







Research Article

Occurrence and Pathogenicity of Indigenous Entomopathogenic Fungi Isolates to Fall Armyworm (*Spodoptera frugiperda* J. E. Smith) in Western Amhara, Ethiopia

Mequanint Andualem Mekonnen ¹, Gebremariam Asaye Emirie ¹,
Solomon Yilma Mitiku ², Bogale Nigir Hailemariam ³,
Melkamu Birhanie Mekonnen ¹ and Anteneh Ademe Mengistu ¹

¹Adet Agricultural Research Center, P.O. Box 08, Bahir Dar, Ethiopia

²Ambo Agricultural Research Center, P.O. Box 37, Ambo, Ethiopia

³Bioversity International: Alliance of Bioversity International and International Center for Tropical Agriculture, Addis Ababa, Ethiopia

Correspondence should be addressed to Mequanint Andualem Mekonnen; mequanint09@gmail.com

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The fall armyworm (FAW) (*Spodoptera frugiperda* J. E. Smith) is widely recognized as an invasive lepidopteran pest in Africa. Adoption of synthetic pesticides remains an option for emergency control of the FAW, but their large-scale use by small-scale farmers is costly and environmentally unsafe. Management options based on entomopathogenic fungi could minimize risks for health and the environment. In our study, the occurrence of entomopathogenic fungi in different habitats along with their pathogenicity to fall armyworm was examined. A total of 56 soil samples were collected from three locations. A standard isolation method, baited with the wax moth larvae (*Galleria mellonella*), was used for the isolation of entomopathogenic fungi from soil. Twelve entomopathogenic fungi were isolated from the total soil samples collected. It was observed that *Metarhizium* spp. was more frequent and widespread than *Beauveria* spp. and their occurrence was high in the forest habitat. Pathogenicity of indigenous entomopathogenic fungi isolates on fall armyworm larvae was tested by applying conidial suspension containing 1×10^8 spores/ml. All of the isolates tested were pathogenic to larvae of fall armyworm, with mortalities varying from 30 to 80% at 10 days posttreatment. Our results suggest that native entomopathogenic fungi could be integrated for the management of *Spodoptera frugiperda* larvae.

1. Introduction

The fall armyworm (*Spodoptera frugiperda* (J. E. Smith) *Lepidoptera: Noctuidae*) is a native agricultural pest in the Americas' tropical and subtropical regions [1]. In Africa, the pest was first detected in late 2016 [2] due to unsafe quarantine practice [3]. Since then, the fall armyworm (FAW) has spread to nearly every country in sub-Saharan Africa [4]. Its superior biological characteristics, such as the absence of diapause, short generation time, high fecundity, high polyphagy, and long-distance migration ability [5], allow FAW to spread easily across a country. Following the

arrival and rapid spread of FAW in Africa, several reports on its impact, particularly crop losses, have been published. Based on the survey study, the highest maize yield loss (77%) has been reported from Zambia [6], followed by 47% and 32% in Kenya and Ethiopia, respectively [7]. According to Baudron et al. [8], the direct measurement of the maize yield loss caused by FAW in Zimbabwe during 2018 was 11.6%. Annual maize yield losses of 8.3 to 20.6 million tons were estimated in 12 African maize-producing countries [3, 9].

FAW is capable of causing damage to maize plants at all developmental stages, from seedling emergence to maturity, and defoliates the entire plant, resulting in significant yield

loss if not controlled. This pest attacks the host plant's aboveground foliage, including the leaves, stem, and reproductive parts [10]. Plants infested with fall armyworm at an early stage suffered severe defoliation; while at later stages of development, the cob was exposed to mycotoxin-producing microorganisms in addition to direct feeding of kernels by FAW. The highest infestation associated with favorable agroecological conditions for FAW proliferation poses a threat to African food security [9]. Also, the arrival of FAW might lead to the displacement of stem borers from maize, its preferred host to sorghum.

Because of the sudden arrival and rapid spread of FAW, as well as the pest's ability to cause high potential losses, African governments decide to apply insecticides as soon as the first symptoms are observed. However, large-scale application by small-holder farmers is neither feasible nor cost-effective. Correspondingly, the continuous use of chemical products has resulted in the development of insecticide resistance, the extinction of natural enemies, and environmental contamination [11, 12]. Hence, there is a need to develop cost-effective, sustainable, and environmentally safe FAW management options. One of the safe alternatives to control *S. frugiperda* is developing and using pesticides based on microbial agents. These microbes are insect pathogens, which are target-specific, and have the potential to reduce the use of hazardous chemical pesticides [13]. Among insect pathogenic microbes, entomopathogenic fungi have been considered as a potential biocontrol agent against several insect pest species [14–17], due to their epizootics and pathogenicity [18]. Fungal biocontrol agents are the most important ones due to their broad host range, pathogenicity route, and ease of delivery [19, 20]. Hence, isolation and identification of indigenous fungal entomopathogens from the soil environment with the prospect of getting isolates with insecticidal properties is one of the research priorities in the pest management. The objectives of this study were therefore to search for locally present entomopathogenic fungi and determine their pathogenicity level to fall armyworm.

2. Materials and Methods

2.1. Soil Sampling Procedure and Survey Area. Fifty-six soil samples were collected at different habitats in the South Gondar, Awi, and West Gojjam administrative zones of the Amhara regional state from October to December 2019 (Figure 1). Geographically, the South Gondar zone is located in between 11°02'–12°33' latitude and 37°25'–38°43' longitude. The zone has a bimodal rainfall pattern varies from 900 mm to 1599 mm. The average temperature in the zone is 17°C [21]. The Awi zone is located between 10°30'0.00" to 12°00'0.00" latitude and 36°00'0.00" to 37°00'0.00" longitude, with elevations ranging from 700 to 2900 m.a.s.l. It has a better annual rainfall distribution (800 to 2700 mm/year) [22]. The annual minimum and maximum temperatures range from 5°C to 27°C [23]. West Gojjam is located between 36°30' and 37°5' longitudes and 10°16' and 11°54' latitudes, with elevations ranging from 1500 to 3500 m.a.s.l. The zone has ambient temperatures ranging from 15 to 27°C [24].

Soil samples were collected using a shovel by digging to a depth of 10–15 cm after removing surface debris. There were 26 samples from agricultural habitats (maize fields), and 30 samples were from forest habitats (17 samples from natural forests and 13 from plantation forests). One kg of composite soil samples was taken at every sampling site and put in clear polyethylene plastic bag (30 by 40 cm) sealed with a rubber band. Each sample was provided with a unique reference code to identify the sampling site. Data on the sampling site using global positioning system (GPS) equipment, location, place, habitat type, and date were recorded. The collected soil samples were transported to the insect biocontrol laboratory at the Ambo Agricultural Research Center for further processing.

2.2. Isolation of Entomopathogenic Fungi. The standard isolation method of *Galleria* bait method was used as selective medium for isolation of entomopathogenic fungi from soil. Because of their susceptibility to such fungi, larvae of the wax moth, *Galleria mellonella*, were used as a bait insect. *Galleria mellonella* were reared following the method of Meyling [25]. *G. mellonella* adults were obtained from the Ambo Agricultural Research Center in Ethiopia. Adult moths (one female for every one male) were kept in a flask with honey and water. In addition, a folded tissue paper was placed for oviposition. The paper was removed with the eggs attached and placed in a new plastic jar with the feed ingredient. The feed composition was 180 g of honey, 180 g of glycerine, and 50 g of wheat bran. The feed and egg-attached tissue papers were transferred in a bigger jar for rearing inside an incubator at 20°C. The feed was changed periodically based on the larval growth stage of *G. mellonella*.

Larvae of medium sized approximately, 2.5–3 cm in length (4 weeks after hatching), were used for baiting soil samples [25]. To prevent webbing, larvae were immersed in boiled water (at 56°C) for 10 seconds before being transferred for cooling in a running bath water for 30 seconds. Plastic pots with the capacity of 500 ml were filled by collected composite soil samples. Five (5) to ten (10) larvae were released in each plastic pot. The samples were incubated at 20–22°C in the dark by inverting the individual containers every day to ensure the larvae moved through the soil regularly. After the incubation period, the soil was examined for dead larvae. When found, larval cadavers were immediately removed and surface-sterilized with 1% sodium hypochlorite for 3 minutes, washed three times with sterile distilled water, placed on sterile filter paper, and then sealed in Petri dish. Finally, Petri dishes containing the cadavers and sealed with parafilm were incubated at 25°C for 12 days [26]. The Petri dishes were inspected daily for the presence of fungal mycelium, which was isolated and identified based on morphological characteristics. Identification was done based on the macroscopic and microscopic characters of fungi. Colony characteristics, such as the color on the upper and lower surfaces of the Petri dishes, are among the macroscopic traits observed, while the microscopic characteristics are based on the size, shape, and arrangement of the hyphae, conidiophores, and conidia [27–29].

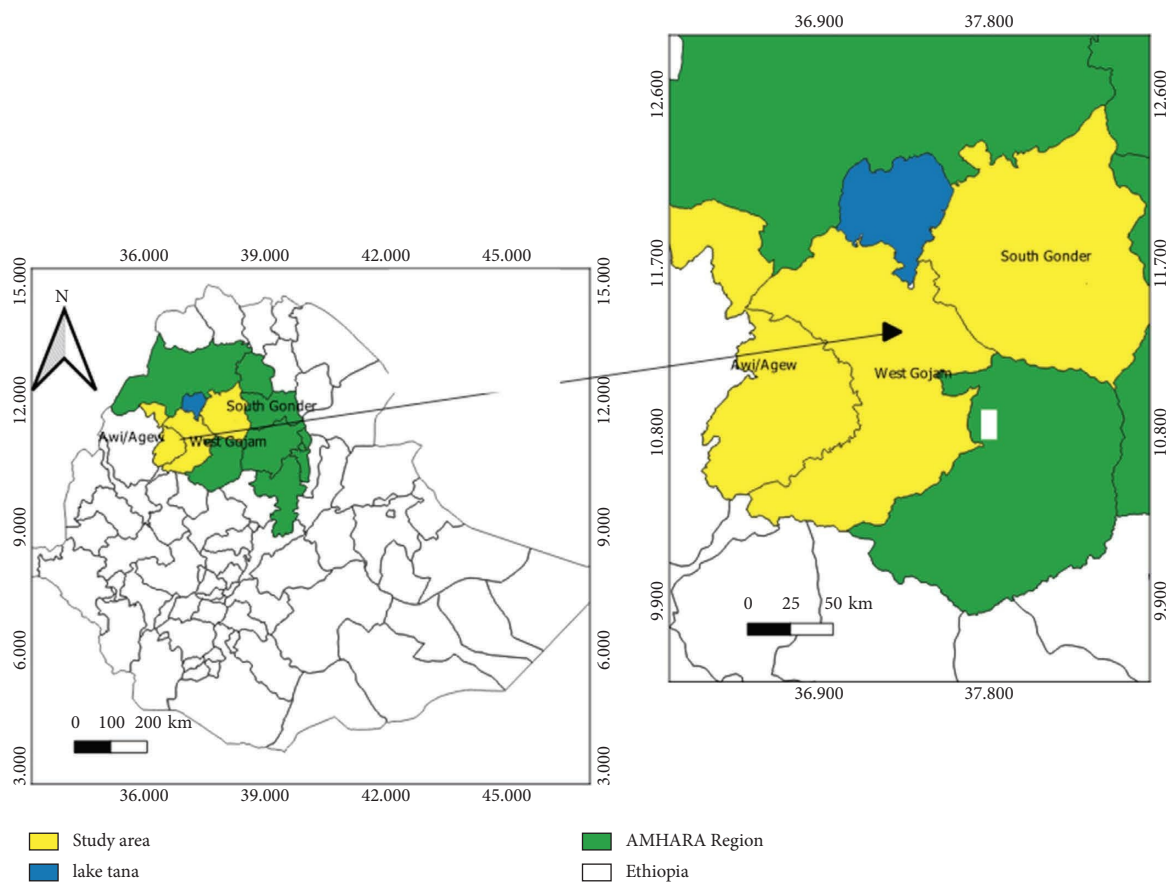


FIGURE 1: Survey area details.

2.3. Rearing of Fall Armyworm (FAW). Insect rearing was conducted as per Tefera et al. [10], with some modifications. An initial colony of FAW was collected from farmers' maize fields. The larvae were placed in a ventilated rectangular plastic cage and fed on fresh maize leaves harvested from maize plants grown under a screen house. The prepupal stage was transferred to a plastic cage filled with soil to a depth of 10 to 15 cm to facilitate pupation. All the emerging adult moths were then transferred to insect rearing netted cages of a size 85 cm height, 50 cm length, and 50 cm width, each containing 30 days old maize seedling grown on the pot to facilitate their mating and egg-laying potential. Sterile cotton soaked in a sugar solution was placed in a Petri dish inside the rearing cage as a food source for the emerging adult moths. Eggs were laid on maize leaves which serve as an immediate food source for the hatched larvae. The greenish young larvae on the maize leaves were collected and placed in a ventilated rectangular plastic cage, provided with tender and fresh maize leaves [30]. The resulting second- to third-instar first-generation (F1) larvae were used for the bioassay.

2.4. Pathogenicity Test. Insecticidal activities of one *Beauveria* (S11) and fourteen *Metarhizium* (S5, S14, S20, S24, S26, S32, S33, S36, S46, S50, S54, S73, S75, and S80) isolates were preliminarily bioassayed at the Adet Agricultural Research

Center, entomology laboratory, from mid-July to August 2020. Twelve of the fifteen isolates were isolated from our soil sample, and three *Metarhizium* isolates were accessed from the Ambo Agricultural Research Center. The bioassay method, as followed by Ramanujam et al. [31], was used. All treatments including untreated control were replicated four times. A total of 20 larvae were used for each treatment. The treatments were arranged in a completely randomized design (CRD). For the assay, isolates showing clearly abundant sporulation on agar plates were used. Fungal spores obtained on solid medium were harvested by a sterile spatula and suspended in sterile distilled water with Tween 80 at 0.02%. The second-instar first-generation (F1) *S. frugiperda* larvae were gently removed from the colony and immersed in a spore suspension of 1×10^8 spores/ml. The number of conidia was determined by using a hemocytometer and a compound microscope following the serial dilution method. Untreated control insects were immersed in sterile distilled water containing 0.02% Tween 80. All the treated and untreated larvae were then placed on 150 mm diameter sterilized filter paper to trap water condensation. Following this, each of the treated larvae was placed in a single Petri dish containing fresh maize leaves. The treated and control larva were provided with fresh maize leaves daily after frass and leaf debris were removed. Larvae mortality was recorded every 48 h for 10 days, and the percent mortality of larvae was computed.

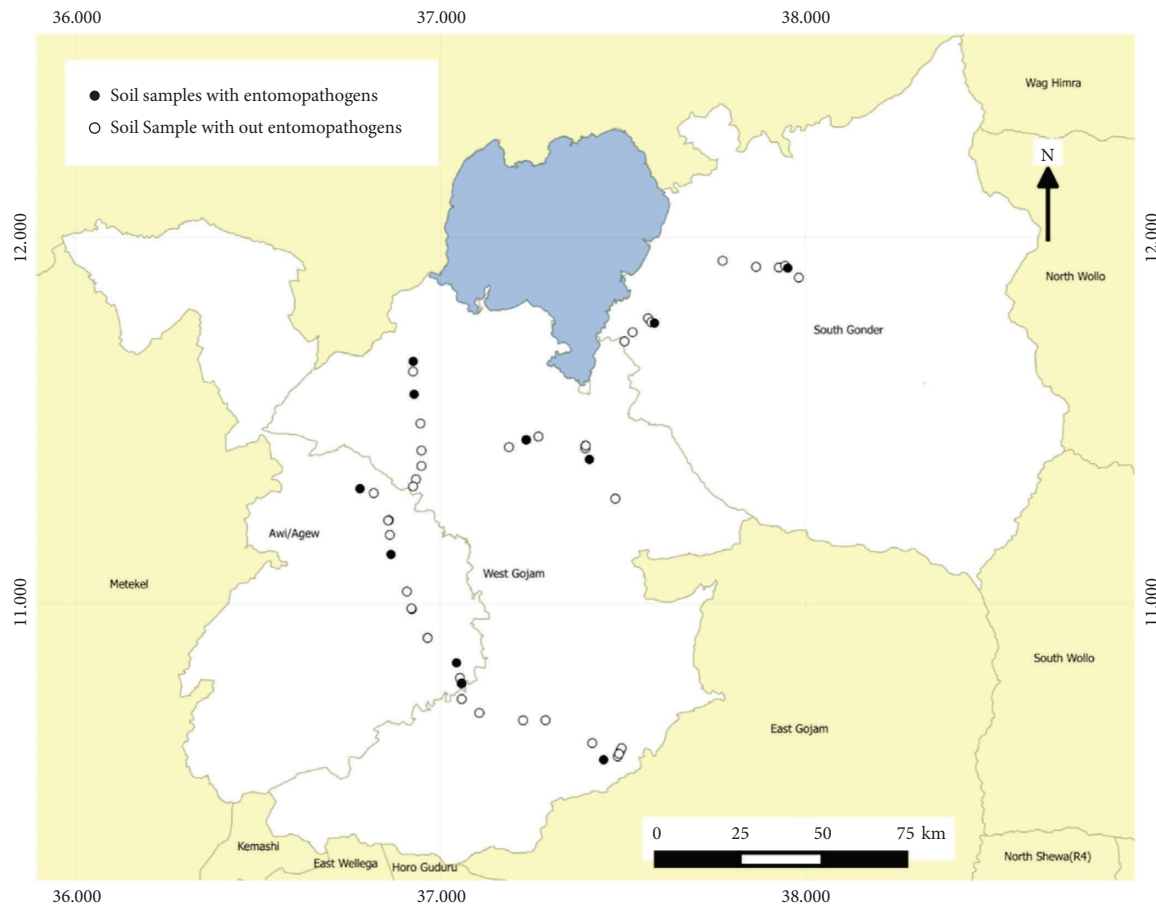


FIGURE 2: Occurrence of entomopathogenic fungi across different administrative zones.

2.5. Statistical Analysis. The effect of geographical location and habitat type on the occurrence of entomopathogenic fungi obtained from waxworm larvae baited in soil samples was examined using descriptive analysis. ANOVA was performed on the percentage mortality data using SAS 9.4 statistical software. The mean values were separated by the LSD test.

3. Results and Discussion

3.1. Occurrence and Distributions of Entomopathogenic Fungi (EPF) in Different Habitats and Altitudes. Fungal entomopathogens are frequently found in soil as saprophytes because it is protected from UV radiation and other harmful abiotic and biotic factors [32]. This result confirmed the presence of EPF (*Metarhizium* spp. and *Beauveria* spp.) in soils sampled from Gojjam and South of Gonder. Twelve soil samples (21.4%) out of the 56 that were collected were positive for entomopathogenic fungi (Figure 2). The number of entomopathogenic fungi that we detected there was lower than usual compared to other studies conducted in Ethiopia. Ayele et al. [33] detected a total of 48% EPF isolates from the collected soil samples using *Galleria* baiting, while Gebremariam et al. [34] found 54.2% EPF from the total samples collected.

Eleven (19.6%) of the isolates were identified as *Metarhizium* spp., but only 1 (1.7%) isolate was identified as *Beauveria* spp (Table 1). These fungal species are prevalent in Ethiopia. Gebremariam et al. [34] reported the presence of *Metarhizium* spp. and *Beauveria* spp. in Shoa. Ayele et al. [33] detected *Metarhizium* spp. in soil samples collected in Ethiopia's central rift valley, whereas Belay et al. [35] reported *Beauveria* spp. in the Jima zone coffee growing area. The occurrence and distributions of entomopathogens are influenced by environmental factors such as geographic location, climatic conditions, habitat type, cropping system, and soil properties [36–38].

The entomopathogenic fungi *Metarhizium* spp. was widespread in the sampled areas, but their frequency of occurrence and distribution varied by habitat. More *Metarhizium* spp. isolates (26.6%) were retrieved in forest soil than agricultural soil (Table 1). The EPF isolate of *Beauveria* spp. was obtained in habitats with cypress plantation forest. Similarly, Quesada-Moraga et al. [37] and Wakil et al. [39] reported a high occurrence of entomopathogenic fungi in forest habitats. This could be due to the high organic matter content of forest soil, which increases the diversity and abundance of potential hosts available to EPF [40, 41]. The occurrence of entomopathogenic fungi was strongly related to soils with high organic matter content [37, 42].

TABLE 1: Occurrence of entomopathogenic fungi by habitat.

Habitat type	Total no. of soil samples	<i>Metarhizium</i> spp.		<i>Beauveria</i> spp.	
		No. of soil samples with fungi	% of soil samples with fungi.	No. of soil samples with fungi.	% of soil samples with fungi.
Agricultural soil	26	3	11.5	0	0
Forest soil	30	8	26.6	1	3.3

TABLE 2: Occurrence of entomopathogenic fungi at different altitude ranges.

Altitude	Total no. of soil samples	<i>Metarhizium</i> spp.		<i>Beauveria</i> spp.	
		No. of soil samples with fungi	% of soil samples with fungi	No. of soil samples with fungi	% of soil samples with fungi
<2300	42	8	19	0.0	0.0
>2300	14	3	21.4	1	7.1

TABLE 3: Pathogenicity of *Metarhizium* spp. and *Beauveria* spp. isolates against FAW.

Treatments		% mortality (% SE) 6 DPI	% mortality (% SE) 8 DPI	% mortality (% SE) 10 DPI
<i>Beauveria</i> spp.	S11	45 (± 12.6) ^{ab}	65 (± 15.0) ^{ab}	80.0 (± 14.1) ^a
	S5	30 (± 12.9) ^{abcd}	45 (± 9.6) ^{abcd}	60.0 (± 8.2) ^{abc}
	S14	15 (± 5.0) ^{cde}	35 (± 15.0) ^{bcde}	45.0 (± 9.6) ^{abc}
	S20	15 (± 5.0) ^{cde}	20 (± 8.2) ^{edf}	60.0 (± 8.2) ^{abc}
	S24	15 (± 5.0) ^{cde}	30 (± 5.8) ^{cdef}	35.0 (± 5.0) ^{bcd}
	S26	20 (± 8.2) ^{bcde}	35 (± 9.6) ^{bcde}	35.0 (± 9.6) ^{bcd}
	S32	10 (± 10.0) ^{ed}	25 (± 12.6) ^{edf}	55.0 (± 17.1) ^{abc}
	S33	10 (± 10.0) ^{ed}	40 (± 18.3) ^{abcde}	60.0 (± 23.1) ^{abc}
<i>Metarhizium</i> spp.	S36	0 (± 0.0) ^e	10 (± 5.8) ^{fe}	30.0 (± 12.9) ^{cd}
	S46	25 (± 15.0) ^{bcde}	40 (± 16.3) ^{abcde}	45.0 (± 17.1) ^{abc}
	S50	0 (± 0.0) ^e	10 (± 10.0) ^{fe}	40.0 (± 24.5) ^{bcd}
	S54	55 (± 9.6) ^a	70 (± 10.0) ^a	80.0 (± 8.2) ^a
	S73	40 (± 8.2) ^{abc}	50 (± 5.8) ^{abcd}	70.0 (± 12.9) ^{ab}
	S75	30 (± 17.3) ^{abcd}	60 (± 11.5) ^{abc}	80.0 (± 8.2) ^a
	S80	15 (± 9.6) ^{dce}	60 (± 8.2) ^{abc}	70.0 (± 10.0) ^{ab}
	Cont.	0 (± 0.0) ^e	0 (± 0.0) ^f	5.0 (± 5.0) ^d

SE = standard error; DPI = date postinoculation; means within a column followed by different letters are significantly different at $P < 0.05$ (LSD test).

Concerning altitude, soil samples were collected at elevations ranging from 1800 to 2600 meters. The EPF was present at almost all altitudes (Table 2). According to Quesada-Moraga et al. [37], entomopathogenic fungi were found at a wide range of altitudes.

3.2. Pathogenicity to FAW. In the bioassays, all of the tested entomopathogenic fungi isolates were found to be pathogenic to *S. frugiperda* larvae, but there were significant differences in larval mortality among the entomopathogenic fungi isolates. Out of 15 EPF isolates, S54 caused greater mortality (55%) at 6 days postinoculation (DPI), but it did not differ significantly from three *Metarhizium* spp. isolates (S5, S73, and S75) and the one *Beauveria* spp. isolate (S11) (Table 3). Larval mortality in the rest of the EPF isolates ranged from 10% to 25% at 6 DPI. However, no larval mortality was observed in isolates of S50 and S36-treated larvae at 6 DPI. Larval mortality was doubled in most EPF isolates at 8 DPI, with 10% mortality recorded in isolates S50 and S36, which had no larval mortality at 6 DPI (Table 3). At 8 DPI, the mortality of larvae caused by seven isolates (S54,

S75, S80, S11, S73, S5, and S33) was significantly higher than the rest of the EPF isolates. Among the EPF isolates tested against *S. frugiperda*, isolates S11, S54, and S75 caused the greatest mortality (80%) at 10 DPI (Table 3). S73 and S80 were the second-most active isolates against *S. frugiperda*, with similar 70% mortality. EPF isolates S5, S20, and S33 all caused similar 60% larvae mortality at 10 DPI (Table 3). The rest of isolates caused less than 45% larvae mortality. Mortality in the control was 5% at 10 DPI, but no larvae mortality was recorded at 6 and 8 DPI.

In general, all of the tested native isolates of fungal entomopathogens were pathogenic to *S. frugiperda* larvae (Table 3). This finding confirms the previously reported susceptibility of FAW to entomopathogenic fungi [43–45]. However, there was overall significant variability in the pathogenic activity of the entomopathogenic fungi isolates obtained from the different habitats. The result of the study showed that native isolates of *Metarhizium* spp. and *Beauveria* spp. were effective against FAW larvae with 30–80% mortality at 10 days postinoculation. Similarly, Ramanujam et al. [31] reported 10–67% larvae mortality with *B. bassiana* and *M. anisopliae* strains. An extensive

review conducted by Khan et al. [46] revealed that a number of different genes were involved in the pathogenicity caused by entomopathogenic fungi. Some of these genes are involved in the degradation of the cuticle, while others are involved in adhesion, which mediates spore attachment and fungal differentiation. According to Fang et al. [47], entomopathogenic fungal enzymes play an important role in the virulence of insect pests, which varies from strain to strain. The mortality of larvae increases as the number of days after inoculation increases in all EPF isolates.

4. Conclusion

The present study indicated the presence of indigenous entomopathogenic fungi (*Metarhizium* spp. and *Beauveria* spp.) and their pathogenicity against FAW under controlled conditions. It was observed that *Metarhizium* spp. were more frequent widespread than *Beauveria* spp. The occurrence of EPF was also more prevalent in forest habitats than in agricultural soils. EPF isolates S11, S54, and S75 cause 80% larval mortality. Therefore, based on the bioassay results, EPF isolates causing high mortality of larvae were considered as potential biological control agents of *S. frugiperda* larvae. This is an essential first step in the development of a management strategy which integrates entomopathogenic fungi. However, their increased utilization will require improvements in formulation that enable ease of application, increased environmental persistence, and longer shelf life. Future perspectives of this work will also include further morphological and molecular identification of the effective EPF strains.

Data Availability

The datasets that support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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