VIRULENCE OF ENTOMOPATHOGENIC FUNGI ON IMMATURE STAGE (3RD INSTAR) OF FALL ARMYWORM *SPODOPTERA FRUGIPERDA* (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

The fall armyworm, *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae), is a notorious polyphagous pest. Within a short period, this pest can cause significant damage to maize. Farmers are using widely synthetic pesticides to control fall armyworm. Further, the resistance development in insects and the non-target effect of chemicals on the environment and humans pose serious threats to using insecticides. The use of entomopathogenic fungi (EPF) is considered an important tool in integrated pest management programs. The main objective of this study was to check the impact of different conidial concentrations of fungus on mortality of *S. frugiperda*. From lower to higher concentrations after 2-8 days mortality rate due to *Isaria fumosorosea*, *Aspergillus* sp. *Metarhizium anisopliae,* and *Beauveria bassiana* ranges from 0.16%-53.50%, 0.23%-66.90%, 3.59%-73.59% and 6.88%-80.21%, respectively at lowest to highest concentration 1×10^4 to 1×10^8. After 2-8 days LC_{50} values of *M. anisopliae* range from 1.12 x 10^{11}-1.03 x 10^6 (spores/mL) *B. bassiana* ranges from 1.68 x 10^9-8.03 x 10^6(spores/mL) *Aspergillus* sp ranges from 6.8 x 10^9-1.8 x 10^7(spores/mL) *I. fumosorosea* ranges from 1.19 x 10^9-8.1 x 10^7 (spores/mL)**.** The tested fungal isolates could play an essential role as microbial biopesticides in suppressing the *S. frugiperda* population after further investigations on their efficacy are obtained in the field.

Keywords: *Spodoptera frugiperda*, *Beauveria bassiana*, *Metarhizium anisopliae*, *Isaria fumosorosea, Aspergillus*, virulence, mortality

INTRODUCTION

The fall armyworm (FAW), *Spodoptera frugiperda*, causing significant yield losses in maize, is the most significant pest in the world (Chisonga *et al*., 2023; Nget *et al*., 2024). As a pest that devours its prey, FAW was first identified in 1797 as being endemic to subtropical and tropical areas of America (Yan *et al*., 2022; Tay *et al*., 2023; Nget *et al*., 2024). It was first observed on the African continent and is a member of the Noctuidae family within the Lepidoptera order (Goergen *et al*. 2016; Keegan *et al*., 2021; Ajene *et al*., 2024). This pest's high level of adaptability, migratory habits, and rapid reproduction rate enable it to spread to more than 70 different nations worldwide (CABI, 2022; Mishra *et al*., 2023; Demis and Jemal, 2024). Over 350 different plant species, including important economic and staple crops like sorghum, corn, barley, rice, soybean, tobacco, tomato, and peanut, could be harmed by the larvae of *S. frugiperda* (Montezano *et al*., 2018; Chen *et al*., 2022; Tao *et al*., 2024).

The maize production has been scared by fall armyworm (FAW), *S. frugiperda*, which is highly ranked as the most devastating pests of the maize crop. Entomopathogenic fungi (EPFs) use enzymes to damage the cuticles of insects and gain entry into insects' bodies (Rajula *et al*. 2020; Demis and Jemal, 2024; Ma *et al*., 2024). There are two genetic strains of FAW: the "rice strain," which favors rice and other grass species, and the "maize strain," which primarily feeds on maize and occasionally on sorghum (Akeme *et al*., 2021; Demis and Jemal, 2024). Due to its biology, ability for airborne migration, and high fecundity rate, its population is expanding quickly (Prasanna *et al*. 2018; Jiang *et al*., 2022; Dessie *et al*., 2024). This invasive pest can potentially cause damage to tobacco fields if its population reaches its peak (Xu *et al*. 2019; Dessie *et al*., 2024; Sisay *et al*., 2024). All over the world, the pest

has caused an annual economic loss of between 22 and 67 percent (Baudron *et al*., 2019; Ullah *et al*., 2023; Dessie *et al*., 2024).

Although the use of insecticide is a quick method to manage insect pests in the field (Hajjar *et al*., 2023; Abd-El-Azim *et al*., 2024; Barathi *et al*., 2024). *S. frugiperda* is very difficult to control with insecticide because of the way it feeds, as the inner leaves of the plants contain larval excrement that shields them from toxins (Paredes-Sánchez *et al*. 2021; Guo *et al*., 2022; Zhang *et al*., 2024). To effectively combat this pest, it is therefore necessary to reduce the use of insecticides and discover some sustainable IPM techniques. According to research by Idrees *et al*., (2021) and Abd-El-Azim *et al*. (2024) the fall armyworm is most vulnerable to entomopathogens like bacteria, viruses, nematodes, and fungi, and the use of entomopathogens as a management strategy during severe outbreaks has been demonstrated to be effective (Smagghe *et al*., 2023; Abd-El-Azim *et al*., 2024; Wang *et al*., 2024). Fungal biocontrol agents have a distinctive mode of infection; unlike bacteria and viruses, they can directly infect their host through the cuticle.

According to a laboratory investigation by Zhang *et al*. (2023), *M. anisopliae* and *B. bassiana* were efficient inhibitors of early instar FAW. Both *Metarhizium* and *Beauveria* spp. isolates were tested against FAW (Apirajkamol *et al*., 2023; Abd-El-Azim *et al*., 2024; Wang *et al*., 2024). More research on removing fungi from the host niche aids in the creation of a sustainable, safe, and environmentally beneficial bio-insecticide for the control of FAW. Thus, the study's goals were to separate locally accessible fungi from the soil of a maize-growing region and evaluate, in a lab setting, how well they inhibited *S. frugiperda* larvae.

MATERIALS AND METHODS

Spodoptera frugiperda rearing

Larvae and egg batches of *S. frugiperda* were collected from maize field (32° 07′ 53.8″ N 72° 41′ 34.4″ E). Then collected population of *S. frugiperda* larvae was brought to the laboratory for rearing. Larvae of *S. frugiperda* were kept individually in small plastic Petri plates (90×15 mm). Fresh leaves of maize were provided to the larvae daily. The diet was replaced daily, and the feces were removed from the Petri plates daily. After the pupation, the pupae were placed in small plastic cages for adult emergence. The male and female adults were introduced in jars for mating and egg-laying. Honey solution (10%) as soaked cotton plugs was provided to adults. The stripes of muslin clothes were hung in jars for egg-laying. The culture was maintained under controlled conditions of 26 ± 2 °C and 65 ± 5% R.H. The culture was maintained up to F_3 generations to be used in further experiments.

Entomopathogenic fungus

The EPF strains were tested against 3rd instar larvae of *S. frugiperda*. Laboratory culture of EPF was grown on Potato Dextrose Agar (PDA) at 25 ± 2 °C, 70% RH, and 12:12 h (L:D) photoperiod. The conidia quality was determined using a Neubauer chamber hemocytometer. The germination was determined on PDA plates (based on the counts of 200 random conidia/plate), 18 h post-incubation at 25 ± 2 °C and in bioassay, suspension of 90% conidial germination was used. The conidial suspension was adjusted to different concentrations of 1×10^5, 1×10^6, 1×10^7, 1×10^8, and 1×10^9 spores/mL in distilled water with 0.05% Tween 80. Sterile distilled water with Tween 80 was used as a positive control.

Statistical analysis

Larval mortality data were corrected using Abbott's formula. Natural mortality and its normality have been previously tested using the Shapiro-Wilk test. A one-way analysis of variance (ANOVA) is performed. If there is data Since these are not normally distributed, they were further arcsine transformed before analysis. Treatments were significantly different ($p<0.05$) and mean values were separated using Tukey HSD test. All data analyses including reduced food intake in 3-instar *S. frugiperda* larvae were run with SPSS (version 16.0) and characters were computed with Statistix. 8.1 Software.

RESULTS

Effect of fungal isolates on third instar of *S. frugiperda* larvae

After 2 days of post-treatment application, at a concentration of 1×10^8 (Spores/mL) *B. Bassiana, Metarhizium anisopliae, Aspergillus* sp,, *Isaria fumosorosea* caused significantly ($F=14738$; $df=17$; $p<0.0033$) ($F=619$; $df=17$; $p<0.0033$) ($F=769$; $df=17$; $p<0.0033$) ($F=587$; $df=17$; $p<0.0033$) mortality rates 20.21%, 17.26%, 16.90% and 10.16%, respectively (Table 1). While the lowest mortality of 3.59%, 6.88%, 0.23%, and 0.16% at concentration

1×10^4 respectively. However at concentrations 1×10^5 to 1×10^7 mortality ranges (from 6.92% - 13.59%) (10.21% - 16.88%) (3.56% – 10.23%) and (0.16% - 3.50%), respectively (Table 1).

The result revealed that after 4 days of application at a concentration of 1×10^8(Spores/mL) *B. Bassiana, Metarhizium anisopliae, Aspergillus* sp., *Isaria fumosorosea* occurs specific (F=8110; df = 17; p<0.0033) (F=1561; df = 17; p<0.0033) (F=1585; df = 17; p<0.0033) (F=1760; df = 17; p<0.0033) induced egg mortality rates 36.88%, 30.26%, 26.90%, and 30.26% individually. At low concentrations, 1×10^4 mortality rates vary 13.55%, 6.92%, 3.55%, and 3.50% singly. But from concentration 1×10^5 to 1×10^7 mortality ranks (16.88% - 30.21%) (10.26% - 23.59%) (10.23% – 20.26%) and (6.83% - 16.88%), respectively (Table 1).

The result showed that after 6 days of application at a concentration of 1×10^8(Spores/mL) *B. Bassiana, Metarhizium anisopliae, Aspergillus* sp, *Isaria fumosorosea* caused specific (F=3030; df = 17; p<0.0033) (F=3906; df = 17; p<0.0033) (F=2422; df = 17; p<0.0033) (F=4120; df = 17; p<0.0033) induced egg mortality rates 60.21%, 50.26%, 33.59%, and 20.26% individually. At low concentrations, 1×10^4 mortality rates vary 20.21%, 13.59%, 6.90%, and 3.59% singly. But from concentration 1×10^5 to 1×10^7 mortality ranks (26.88% - 43.55%) (20.26% - 33.59%) (16.98% – 26.90%) and (13.50% - 23.59%), respectively (Table 1).

After 8 days of application at a concentration of 1×10^8 (Spores/mL) *B. Bassiana, Metarhizium anisopliae, Aspergillus* sp, *Isaria fumosorosea* caused specific (F=821; df = 17; p<0.0033) (F=8311; df = 17; p<0.0033) (F=9259; df = 17; p<0.0033) (F=11787; df = 17; p<0.0033) were pathogenic to the larvae of *S. frugiperda* but overall mortality ranged 80.21%, 73.59%, 66.90%, and 53.50% individually. At low concentrations, 1×10^4 mortality rates vary from 33.55%, 20.26%, 13.59%, and 10.16% singly. But from concentration 1×10^5 to 1×10^7 mortality ranks (40.21% - 63.55%) (33.59% - 53.59%) (26.88% – 43.55%) and (23.50% – 36.83%), respectively (Table 1).

Table 1. Effects of fungal species on percent mortality of the 3rd instar *S. frugiperda* larvae at five different concentrations for different days.

EPF	Days	Mortality ± S.E.					
		1×10^4	1×10^5	1×10^6	1×10^7	1×10^8	Control
Metarhizium anisopliae	2 days	3.59 ± 0.18^{E}	6.92 ± 0.27^{D}	10.26 ± 0.33^{C}	13.59 ± 0.37^{B}	17.26 ± 0.40^{A}	3.59 ± 0.18^{E}
	4 days	6.92 ± 0.27^{E}	10.26 ± 0.33^{D}	16.92 ± 0.39^{C}	23.59 ± 0.42^{B}	30.26 ± 0.45^{A}	3.59 ± 0.18^{F}
	6 days	13.59 ± 0.37^{E}	20.26 ± 0.40^{D}	23.59 ± 0.42^{C}	33.59 ± 0.45^{B}	50.26 ± 0.48^{A}	3.59 ± 0.18^{F}
	8 days	20.26 ± 0.40^{E}	33.59 ± 0.45^{D}	36.92 ± 0.46^{C}	53.59 ± 0.50^{B}	73.59 ± 0.58^{A}	6.92 ± 0.27^{F}
Beauveria bassiana	2 days	6.88 ± 0.27^{D}	10.21 ± 0.33^{C}	10.21 ± 0.33^{C}	16.88 ± 0.38^{B}	20.21 ± 0.40^{A}	3.55 ± 0.17^{E}
	4 days	13.55 ± 0.18^{E}	16.88 ± 0.39^{D}	20.21 ± 0.40^{C}	30.21 ± 0.45^{B}	36.88 ± 0.46^{A}	3.55 ± 0.17^{F}
	6 days	20.21 ± 0.40^{E}	26.88 ± 0.42^{D}	33.55 ± 0.45^{C}	43.55 ± 0.47^{B}	60.21 ± 0.53^{A}	3.55 ± 0.17^{F}
	8 days	33.55 ± 0.45^{E}	40.21 ± 0.46^{D}	46.88 ± 0.47^{C}	63.55 ± 0.55^{B}	80.21 ± 0.63^{A}	3.55 ± 0.17^{E}
Aspergillus sp.	2 days	0.23 ± 0.90^{E}	3.56 ± 0.18^{D}	6.90 ± 0.27^{C}	10.23 ± 0.33^{B}	16.90 ± 0.38^{A}	0.23 ± 0.90^{E}
	4 days	3.55 ± 0.17^{E}	10.23 ± 0.33^{D}	13.55 ± 0.18^{C}	20.26 ± 0.40^{B}	26.90 ± 0.42^{A}	3.55 ± 0.17^{E}
	6 days	6.90 ± 0.27^{E}	16.98 ± 0.38^{D}	20.23 ± 0.40^{C}	26.90 ± 0.42^{B}	33.59 ± 0.45^{A}	3.56 ± 0.17^{F}
	8 days	13.59 ± 0.37^{E}	26.88 ± 0.42^{D}	30.26 ± 0.45^{C}	43.55 ± 0.47^{B}	66.90 ± 0.55^{A}	3.56 ± 0.17^{F}
Isaria fumosorosea	2 days	0.16 ± 0.85^{C}	0.16 ± 0.85^{C}	0.16 ± 0.85^{C}	3.50 ± 0.18^{B}	10.16 ± 0.33^{A}	0.16 ± 0.85^{C}
	4 days	3.50 ± 0.18^{E}	6.83 ± 0.27^{D}	10.26 ± 0.33^{C}	16.88 ± 0.38^{B}	20.26 ± 0.40^{A}	3.59 ± 0.18^{E}
	6 days	3.59 ± 0.18^{E}	13.50 ± 0.37^{D}	16.92 ± 0.39^{C}	23.59 ± 0.42^{B}	30.26 ± 0.45^{A}	3.59 ± 0.18^{E}
	8 days	10.16 ± 0.33^{E}	23.50 ± 0.42^{D}	26.83 ± 0.43^{C}	36.83 ± 0.46^{B}	53.50 ± 0.50^{A}	3.59 ± 0.18^{F}

Mean ± SE values within a column not sharing a common letter are significantly different at $p < 0.05$ using Tukey's test.

Toxicity of the tested EPF strains

Data presented in (Tables 2-5) showed the effectiveness of entomopathogenic fungus on the 3rd instar larvae of *S. frugiperda*. After 2 days of post-treatment, *M. anisopliae* least effective entomopathogenic fungus than *B. Bassiana, Aspergillus* sp., and *Isaria fumosorosea* where LC_{50} values were 1.12 x 10^{11} (spores/mL), 1.68 x 10^9 (spores/mL), 6.8 x 10^9 (spores/mL) and 1.19 x 10^9(spores/mL) respectively. The obtained data of biological aspects after 4 days on the effects of *M. anisopliae, B. bassiana, Aspergillus* sp., and *Isaria fumosorosea* at their LC_{50} values on the 3rd instar larvae showed 2.34 x 10^9 (spores/mL), 2.2 x 10^9 (spores/mL), 2.3 x 10^9 (spores/mL) and 5.1 x 10^9 (spores/mL). The value of LC_{50} of *M. anisopliae, B. bassiana, Aspergillus* sp and *Isaria fumosorosea* against the 3rd instar after 6 days is followed as 3.11 x 10^7(spores/mL), 1.73 x 10^8(spores/mL), 1.73 x 10^9(spores/mL) and 1.40 x

10^9(spores/mL) which is more toxic than day 2 and day 4, LC_{50} values. The obtained data after 8 days on the effects of, *M. anisopliae, B. bassiana, Aspergillus* sp., and *Isaria fumosorosea* at their LC_{50} values on the 3rd instar larvae showed 1.03 x 10^6 (spores/mL), 8.03 x 10^6 (spores/mL), 1.8 x 10^7 (spores/mL) and 8.1 x 10^7(spores/mL) (Tables 2-5).

Table 2. LC_{50} values (spores/mL) of *Metarhizium anisopliae* against *S. frugiperda* after different exposure intervals.

Days	LC_{50} (spores/mL)	FD Limit	Slope ± S.E.	χ^2	D.F.	P
Day 2	1.12 x 10^{11}	2.83 x 10^9 - 1.83 x 10^{16}	0.178 ± 0.05	1.08	3	0.781
Day 4	2.34 x 10^9	2.72 x 10^8 -2.80 x 10^{11}	0.15 ± 0.03	0.517	3	0.915
Day 6	3.11 x 10^7	1.19 x 10^7 -1.23 x 10^8	0.17 ± 0.03	0.568	3	0.904
Day 8	1.03 x 10^6	4.16 x 10^5 2.29 x 10^6	0.16 ± 0.02	1.79	3	0.617

Table 3. LC_{50} values (spores/mL) of *Beauveria bassiana* against *S. frugiperda* after different exposure intervals.

Days	LC_{50} (spores/mL)	FD Limit	Slope ± S.E.	χ^2	D.F.	P
Day 2	1.19 x 10^9	1.0 x 10^8 - 8.3 x 10^{12}	0.29 ± 0.06	2.29	3	0.514
Day 4	2.2 x 10^9	3.72 x 10^8 - 8.4 x 10^{10}	0.22 ± 0.04	1.53	3	0.676
Day 6	1.73 x 10^8	1.18 x 10^7 - 5.23 x 10^8	0.19 ± 0.03	0.689	3	0.876
Day 8	8.03 x 10^6	4.09 x 10^6 - 1.70 x 10^7	0.21 ± 0.02	2.48	3	0.477

Table 4. LC_{50} values (spores/mL) of *EPF (Aspergillus* sp.*)* against *B. dorsalis* after different exposure intervals.

Days	LC_{50} (spores/mL)	FD Limit	Slope ± S.E.	χ^2	D.F.	P
Day 2	6.8 x 10^9	1.0 x 10^8 - 8.3 x 10^{12}	0.29 ± 0.06	2.29	3	0.514
Day 4	2.3 x 10^9	4.2 x 10^8 - 7.3 x 10^{10}	0.26 ± 0.04	4.41	3	0.22
Day 6	1.73 x 10^9	2.80 x 10^8 -7.23 x 10^{10}	0.19 ± 0.03	4.17	3	0.243
Day 8	1.8 x 10^7	9.05 x 10^6 4.30 x 10^7	0.22 ± 0.02	3.01	3	0.389

Table 5. LC_{50} values (spores/mL) of *(Isaria fumosorosea)* against *B. dorsalis* after different exposure intervals.

Days	LC_{50} (spores/mL)	FD Limit	Slope ± S.E.	χ^2	D.F.	P
Day 2	1.68 x 10^9	3.3 x 10^8 - 1.4 x 10^{11}	0.73 ± 0.22	0.976	3	0.807
Day 4	5.1 x 10^9	7.14 x 10^8 - 4.29 x 10^{11}	0.28 ± 0.06	3.48	3	0.323
Day 6	1.40 x 10^9	2.90 x 10^8 - 2.73 x 10^{10}	0.23 ± 0.04	6.84	3	0.077
Day 8	8.1 x 10^7	3.15 x 10^7 - 3.30 x 10^8	0.20 ± 0.02	3.28	3	0.349

DISCUSSION

Beauveria bassiana (the entomopathogenic Hyphomycetous fungi) are naturally soil dwellers (Altaf *et al*., 2023; Samal *et al*., 2024). Hence, they proved themselves successful biocontrol agents against serious pests without threat to beneficial insects (Bamisile *et al*., 2021; Samal *et al*., 2024). *B. bassiana* and *Isaria fumosorosea* are successful biological agents in the control of multiple species pests (Robles-Acosta *et al*., 2019). In a previous study, *B. bassiana* and *B. bassiana* showed high efficacy against the fall webworm larvae (Bai *et al*., 2015; Hu *et al*., 2021; Samal *et al*., 2024). The use of EPF plays an important role in IPM strategies due to the great effect on soil-dwelling pests life stages and the significant decrease in the reproductive ability of insect pests (Bamisile *et al*., 2021; Deka *et al*., 2021; Idrees *et al*., 2021; Fakeer *et al*., 2024; Samal *et al*., 2024).

Several microbial pathogens cause disease in FAW, including fungi, bacteria, and viruses (Gardner *et al*., 1984), but only some pathogens between them cause infection of pests (Polanczyk *et al*., 2000; Garcia *et al*., 2008; Negrisoli *et al*., 2010; Behle and Popham, 2012; Salvadori *et al*., 2012; Gómez *et al*., 2013).

The use of entomopathogens as biocontrol agents is a promising and alternative approach in the integrated management of different insect pests (Deka *et al.,* 2021). The impact of various concentrations of *M. Anisopliae, B. Bassiana, Aspergillus* sp, and *Isaria fumosorosea* was studied on S. frugiperda. During the penetration, these entomopathogenic fungi types secrete the proteins such as trypsins, subtilisins, and chymotrypsins that digest the protein-rich procuticle of arthropods (Wang *et al*., 2008). Due to the penetration of EPF, the utilization of food biomass of FAW larvae was decreased in terms of ECI and RCR. Obtained values are supported by previous studies that reported a reduction in ECI and RCR values of treated insects than in untreated with the application of entomopathogens (Moorthi *et al*., 2015). Due to indiscriminate food utilization or not storing food in the intestine of larvae to get energy and nitrogen the AD values were also decreased (Hussain *et al*., 2009). Moorthi *et al*. (2015) reported a reduction in feeding limit including RCR, RGR, ECI, and AD of *S. litura* (Fabricius) after the application of EPF and the results are the same as this. Overall, the reduction in food consumption of insects is due to toxic substances secreted by EPF inside the host's body that eventually damage the structural integrity (Tefera and Pringle, 2003). All the feeding indices parameters were decreased by increasing the concentration of EPF. Thus the effect of EPF is directly dependent on the conidial concentration (El Husseini, 2019). Using higher concentrations is not a problem as the EPFs are not toxic to humans and the environment (Yasin *et al*., 2019). To handle the larval population of *S. Frugiperda* higher concentration of EPF can be successful.

As (Romero-Arenas *et al*., 2014) reported, 72.5% mortality of *S. frugiperda* larvae (3rd instar) with the application of *M. anisopliae*, was at the dose rate of 5.3×10^5 conidia/ml. (Mahmood *et al*., 2019) also reported considerable mortality of *Sitobion avenae* (Fab.) (Hemiptera: Aphididae) with *B. bassiana* application. Due to exposure to a pathogen, the immunity of insects influences that led to a reduction in survival (Jensen *et al*., 2019). The effect of EPFs on the successive generation of *S. frugiperda* would be more helpful for designing an IPM technique for this pest (Altaf *et al*., 2023).

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