Table 2).

Efficacy of different fungal isolates on larval instars and pupae of Fall Armyworm (FAW)

According to the results, *B. bassiana* caused the highest mortality rate of 34.09% in 1st instar larvae, while *L. lacanii* recorded the lowest mortality rate of 11.51% over a 10-day treatment period. The statistical analysis ($F_{5, 89} = 1648.23$; $p = 0.0000$) highlights the significance of these mortality rates. Based on the results, *B. bassiana* induced the highest mortality rate at 23.43% in 2nd instar larvae, while *L. lacanii* recorded the lowest mortality rate at 10.18% over a 10-day treatment period ($F_{5, 89} = 751.82$; $p = 0.0000$). *B. bassiana* triggered the highest mortality rate at 22.76% in 3rd instar larvae, with *L. lacanii* recording the lowest mortality rate at 7.51% over a 10-day treatment period. The statistical analysis ($F_{5, 89} = 1024.67$; $p = 0.0000$) emphasizes the

significance of these observed mortality rates. According to the results, *B. bassiana* caused the highest mortality rate of 29.02% in 4th instar larvae, while *L. lacanii* had the lowest mortality rate of 9.10% during a 10-day treatment period ($F_{5, 89}$ = 1024.67; p = 0.0000). The same pattern was observed for the fall armyworm's 5th and 6th instar larvae. The findings indicate that *B. bassiana* resulted in the highest mortality rate of 18.76% in fall armyworm pupae, while *L. lacanii* exhibited the lowest mortality rate at 8.85% during a 10-day treatment period. The statistical analysis ($F_{5, 89}$ = 435.37; *p* = 0.0000) emphasizes the significance of these observed mortality rates (Table 2).

Factorial analysis of variance

The factorial analysis of variance (ANOVA) showed significant interactions between spore concentrations and treatments for various developmental stages of fall armyworm. The results are as egg mortality: $F_{20, 89}$ = 192.27; p = 0.0000, 1st instar larvae: $F_{20, 89}$ = 300.44; p = 0.0000, 2nd instar larvae: $F_{20, 89}$ = 232.96; p = 0.0000, 3rd instar larvae: $F_{20, 89}$ = 289.03; p = 0.0000, 4th instar larvae: $F_{20, 89}$ = 300.44; p = 0.0000, 5th instar larvae: $F_{20, 89}$ = 316.90; p = 0.0000, 6th instar larvae: $F_{20, 89}$ = 289.03; p = 0.0000 and Pupae: $F_{20, 89}$ = 84.84; p = 0.0000. These findings suggest that the combination of spore concentrations and treatments significantly impacted mortality rates across different stages of fall armyworm development (Figures 1-8).

Table 1. Percent mortality of different life stages of fall armyworm (*Spodoptera frugiperda*) treated with different concentrations of fungal isolates at 10 days post treatment.

Concentrations (spores/ml)	Percent Mortality (± Std. Error of Means)							
	Eggs	1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar	6th Instar	Pupae
1×10^6	4.02 ± 0.33^{D}	4.02 ± 0.33^{D}	4.57 ± 0.40^{D}	4.02 ± 0.33^{D}	1.61 ± 0.33^{D}	1.51 ± 0.31^{D}	2.90 ± 0.33^{D}	4.02 ± 0.33^{D}
1×10^7	27.35 ± 1.28^{C}	15.13 ± 1.91^{C}	9.57 ± 0.75^{C}	11.24 ± 1.31^{C}	12.72 ± 1.91^{C}	6.06 ± 0.75^{C}	10.12 ± 1.31^{B}	10.13 ± 0.94^{C}
1×10^8	35.68 ± 1.97^{B}	27.91 ± 2.89^{B}	19.02 ± 1.20^{B}	22.35 ± 2.32^{B}	25.50 ± 2.89^{B}	15.51 ± 1.20^{B}	11.23 ± 2.32^{B}	16.79 ± 1.48^{B}
1×10^9	49.57 ± 2.78^{A}	46.24 ± 2.97^{A}	42.91 ± 3.18^{A}	34.02 ± 2.97^{A}	43.83 ± 2.97^{A}	39.40 ± 3.18^{A}	32.90 ± 2.97^{A}	25.68 ± 1.60^{A}
Control	3.46 ± 0.13^{D}	3.46 ± 0.13^{D}	4.02 ± 0.33^{D}	3.46 ± 0.13^{D}	1.05 ± 0.13^{D}	1.19 ± 0.24^{D}	2.34 ± 0.13^{D}	3.46 ± 0.13^{D}

Means followed by the same letters are not significantly different by Tukey's test at $p < 0.05$.

Table 2. Percent mortality of different life stages of fall armyworm (*Spodoptera frugiperda*) treated with different fungal isolates after 10 days post treatment.

Fungal species	Percent Mortality (± Std. Error of Means)							
	Eggs	1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar	6th Instar	Pupae
Beauveria bassiana	34.09 ± 3.07^{A}	31.43 ± 6.68^{A}	23.43 ± 5.69^{A}	22.76 ± 5.09^{A}	29.02 ± 6.68^{A}	19.92 ± 5.69^{A}	21.64 ± 5.09^{A}	18.76 ± 3.46^{A}
Metarhizium anisopliae	25.44 ± 2.45^{B}	22.77 ± 4.93^{B}	18.77 ± 4.57^{B}	13.44 ± 3.19^{B}	20.36 ± 4.93^{B}	15.43 ± 4.53^{B}	12.32 ± 3.19^{B}	12.77 ± 2.35^{B}
Trichoderma viride	22.80 ± 2.36^{C}	18.80 ± 4.20^{C}	14.13 ± 3.45^{C}	10.80 ± 2.67^{C}	16.39 ± 4.20^{C}	10.87 ± 3.39^{D}	9.68 ± 2.67^{C}	10.80 ± 1.99^{BC}
Isaria fumosorosea	19.99 ± 2.27^{D}	16.66 ± 3.87^{CD}	12.66 ± 3.06^{D}	8.66 ± 2.01^{CD}	14.25 ± 3.87^{CD}	9.42 ± 3.00^{D}	7.54 ± 2.01^{CD}	9.33 ± 1.73^{BC}
Lecanicillium lecanii	18.18 ± 2.15^{D}	11.51 ± 2.68^{E}	10.18 ± 2.04^{E}	7.51 ± 1.32^{D}	9.10 ± 2.68^{E}	6.95 ± 1.97^{E}	6.39 ± 1.32^{D}	8.85 ± 1.45^{C}
Aspergillus Niger	23.60 ± 2.68^{BC}	14.93 ± 3.97^{D}	16.93 ± 4.61^{BC}	14.93 ± 4.05^{B}	12.52 ± 3.97^{D}	13.80 ± 4.54^{CD}	13.81 ± 4.05^{B}	11.60 ± 2.68^{B}

Means followed by the same letters are not significantly different by Tukey's test at $p < 0.05$.

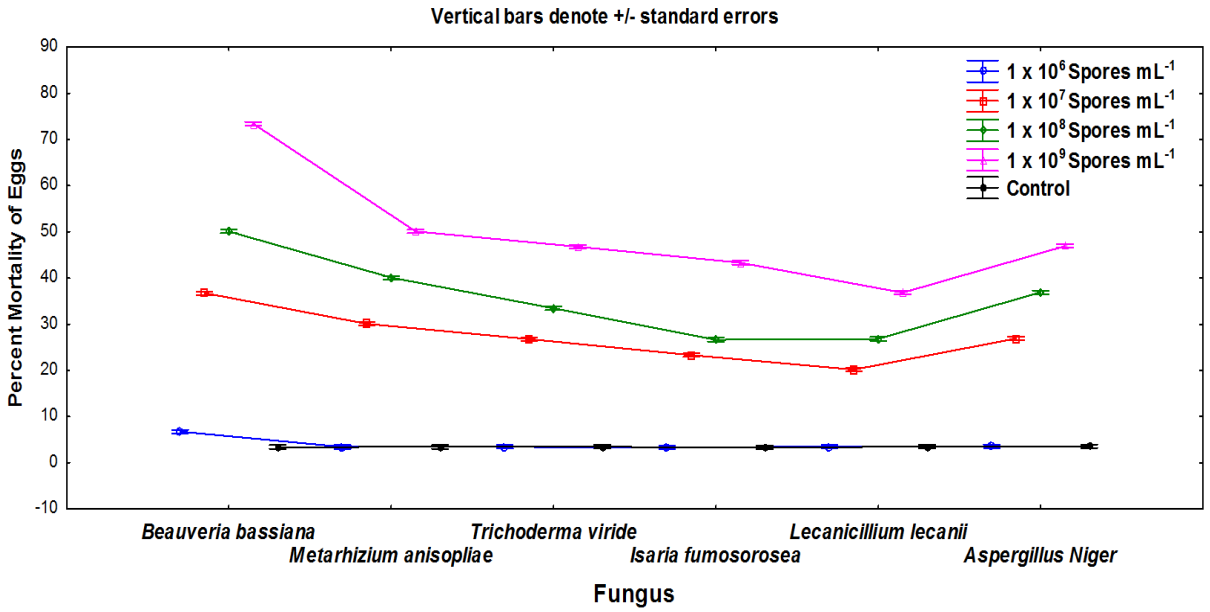

Figure 1. Impact of entomopathogenic fungi (EPF) on mortality of eggs of fall armyworm evaluated with different concentrations at 10 days post-treatment.

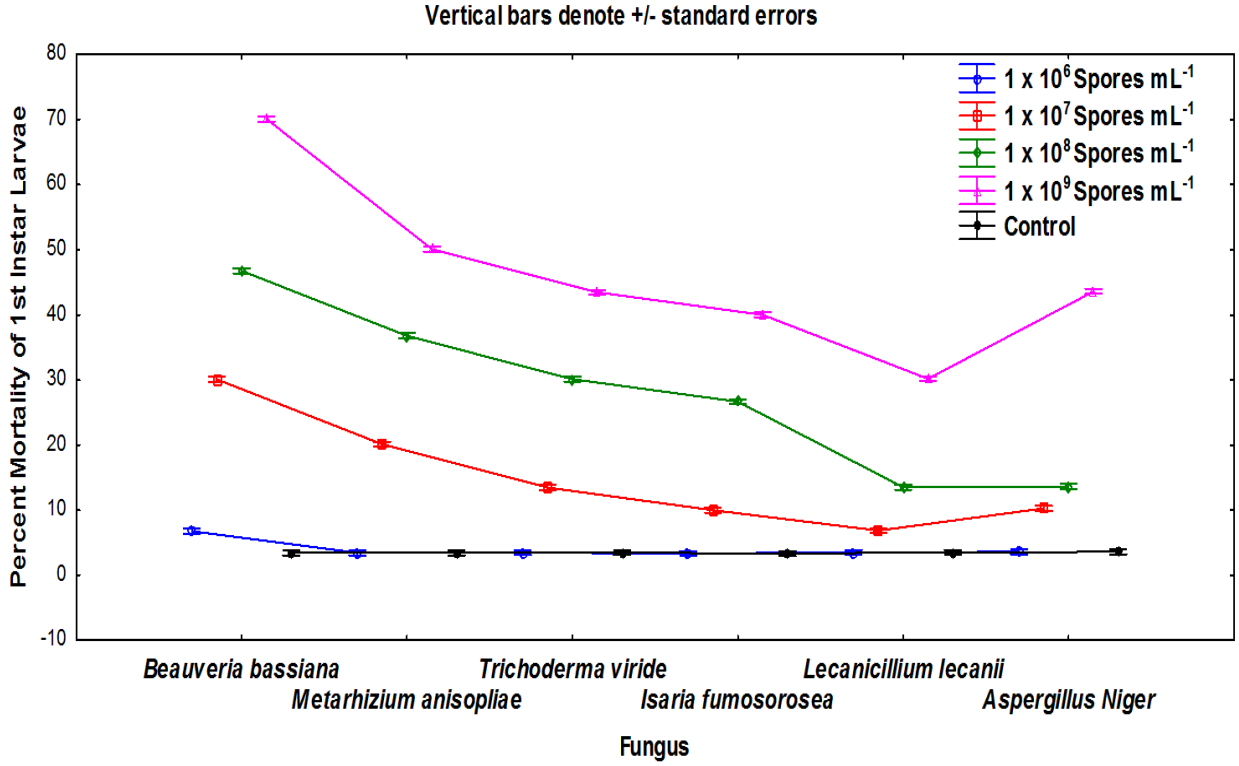

Figure 2. Impact of entomopathogenic fungi (EPF) on mortality of 1st instar larvae of fall armyworm evaluated with different concentrations at 10 days post-treatment

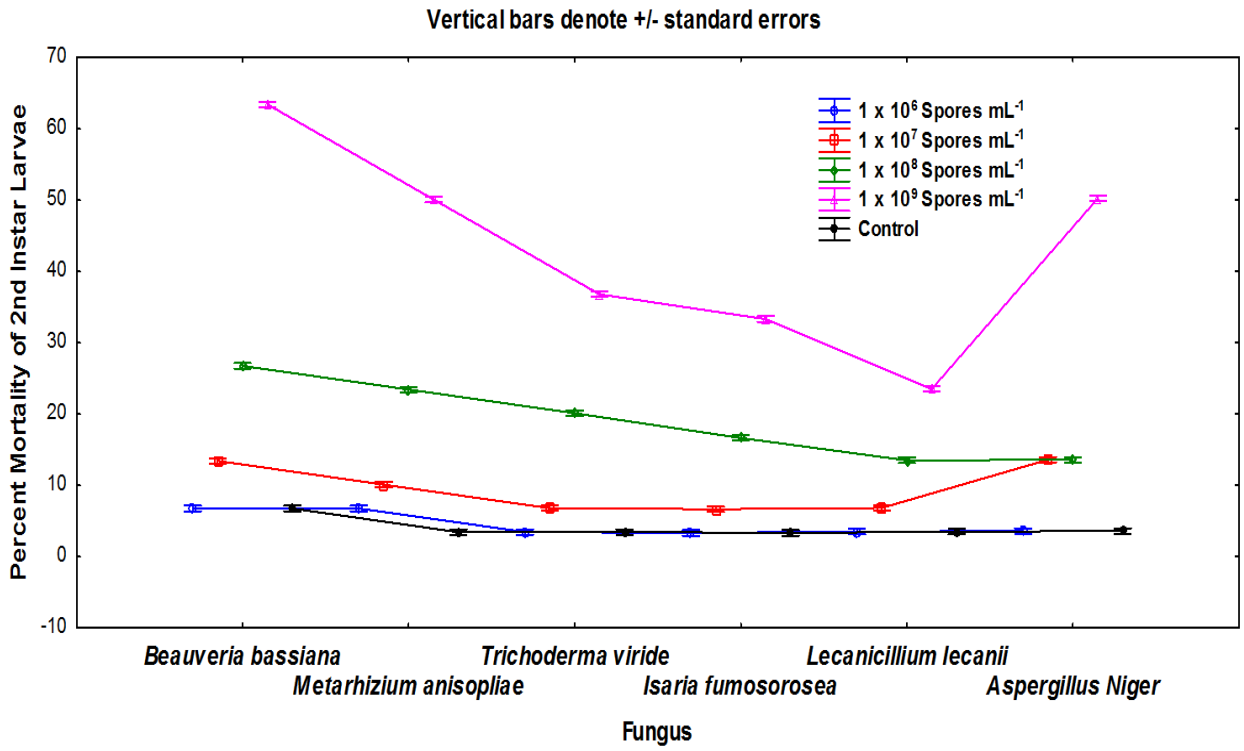

Figure 3. Impact of entomopathogenic fungi (EPF) on mortality of 2nd instar larvae of fall armyworm evaluated with different concentrations at 10 days post-treatment

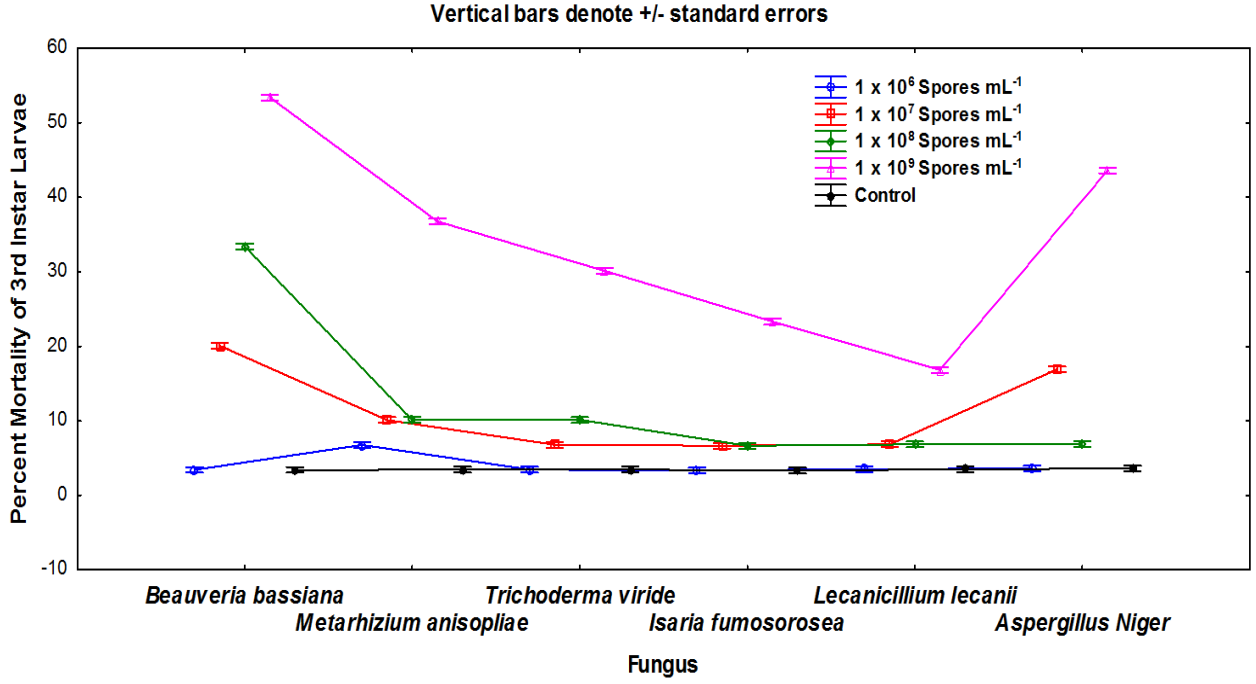

Figure 4. Impact of entomopathogenic fungi (EPF) on mortality of 3rd instar larvae of fall armyworm evaluated with different concentrations at 10 days post-treatment

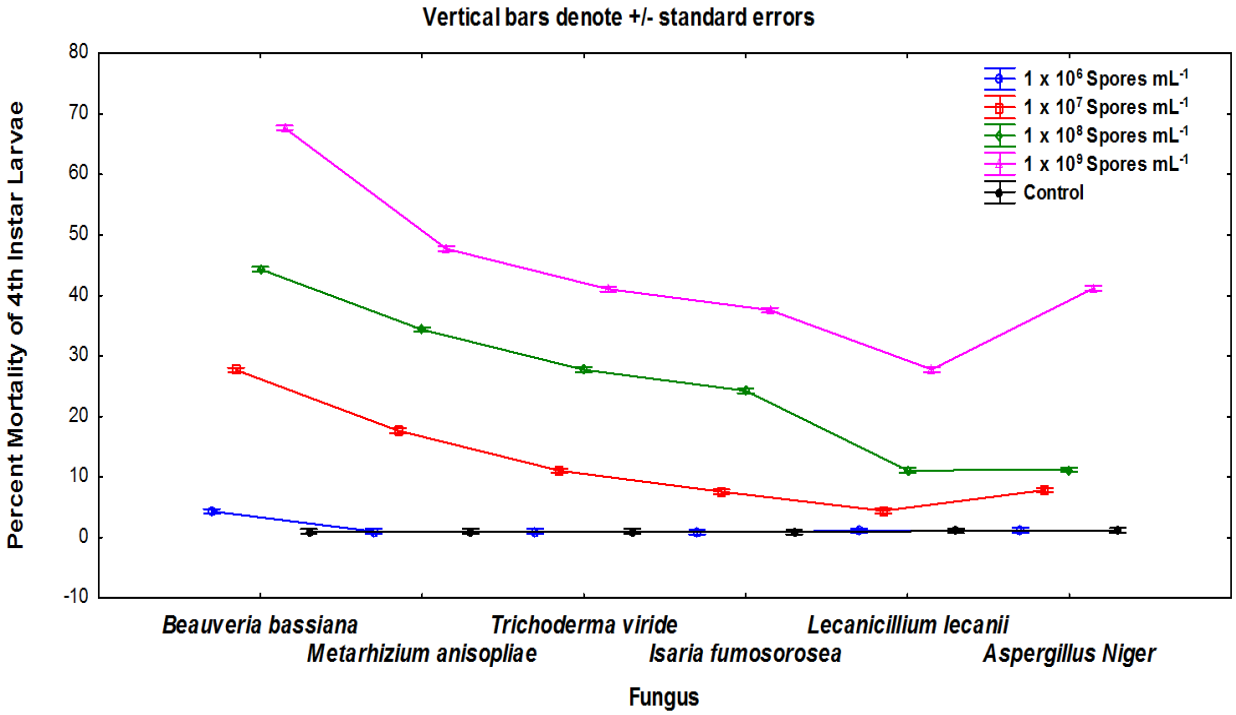

Figure 5. Impact of entomopathogenic fungi (EPF) on mortality of 4th instar larvae of fall armyworm evaluated with different concentrations at 10 days post-treatment

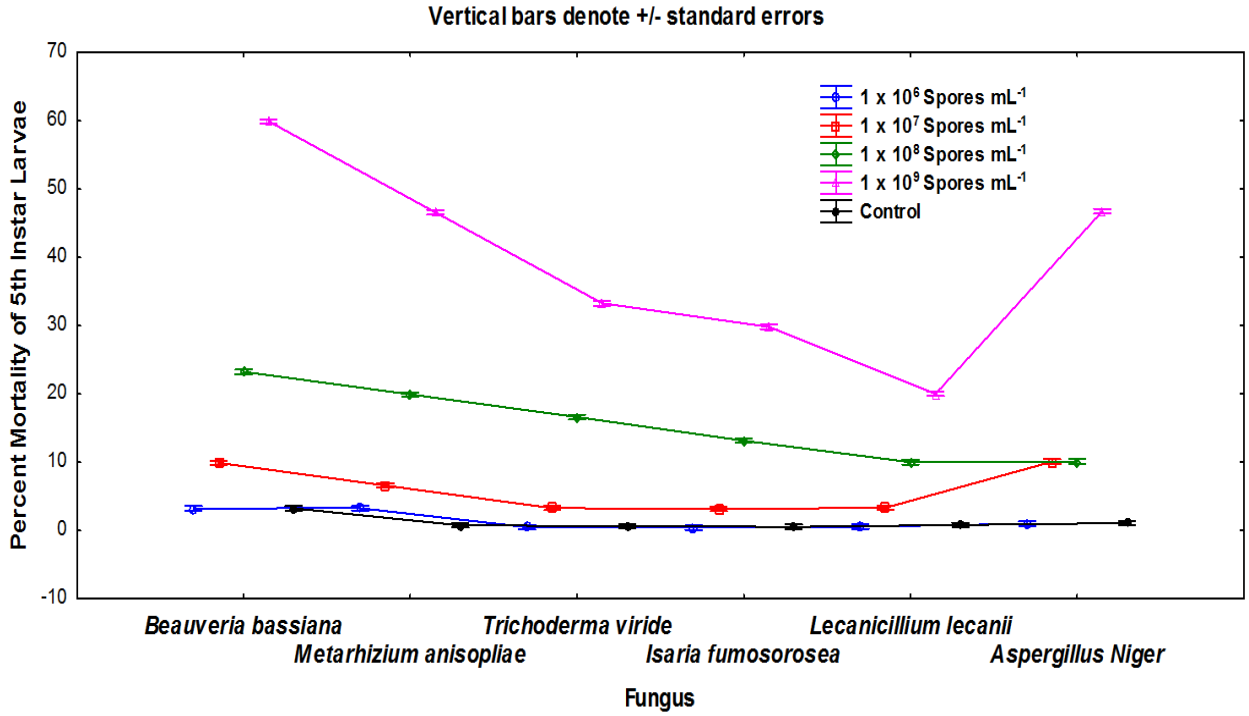

Figure 6. Impact of entomopathogenic fungi (EPF) on mortality of 5th instar larvae of fall armyworm evaluated with different concentrations at 10 days post-treatment

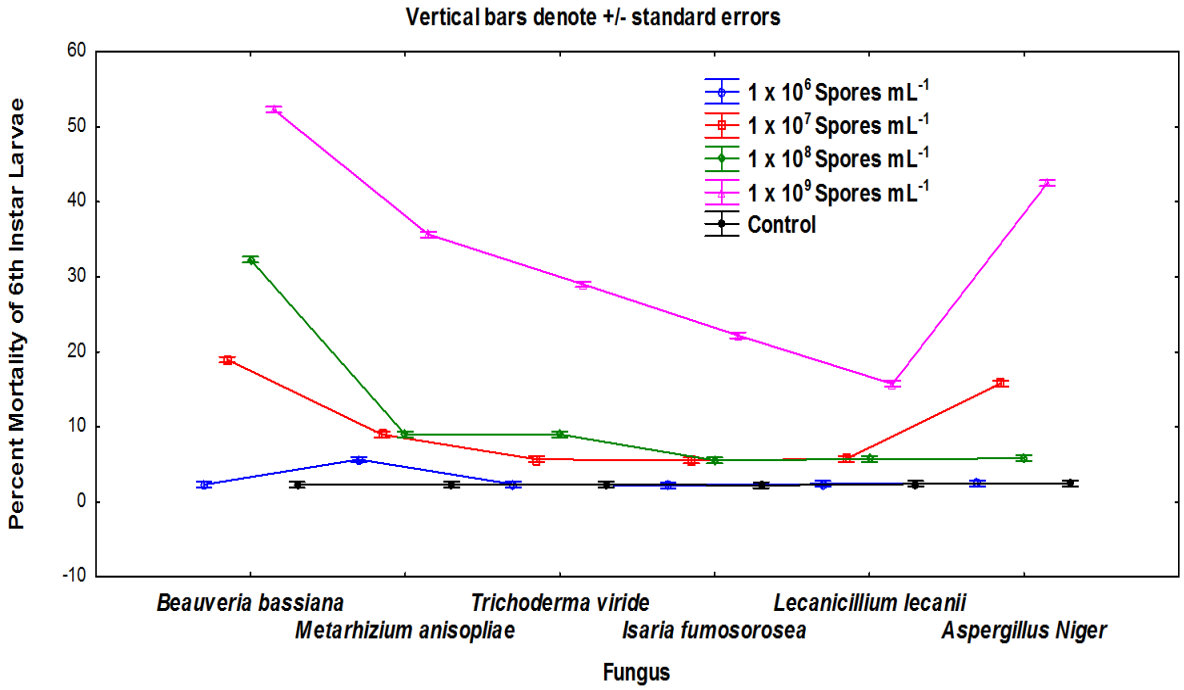

Figure 7. Impact of entomopathogenic fungi (EPF) on mortality of 6th instar larvae of fall armyworm evaluated with different concentrations at 10 days post-treatment.

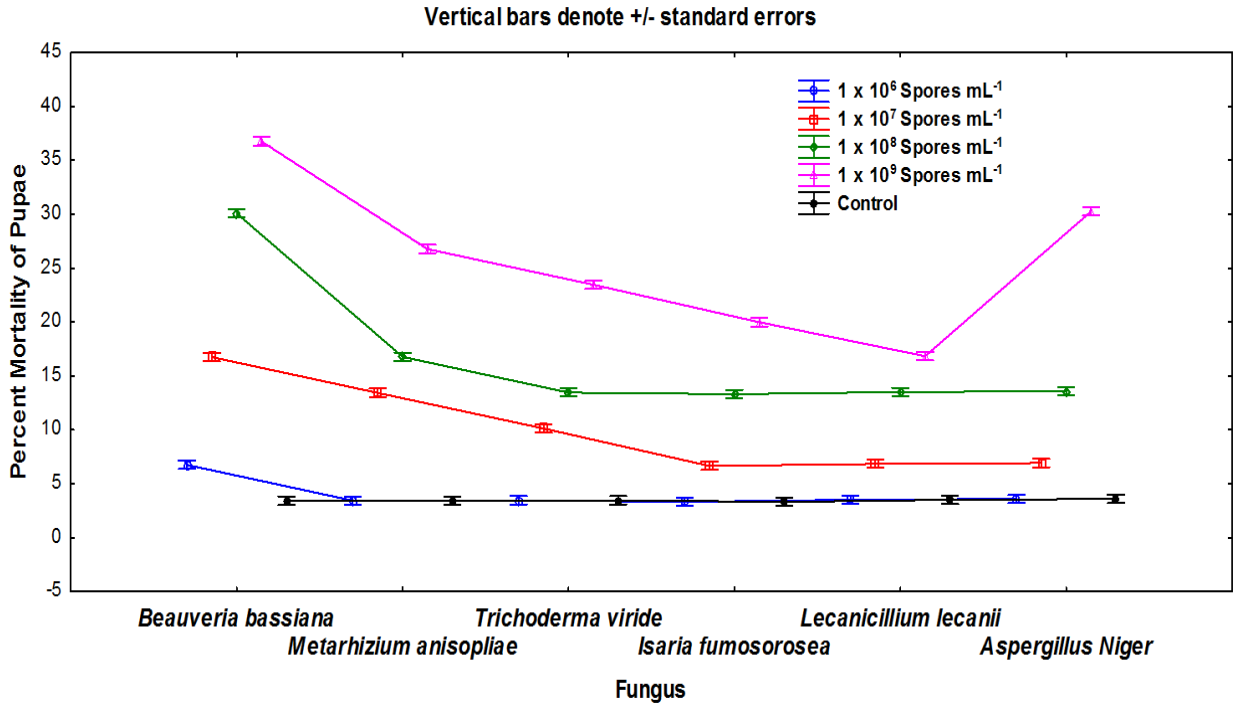

Figure 8. Impact of entomopathogenic fungi (EPF) on mortality of pupae of fall armyworm evaluated with different concentrations at 10 days post-treatment.

Discussion

It's fascinating how the study delves into the diverse microbial pathogens associated with Fall Armyworm (FAW), encompassing fungi, bacteria, and viruses (Gardner et al., 1984). However, the research highlights that only a few pathogens are responsible for effectively infecting these pests (Gómez et al., 2013). Among them, the FAW nuclear polyhedrosis virus (NPV) stands out as a crucial pathogen, inducing substantial mortality in the pest.

The study undserscores the importance of entomopathogens capable of initiating infections in pests before they reach their destructive stage. This emphasis on controlling insect pests at any susceptible life stage could be a key strategy in effective pest management. It is worth noting that eggs require maximum nutrients for development and are highly susceptible to microbial infections. This sensitivity to pathogenic microorganisms has been observed in various studies. The current study's findings align with this pattern, highlighting that Fall Armyworm (FAW) eggs were the most vulnerable to the tested fungal isolates. This vulnerability of eggs to fungal isolates underscores their potential as a strategic target for effective pest management. Results align with previous studies. The effectiveness of isolates such as *B. bassiana* and *M. anisopliae* in inducing significant fall armyworm (FAW) egg mortality, as reported in studies by Akutse *et al.,* (2019) and Idrees *et al.,* (2021), adds weight to our findings. Moreover, the virulence of *B. bassiana* against *Helicoverpa armigera* egg mortality, as dyed by Bahar *et al.,* (2011), further supports the potential of these isolates in pest management strategies. This collective evidence underscores the promising role of these fungal isolates in combating pest populations. It's intriguing to see the parallels between your study and the findings of Idrees *et al.,* (2021), where isolates of *Aspergillus* sp. did show significant induction of Fall Armyworm (FAW) egg mortality. Interestingly, these observations are also supported by Anand and Tiwary (2009), who reported the highest egg mortality in *Spodoptera litura* when treated with isolates of *Aspergillus* sp. Such outcome variations highlight the complexity of interactions between different isolates and insect species, underscoring the importance of understanding specific host-pathogen dynamics for effective pest management strategies.

The finding of our research was also supported by past studies. The effectiveness of *B. bassiana* and *M. anisopliae* isolates in inducing significant Fall Armyworm (FAW) larval mortality, as observed by Akutse *et al.,* (2019), aligns with our study. Similarly, the lack of significant mortality against FAW larvae of FAW with isolates of *Trichoderma viride, Isaria fumosorosea,* and *Lecanicillium lecanii*, as reported by Idress et al. (2021), further supports our research outcomes. This collective body of evidence reinforces various isolates' specificity and differential impact on FAW at different life stages.

In our study, it's intriguing to note the specificity of the tested entomopathogenic fungi (EPF) against second instar larvae of Fall Armyworm (FAW). The ineffectiveness of isolates of *L. lecanii* in causing significant larval mortality in FAW aligns with findings from Idrees et al. (2021) ; Shahriari et al. (2021), where similar observations were made for both FAW and *Chilo suppressalis*. Additionally, the contrasting effectiveness of the isolate *B. bassiana*, found to be the most effective against second instar larvae of *S. litura* as well as effective in causing larval mortality in FAW, as reported by Herlinda et al. (2020), further emphasizes the species-specific nature of EPF interactions with different insect larvae. Interestingly, some fungal isolates are more effective against early instar larvae than mature ones. For instance, the *Cladosporium* sp. isolate was found to cause significant mortality in early instar larvae of *H. armigera* but was less virulent towards mature larvae (Bahar et al., 2011). This highlights the importance of considering the developmental stage of the target insect when assessing the efficacy of fungal isolates. This variation in virulence across different life stages is consistent with broader observations in the

Cladosporium genus, where fewer species were effective against aphids and whiteflies (Gui et al., 2005). Therefore, adopting a tailored and stage-specific approach is crucial when considering fungal isolates for pest management strategies.

The understanding from previous research and our study found a common observation that entomopathogenic fungi (EPF) do not infect all stages of lepidopteron pests uniformly. The tendency for EPF to be more effective against the earliest stages of pests compared to later stages suggests a potential development of resistance in later mature stages of the pest, as highlighted by Idrees et al. (2022b) our study's observation that fungal isolates did not cause significant pupal mortality in Fall Armyworm (FAW) aligns with previous research, such as (Anand et al., 2009 ; Asi et al., 2013), where similar results were reported for pupal mortality of *S. litura* within a 10-day post-treatment period. This consistency across studies emphasizes the need for a comprehensive understanding of the specific interactions between EPF and different developmental stages of pests for effective pest management strategies.

Conclusion

The entomopathogenic fungi (EPF) *Beauveria bassiana*, *Metarhizium anisopliae*, *Trichoderma viride*, *Isaria fumosorosea*, *Lecanicillium lecanii*, and *Aspergillus Niger* have been evaluated and found to have the potential to infect immature stages of Fall Armyworm (FAW). These fungi are promising candidates for developing microbial pesticides and can be integrated into pest management strategies due to their efficacy. These findings highlight the importance of these fungi in pest control. To improve our knowledge and make the most of their potential, further research could focus on identifying the main toxins that affect the physiological functions of FAW and hinder their feeding performance. This information would help refine and target the use of these EPF, leading to more effective and sustainable methods for controlling FAW populations.

Acknowledgements

It's great to receive recognition for the support provided by the faculty, especially the Department of Entomology. Collaborative efforts and institutional support are crucial for the success of research projects.

References

Ahmed, M., Hussain, S., Ahmad, M., Ahmad, M. Q., & Afzal, M. (2022). Efficacy of plant-derived chemicals against insect pests and diseases: A review. *Acta Ecologica Sinica, 42*(1), 61–71.

Ahmad, S., Shah, F., Khaliq, I., Hussain, S., Akram, H. M., & Mahmood, A. (2020). Status and future challenges of maize production in Pakistan: A review. *Soil & Environment, 39*(2), 167–174.

Akutse, K. S., Fiaboe, K. K. M., Van den Berg, J., & Ekesi, S. (2019). Fungal entomopathogens isolated from invasive fall armyworm in Africa and their virulence against lepidopteran pests. *PLoS One, 14*(9), e0222332.

Akutse, K. S., Kimathi, E. K., Fiaboe, K. K. M., Agbodzavu, K. M., & Subramanian, S. (2020). Review of fall armyworm (*Spodoptera frugiperda*), biology, ecology, and management strategies in Africa. *International Journal of Tropical Insect Science, 40*(1), 5–19.

Anand, T., & Tiwary, M. (2009). Evaluation of entomopathogenic fungi against *Spodoptera litura* Fab. in vitro. *Journal of Biopesticides, 2*(1), 103–106.

Asi, M. R., Bashir, M. H., & Hussain, D. (2013). Comparative efficacy of different isolates of *Metarhizium anisopliae* against *Spodoptera litura* (Fabricius) (*Lepidoptera*: Noctuidae). *International Journal of Agronomy and Plant Production, 4*(4), 838–842.

Bahar, M. H., Pathan, A. K., & Zafar, Y. (2011). Identification and virulence of entomopathogenic fungi against *Helicoverpa armigera* (*Lepidoptera*: Noctuidae). *Pakistan Journal of Zoology, 43*(3), 533–541.

Day, R., Abrahams, P., Bateman, M., Beale, T., Clottey, V., Cock, M., Colmenarez, Y., Corniani, N., Early, R., Godwin, J., Gomez, J., Jadhav, D., Redak, R., Salum, K., Sergine, S., & Witt, A. (2017). Fall armyworm: Impacts and implications for Africa. *Outlooks on Pest Management, 28*(5), 196–201.

Erasmus, A. (2019). Why the fall armyworm has scientists worried. *The Conversation.* http://theconversation.com/why-the-fall-armyworm-has-scientists-worried-117573

Gardner, W. A., Bostian, E. L., & Travis, J. W. (1984). Role of microorganisms in the ecology of the southern armyworm, *Spodoptera eridania*. *Environmental Entomology, 13*(3), 655–660.

Gilal, M. A., Khuhro, R. D., Soomro, A. N., Mahar, A. R., Rajper, A. A., Kalar, Z. A., & Wassan, M. (2020). Management strategies for the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) infesting maize crop: A review. *Sarhad Journal of Agriculture, 36*(2), 683–692.

Goergen, G., Kumar, P. L., Sankung, S. B., Togola, A., & Tamo, M. (2016). First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J E Smith) (*Lepidoptera*, Noctuidae), a new alien invasive pest in West and Central Africa. *PLoS One, 11*(10), e0165632.

Gutiérrez-Moreno, R., Mota-Sanchez, D., Blanco, C. A., Whalon, M. E., Terán-Santofimio, H., Rodríguez-Maciel, J. C., & López-Arroyo, J. I. (2019). First report of field-evolved resistance to *Bacillus thuringiensis* toxin Cry1F in fall armyworm. *Scientific Reports, 9*(1), 1–10.

Herlinda, S., Ningsih, L. D. R., & Tarigan, M. (2020). Biological activity of *Beauveria bassiana* and *Metarhizium anisopliae* against *Spodoptera litura* Fabricius (*Lepidoptera*: Noctuidae) larvae. *Journal of Entomology and Zoology Studies, 8*(4), 77–81.

Idrees, A., Waheed, A., Aziz, A., Bibi, S., Ali, S., & Hussain, A. (2022). Impact of biopesticides on soil-dwelling insect pests: A review. *Journal of Pest Science, 95*(1), 129–143.

IPPC. (2018). International Plant Protection Convention: A pest that threatens food security: Fall Armyworm in Asia. Food and Agriculture Organization of the United Nations. http://www.fao.org/fall-armyworm/en/

Jeyanthi, R., & Kombairaju, S. (2005). Studies on chemical control of *Spodoptera frugiperda* on maize. *Journal of Entomological Research, 29*(4), 345–348.

Kalleshwaraswamy, C. M., Asokan, R., Swamy, H. M. M., Maruthi, M. S., Pavithra, H. B., Rangaswamy, K. T., & Mahadeva Swamy, H. M. (2018). First report of occurrence of fall armyworm, *Spodoptera frugiperda* (J E Smith) (*Lepidoptera*: Noctuidae), an alien invasive pest on maize in India. *Pest Management in Horticultural Ecosystems, 24*(2), 15–21.

Liu, Y., Xue, M., Zhang, J., Dong, L., & Jiang, Y. (2022). Botanical pesticides: Natural substances against insect pests and diseases. *Journal of Economic Entomology, 115*(1), 76–84.

Naeem-Ullah, U., Tofique, M., Iqbal, N., Aslam, M., Zubair, M., & Ayyub, M. A. (2019). First report of fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (*Lepidoptera*: Noctuidae), on maize in Pakistan. *Pakistan Journal of Agricultural Sciences, 56*(1), 223–227.

Opisa, S., Du Plessis, H., Akutse, K. S., Fiaboe, K. K. M., & Ekesi, S. (2018). Effects of entomopathogenic fungi and *Bacillus thuringiensis*-based biopesticides on *Spoladea recurvalis* (*Lepidoptera*: Crambidae). *Journal of Applied Entomology, 142*, 617–626.

Qadir, M. I., Ahmad, J., Khan, M. M., Munir, I., Azam, S., & Ahmad, M. (2021). Potential of botanical extracts in management of stored grain insect pests: A review. *The Journal of Animal & Plant Sciences, 31*(4), 1256–1264.

Quimby, P. C., McManus, M. L., & Swearingin, S. P. (2002). Biological control in IPM systems: Past, present, and future. *Integrated Pest Management Reviews, 7*(1), 3–28.

Ríos-Velasco, C., Rodríguez-Pérez, M. A., Valdez-Carrasco, J., & Ortega-Arenas, L. D. (2010). Entomopathogenic fungi as potential control agents of the corn earworm, *Helicoverpa zea* (Boddie) (*Lepidoptera*: Noctuidae). *Journal of Insect Science, 10*(1), 1–10.

Shahriari, M., Hosseini, M. S., Aghdam, A. R., & Seiedy, M. (2021). Evaluation of virulence of five species of entomopathogenic fungi against fall armyworm, *Spodoptera frugiperda* (*Lepidoptera*: Noctuidae), and beet armyworm, *Spodoptera exigua* (*Lepidoptera*: Noctuidae). *Applied Entomology and Phytopathology, 89*(2), 191–207.

Valicente, F. H., & Cruz, I. (1991). Insecticides resistance in fall armyworm, *Spodoptera frugiperda* (Smith) (*Lepidoptera*: Noctuidae) populations from Central America. *Anais da Sociedade Entomológica do Brasil, 20*(2), 165–174.

West, B. C., & Gwinn, K. D. (1993). Effectiveness of nuclear polyhedrosis virus, Bactospeine, and Bt against the fall armyworm (*Lepidoptera*: Noctuidae) in sweet corn. *Journal of Entomological Science, 28*(3), 342–349.