Hindawi
Psyche: A Journal of Entomology
Volume 2022, Article ID 3879183, 9 pages
https://doi.org/10.1155/2022/3879183

Research Article

Endophytic Potential and Larvicidal Efficacy of Entomopathogenic Fungi against the Spotted Stem Borer, *Chilo partellus*

Denberu Kebede,[1,2] **Tesfaye Alemu**,[3] **and Tadele Tefera**[1]

[1]*International Center of Insect Physiology and Ecology (ICIPE), Addis Ababa, P.O. Box 5689, Ethiopia*
[2]*Ethiopian Institute of Agricultural Research, Ambo Agricultural Research Center, P.O. Box 37, Ambo, Ethiopia*
[3]*Department of Microbial, Cellular and Molecular Biology, College of Natural & Computational Sciences, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia*

Correspondence should be addressed to Denberu Kebede; kdenberu55@gmail.com

Received 6 July 2022; Revised 28 November 2022; Accepted 15 December 2022; Published 21 December 2022

Academic Editor: Luciano Toma

Entomopathogenic fungi (EPF) of different genera are known to have the potential to engage in fungus-plant interactions as fungal endophytes. This hidden endophytic interaction offers several advantages to host plants, such as insect pest management. Hence, this study aimed to explore the endophytic potential and virulence of EPF collections after artificial inoculation. A total of 27 EPF isolates from the genera *Beauveria* and *Metarhizium* were screened for virulence. Two inoculation methods (leaf and seed dressing) were used to study the endophytic colonisation potential of the selected isolates. There was a significant variation among the tested isolates in their ability to kill *C. partellus* larvae. Lower mean percentage mortality was recorded for isolates B4, DS-51-21, and B1,9 which scored 28.01%, 32.29%, and 34.58%, respectively. All the screened EPF isolates were able to colonise maize tissues after artificial inoculation, except for APPRC-34GM. The percent colonisation of maize tissues varied with strains, and delivery methods ranged from a minimum of 0% to a maximum of 53%, where the maximum was recorded by S#10H. Larval mortality after feeding maize leaves inoculated with EPF ranged from 18% to 60%. The findings of this study indicated that *Beauveria* spp. and *Metarhizium* spp. have the potential to colonise maize after artificially inoculating and translocating from the site of infection. Hence, the potential to move from the site of infection and larvicidal activity after colonisation may give the advantage to manage insect pests acting on the different parts of maize.

1. Introduction

Insect pests play a significant role in reducing crop production. Among cereals, maize is highly damaged by stem borer species because it contains high concentrations of amino acids and sugars and emits more volatile compounds than other gramineous hosts [1]. Maize is produced by lepidopteran stem borers that feed on plant stems, causing approximately 80% yield loss in Africa [2]. Among lepidopteran stem borers, the spotted stem borer, Chilo partellus (swinhoe), causes up to 45% of maize yield loss in East Africa [1]. The larval stage of C. partellus is the damaging stage that can attack the entire plant, except for the root. In Ethiopia, the pest is highly abundant in semiarid ecozones between 1,200 and 1,985 m above sea level and causes significant loss of yield in maize and sorghum [3, 4]. Various management approaches, including cultural, chemical, and biological approaches, have been used to reduce the damaging effect of *C. partellus*. Moreover, pesticides are still frequently used to kill insect pests, especially to eradicate natural enemies.

Among biological control agents, entomopathogenic fungi (EPF), particularly those of the genera Beauveria and Metarhizium, have been extensively reported to infect a wide range of arthropods. These fungi are predominantly found in soil in different habitats, including oak forests, agricultural soils, pine reforestations, and chaparrals [5]. These two

groups are the most dominant entomopathogenic fungi found in the soil and have the potential to coexist in the same environment without harming each other [6]. However, recent research suggests that EPF under different genera have the potential to engage in fungus-plant interactions as fungal endophytes and are naturally isolated from different plant species, such as coffee [7], maize [8], sugarcane [8], and tomato [9]. Wanger and Lewis [10] showed through germ tube development that conidia of *B. bassiana* enter maize tissue directly through the plant cuticle, and hyphal growth occurs within the apoplast to later extend to the xylem elements. Entomopathogenic endophytes have also been found to colonise both the above and below-ground tissues of their host plant [11].

The virulence determinant traits of EPF are affected by numerous factors, both under laboratory and field conditions. Infection percentage, radial growth, germination potential, and spore-bound Pr − 1 enzyme activity decline with repeated subculturing and nutritional composition [12]. The field efficacy of EPF is also critically affected by environmental conditions, such as unfavourable temperatures, reduced humidity, and high levels of UV radiation. The application of EPF as a plant endophyte has a prior advantage over conventional methods for managing insect pests because its life cycle involves piercing and feeding inside plant parts [13].

This hidden endophytic interaction offers several advantages to host plants. Endophytes have superior biocontrol performance over the symbionts commonly found in soil. *Botryosphaeria quercum* endophytically isolated from healthy pods of the cocoa tree (*Theobroma cacao L.*) showed higher biocontrol indices against phytopathogens than *Trichoderma viride* strains isolated from the rhizosphere soil of cocoa production farms [14]. In addition, endophytic microorganisms confer broad-spectrum host resistance to insect herbivores. Colonisation of plant roots by soil microbes provokes the expression of jasmonic acid and ethylene-dependent genes, which produce induced systemic resistance (ISR) in leaf-chewing insects [15]. N-formaylloline, epoxyjanthitrem, I-naphthalene, and nodulisporic acid A are well-characterizedanti-insect secondary metabolites produced by endophytic fungi [16].

The endophytic colonisation of plants by *Beauveria* and *Metarhizium* protects against insect pests. Entomopathogenic *B. bassiana* endophytically isolated from coffee causes 100% cumulative mortality in adult coffee berry borers [7]. Maize plants inoculated with *B. bassiana* showed an increase in yield and yield parameters such as height, number of leaves, grain weight, and percentage of seed germination, which indicated the additional role of EPF in plant growth [17]. The endophytic presence of *Beauveria* and *Metarhizium* was also investigated to induce upregulation of auxin- and gibberellin-related genes [18]. Application of entomopathogenic fungi was also reported for significant increment on shoot and root biomass of cardamon plant [19]. This supplementary role provided by EPF increases opportunities for multiple uses in integrated pest management strategies. The use of endophytic EPF for insect pest control was found to be compatible with other pest management strategies such as biological agents and chemical insecticides [7]. The field efficacy of entomopathogens is getting restricted due to several factors, including vulnerability to UV light, low moisture, delivery methods, and reaching the target. Therefore, the use of entomopathogens as fungal endophytes may also provide a comparative advantage over the limitations of other delivery methods, such as spraying.

Table 1: List of entomopathogenic fungi (EPF) isolates used for the experiments.

No	Isolate code	Genus	Host/habitat
1	S#34	*Beauveria* spp	Soil
2	DS-51-1	*Metarhizium* spp	Soil
3	S#048BC	*Metarhizium* spp	Soil
4	DS-86-2	*Metarhizium* spp	Soil
5	DS-37-1	*Metarhizium* spp	Soil
6	S#44BC	*Beauveria* spp	Soil
7	S#10H	*Beauveria* spp	Soil
8	DS-51-2	*Beauveria* spp	Soil
9	S#05	*Beauveria* spp	Soil
10	S#53	*Beauveria* spp	Soil
11	APPRC-34GM	*Metarhizium* spp	Soil
12	DS-52-2	*Metarhizium* spp	Soil
13	AF2	*Beauveria* spp	Soil
14	GF4	*Beauveria* spp	Soil
15	BF4	*Beauveria* spp	Soil
16	B4	*Beauveria* spp	Soil
17	APPRC-44BC	*Beauveria* spp	Soil
18	DS-35-2	*Beauveria* spp	Soil
19	M1	*Beauveria* spp	Soil
20	APPRC-27	*Beauveria* spp	Soil
21	KF3	*Beauveria* spp.	Soil
22	B1	*Beauveria* spp	Soil
23	S#41	*Beauveria* spp	Soil
24	B7	*Beauveria* spp	Soil
25	S#45	*Beauveria* spp	Soil
26	APPRC-40GM	*Metarhizium* spp	Soil
27	M2	*Metarhizium* spp	Soil

Most studies on endophytes have been conducted to demonstrate the recovery potential after inoculation, and limited attention has been paid to the possible effects on insects. In addition to their use as biopesticides, there is growing evidence that many EPF species can colonise plant tissues through artificial inoculation or naturally occurring endophytes. There have been many successful attempts to artificially introduce EPF into plants using different techniques [9, 20]. The natural or artificial colonisation of endophytes could be beneficial for plants, as they have been reported to improve plant growth and reduce pest infestation in numerous economic crops [21]. This study was designed to determine the virulence of *Metarhizium* and *Beauveria* isolates against *C. partellus* larvae and assess the endophytic colonisation potential of these isolates after artificial inoculation of maize plants.

2. Materials and Methods

2.1. Screening of EPF Isolates against G. mellonella larvae

2.1.1. Rearing of G. mellonella. The rearing of *G. mellonella* was performed at the Biocontrol Laboratory of the Ambo Agricultural Research Center, Ethiopia. Adult moths were

maintained in 500 ml flasks containing folded tissue paper infused with water and honey solution to allow mating and lay eggs. Tissue papers with laid eggs were picked from the flask and inserted into plastic rearing boxes containing 80 g, 50 g, and 180 g of honey, wheat bran, and glycerol, respectively, as feed for the larvae. The boxes were incubated in the dark at 20°C for up to four weeks to obtain appropriate instars for screening entomopathogenic fungal isolates.

2.1.2. Source of EPF Isolates. The EPF isolates used for the experiment were obtained from the Ambo Agricultural Research Center Bio-control Laboratory and Addis Ababa University Mycology Laboratory, Ethiopia. A total of 27 entomopathogenic Beauveria spp. and Metarhizium spp. were subcultured on Sabouraud dextrose agar (SDA) media and incubated at 26°C for three to four weeks to allow sporulation. All EPF strains were isolated from soil samples collected from different parts of the country using the Galleria bait method (Table 1).

2.1.3. Strain Viability. All 27 isolates were subjected to a germination test to assess the viability of conidia, following the procedures described by Belay et al. [22]. Three to four weeks after inoculation on SDA, the conidia of each fungal isolate were obtained by scraping with a sterile metal spatula and suspended in a test tube containing 10 ml sterile water with Tween 80 (0.01% v/v) to make a stock suspension. Conidial concentration was adjusted to 3×10^6 conidia/ml with an improved Neubauer haemocytometer using a light microscope (Olympus-CH30RF200, Japan) (40x magnification power). Approximately 100 μl of the suspension was spread plated on SDA media in a 90 mm diameter Petri dish, and 1 ml of 70% alcohol was spread on each Petri dish after 24 h of incubation at 26°C to stop over germination. Each Petri plate was pseudotriplicated by placing three sterile coverslips on each Petri dish. The percentage of germination was determined by counting at least 300 conidia under a light microscope (40x magnification). A conidium was considered germinated if it showed germ tube growth as large as its size. Each isolate was replicated three times. The percentage of viability was calculated by dividing the number of germinated spores by the total number of spores examined, multiplied by 100.

2.2. Exposure of G. mellonella larvae to EPF. *G. mellonella larvae* were used to screen for EPF to select relatively virulent strains. Conidia of each Beauveria and Metarhizium strains were harvested from a 2-3-week-old culture plate, and the conidial concentration was adjusted to 1×10^8 conidia/ml. Ten fourth-instar larvae were immersed in a sterile beaker containing the spore suspension for 10–30 seconds and placed on sterile filter paper to prevent suffocation by water. The larvae were then transferred to 9 cm diameter sterile Petri dishes and placed at room temperature (average room temperature 25°C) for ten days. Mortality was recorded daily, and the dead insects were surface disinfected and placed on moist filter paper at room temperature to confirm fungal outgrowth from the cadaver. The control group was treated with sterile distilled water. Each treatment was replicated three times.

2.3. Screening of EPF against C. partellus

2.3.1. Rearing of C. partellus. Larvae of *C. partellus* were reared using its natural host, maize, in the lab at the Ambo Agricultural Research Center. Parasitoid and disease-freefield-collected larvae and pupae were used for rearing. The larvae were transferred to the maize plant, and fresh food was changed every three days until pupation. The pupae were kept in moist Petri dishes until adult emergence. Adult moths were maintained inside a rearing cage with 3-4-week-old maize plants grown in pots and allowed to lay eggs. Eggs were collected daily and kept in sterile Petri dishes. The newly hatched larvae were transferred to a plastic jar containing fresh maize leaves. To feed the larvae regularly, maize plants were grown in a plastic pot and kept in a glasshouse with a photoperiod of 12 h: 12 h light and dark. Second and third-instar larvae were used for the test.

2.4. Pathogenicity of Selected EPF against Larvae of C. partellus. Eight EPF isolates were used to evaluate their pathogenicity in the larvae of *C. partellus*. The isolates were selected based on their viability and virulence in *G. mellonella* larvae. The spore suspensions of each isolate and the desired conidial concentration (1×10^8 conidia/ml) were prepared as described above, and two ml of the suspension was sprayed on 10 s to late-second-instar larvae of C. partellus. The control groups were treated with 2 ml of sterile distilled water with a drop of Tween 80. Fresh three-week-old maize leaves were offered to the larvae after surface disinfection, as described above. Each treatment was replicated thrice and maintained at room temperature. Mortality was recorded daily for 11 days, and the dead larvae were examined for mycosis development to confirm fungal infection, as described above.

2.5. Dose-Response Study of EPF against Larvae of C. partellus

2.5.1. Conidial Suspension Preparation. Three-week-old conidia of three EPF isolates (two *Beauveria spp.* and one *Metarhizium spp.*) were used for multiple concentration assays to determine their lethal concentrations (LC_{50} and LC_{90}) and lethal times (LT_{50} and LT_{90}) to kill 50% and 90% of the insects tested, respectively. This was performed following the procedures described by Tefera and Pringle [23]. Stock conidial suspensions of sporulating fungal cultures were prepared by mixing the conidia with sterile distilled water and drops of 0.1% Tween 80 using a magnetic stirrer. The stock conidial concentration was determined using a haemocytometer, and conidial concentrations were adjusted to four concentration levels: 1×10^8, 1×10^7, 1×10^6, and 1×10^5 conidia/ml.

In each experiment, a batch of 10 C. partellus larvae was placed in a sterile Petri dish (9 cm diameter) and treated with 2 ml of each conidial suspension using a hand-held spray

atomiser. Preweighed pieces of surface-disinfected maize leaves, approximately 5 cm long and 2 cm wide, were offered to the larvae and allowed to feed for 24 h before replacing the leaves with a new one. The negative controls were treated with distilled water containing a drop of Tween 80. The experiment was maintained at room temperature for 11 days. Each treatment was replicated four times. The observations were made daily, and the dead larvae were examined for mycosis development to confirm fungal infection after surface disinfection and placed in moist filter paper.

2.6. Maize Colonization by EPF. Entomopathogenic fungal isolates used for dose-response studies were examined for tissue colonisation. The stock conidial suspension of each isolate was prepared in a test tube containing 10 ml of sterile distilled water containing Tween 80 (0.01% v/v). The suspension conidial concentration was adjusted to 1×10^8 conidia/ml after mixing using a vortex mixer.

2.6.1. Greenhouse Study. Seeds of the Jibat maize variety were used for the potential colonisation study. Sodium hypochlorite (0.5%) and ethanol (70%) were used for surface disinfection of the seeds. To avoid the residue of the disinfectants, the seeds were washed thrice with sterile distilled water. Success of surface sterilisation was confirmed by inoculating the final rinse aliquot on PDA and incubating for up to 3 days at 26°C. Seeds were separated into two groups for the seed and leaf inoculation experiments. The embryonic absence of entomopathogenic fungi from the seed was assessed by plating the crushed seeds on a PDA. Maize was grown in plastic pots (a size of 20 cm × 17 cm) filled with 2.5 kg of a sterile mixture of soil, sand, and compost (2 : 1 : 1).

2.7. Inoculation of EPF. Both leaf and seed inoculation methods were used to infect maize with EPF, as described by Tefera and Vidal [24].

2.7.1. Seed Inoculation. Surface-disinfected seeds were dressed in fungal conidial suspensions by immersing them in 50 ml of the suspension for 10 min. The seeds were planted in pots filled with sterile composite soil (four seeds per pot, of which two were thinned upon emergence), and the plants were maintained in a greenhouse with a 12 h photoperiod. The control seeds were immersed in sterile distilled water.

2.7.2. Leaf Inoculation. Seven days after emergence, seedlings with no seed inoculation were inoculated into the leaf by directly spraying each seedling with a 3 ml fungal conidial suspension (1×10^8 conidia/ml). The control plants were sprayed with sterile distilled water. Each treatment was replicated ten times, and the treatments were arranged in completely randomised designs (CRDs). The two experiments were conducted separately.

2.8. Assessment of EPF from Maize. Twenty days after inoculation, the seedlings were aseptically removed from the pots and assessed for the inoculated isolates. The seedlings were separated into leaf, stem, and root parts, and the surface was disinfected using sodium hypochlorite and ethanol as described above. Four pieces (5 mm × 2 mm) of each plant part per plant replicate were cut from the surface-sterilised sample using a sterile surgical blade and inoculated on SDA to assess the presence of the inoculated EPF isolates. A total of 120 sample pieces (40 pieces per plant part) of each treatment, including the control group, were analysed. The outgrowth of the inoculated strains was recorded after 10 days of incubation at 26°C for fungi. Percent of colonisation was calculated using the formula % of colonisation = number of samples showing isolate growth divided by the total number of analysed samples multiplied by 100 [11].

2.8.1. Endophyte-Insect Interaction. Selected isolates confirmed to colonise maize tissues were used for the endophyte insect interaction study. Insect mortality after feeding with inoculated leaves was conducted by providing the inoculated maize leaves and stems for larvae of C. partellus. In each experiment, a batch of 10°C partellus larvae (2nd instar) was placed in a sterile Petri dish (9 cm diameter) and replicated four times. Preweighed pieces of stem and leaves were offered to the larvae in a Petri dish and allowed to feed for one day before replacing the leaves with a new one. The controls were allowed to feed on maize leaves and stems from sterile distilled water-treated plants. The experiment was kept at room temperature (average 25°C) for 6 days, and each treatment was replicated four times. Mortality data were recorded daily, and the dead larvae were removed and placed on moist filter paper at room temperature to confirm a fungal infection. Mortality due to treatment was determined as described by Abbott [25].

2.9. Statistical Data Analysis. All mortality and germination data were subjected to an ANOVA using SAS software version 9 to determine significant differences among treatments. For viability and pathogenicity tests, the means were separated using Duncan's multiple range test and the least significant difference (LSD) test at $P = 0.05$. LT_{50} and LT_{90} were determined using probit analysis (IBM SPSS Statistics 20). Analysis of variance (ANOVA) was conducted, and the means were separated by LSD.

3. Results

3.1. Strain Viability and Screening on G. mellonella larvae. The viability of 27 EPF strains obtained from different sources was evaluated, and the percentage of conidial germination exceeded 80% (Table 2). The pathogenicity of all 27 EPF isolates was first screened against *G. mellonella larvae* using the galleria-dipping method (Table 2). The percent mortality varied among the tested isolates, causing mortality rates between 13.94% and 90.0%. Isolate S# 048BC was the least virulent isolate, causing 13.94% mortality. Most of the isolates were moderately virulent, inducing larval mortality greater

Table 2: Conidia viability and pathogenicity of entomopathogenic fungi (EPF) isolates against larvae of *G. mellonella*.

Isolate code	Genus	% mortality ± SE	Mean % germination ± SE
S#34	*Beauveria* spp.	80.91 ± 9.09ab	96.35 ± 0.87ab
DS-51-1	*Metarhizium* spp	35.80 ± 913de	94.73 ± 0.66abcdef
S#048BC	*Metarhizium* spp	13.94 ± 13.93e	84.41 ± 3.74j
DS-86-2	*Metarhizium* spp	52.74 ± 5.53bcd	95.06 ± 1.72abcde
DS-37-1	*Metarhizium* spp	58.95 ± 16.50bcd	91.86 ± 1.97efgh
S#44BC	*Beauveria* spp.	73.94 ± 16.06ab	91.73 ± 2.03fgh
S#10H*	*Beauveria* spp.	90 ± 0.00a	91.93 ± 2.66efgh
DS-51-2*	*Beauveria* spp.	90 ± 0.00a	88.99 ± 1.99hi
S#05	*Beauveria* spp.	80.91 ± 9.09ab	95.52 ± 1.43abc
S#53	*Beauveria* spp.	90 ± 0.00a	90.28 ± 1.53ghi
APPRC-34GM*	*Metarhizium* spp	90 ± 0.00a	90.95 ± 1.83gh
DS-52-2	*Metarhizium* spp	77.02 ± 12.98ab	96.72 ± 0.34 a
AF2	*Beauveria* spp	90 ± 0.00a	94.42 ± 0.36abcdef
GF4	*Beauveria* spp	80.91 ± 9.09ab	95.76 ± 0.66abc
BF4*	*Beauveria* spp	90 ± 0.00a	87.63 ± 5.09i
B4*	*Beauveria* spp	90 ± 0.00a	91.94 ± 1.48efgh
APPRC-44Bc*	*Beauveria* spp	90 ± 0.00a	95.94 ± 0.67abc
DS-35-2	*Beauveria* spp	90 ± 0.00a	95.29 ± 0.75abcd
M1	*Beauveria* spp	41.43 ± 11.56cde	90.77 ± 2.04 gh
APPRC-27*	*Beauveria* spp	90 ± 0.00a	95.45 ± 1.36abc
KF3	*Beauveria* spp.	55.19 ± 17.56bcd	89.67 ± 0.59hi
B1*	*Beauveria* spp	90 ± 0.00a	97.65 ± 1.11a
S#41	*Beauveria* spp	90 ± 0.00a	97.16 ± 1.45a
B7	*Beauveria* spp	65.32 ± 13.92abc	93.18 ± 1.58bcdefg
S#45	*Beauveria* spp	53.55 ± 9.91bcd	94.93 ± 0.68abcdef
APPRC-40GM	*Metarhizium* spp	55.19 ± 17.56bcd	92.19 ± 0.42defgh
M2	*Metarhizium* spp	33.24 ± 11.63de	92.94 ± 1.28cdefg

Means with the same letter are not significantly different according to Duncan's multiple range test at $\alpha = 0.05$. *indicate isolates selected for further screening on *C. partellus*.

than 40%. The top eight isolates which caused mortality of greater than 75% and exhibited conidial germination greater than 85% were selected for virulence testing against *C. partellus larvae*.

3.2. EPF Virulence Study against Larvae of C. partellus. The virulence of eight EPF isolates (seven Beauveria and one Metarhizium) was evaluated, with significant variations (Figure 1). Lower mean percentage mortality was recorded for isolates B4, DS-51-21, and B1, which scored 28.01%, 32.29%, and 34.58%, respectively. Isolates APPRC-34GM, S#10H, and APPRC-44BC were the most virulent strains, with mean mortality of 81.39%, 90.00%, and 90.00%, respectively.

3.3. Dose-Response Study. There was a time difference between isolates causing 50% and 90% mortality in larvae (Table 3). The LT_{50} ranged from 3.53 to 4.72 days and 6.15 to 9.05 days for higher and lower concentrations, respectively. S#10H showed a relatively short LT_{50} of 6.15 days. There was a general increase in LT_{50} with a decrease in conidial concentration. The highest LT_{50} (26.69 days) and LT_{90} (9.05 days) were recorded by *Metarhizium sp.* at the least conidial concentration. At the highest conidial concentration (1×10^8 conidia/ml), S#10H caused 90% mortality within 7.46 days, which was nearly equal to the time required by APPRC-34GM, 7.42 days at a conidial concentration of 1×10^6 conidia/ml.

The highest dose required by S#10H to kill 50% and 90% of the larval population was 5.5×10^4 and 2.5×10^7 conidia per ml, respectively; however, the lower LT_{50} and LC_{90} were recorded for APPRC-34GM (1.5×10^4 and 1.73×10^6, respectively). Hence, APPRC-34GM could cause larval mortality with lower conidial concentrations (Table 4).

3.4. Endophytic Colonization of Maize by EPF. All the screened EPF isolates were able to colonise the maize tissues using the two inoculation methods except APPRC-34GM, which was not isolated from roots using the foliar spray method (Figure 2). However, the percentage of colonisation of maize tissues varied with strains, and delivery methods ranged from a minimum of 0% to a maximum of 53%. In the foliar spray method, root colonisation of the three strains was minimal (<13%), indicating low inhabitation and translocation ability from the inoculation site, that is, the leaf, to the bases of the plant. The maximum percentage of colonisation (53%) with foliar spray was recorded by S#10H at the leaf. All the EPF isolates showed relatively good movement from leaf to stem, which may have the possibility to act on stem-dwelling insect pests such as larvae of *C. partellus*. In seed treatment, APPRC-44BC had the

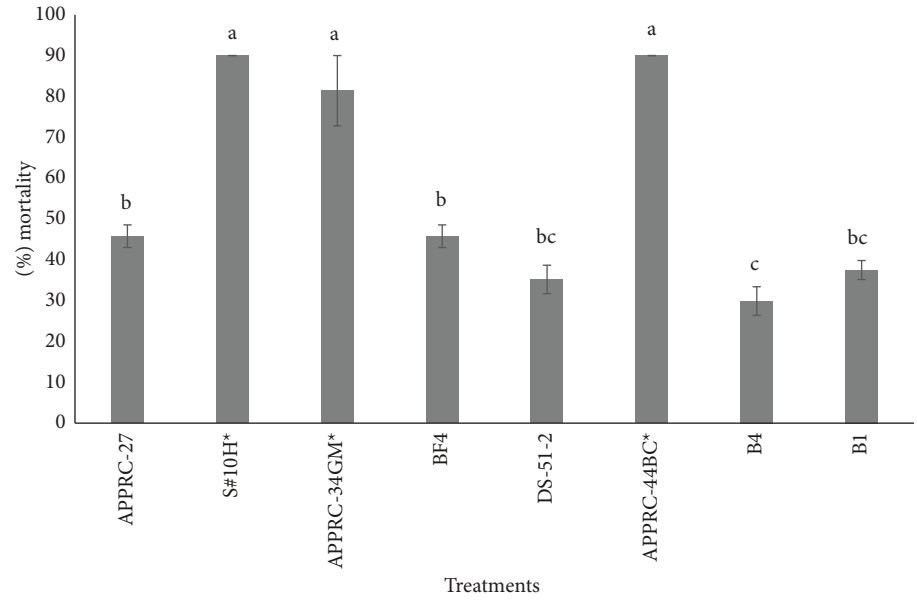

Figure 1: Mean percentage mortality of *Chilo partellus* larvae due to selected entomopathogenic fungi (EPF) strains. Isolates with * were selected for dose response study and colonisation potential study.

Table 3: LT50 and LT90 of *C. partellus* larvae treated with different conidial concentration of *Beauveria* and *Metarhizium* isolates, 11 days after treatment application.

Conidia concentration (ml^{-1})	LT50 (days ± SE)			LT90 (days ± SE)		
	APPRC-44BC	S#10	APPRC-34GM	APPRC-44BC	S#10	APPRC-34GM
1×10^5	7.12 ± 0.42	6.15 ± 0.46	9.05 ± 1.11	16.80 ± 3.16	12.34 ± 0.92	26.69 ± 4.78
1×10^6	6.99 ± 0.55	5.25 ± 0.77	7.42 ± 2.08	16.30 ± 1.87	9.48 ± 1.33	18.90 ± 6.41
1×10^7	4.46 ± 0.34	4.85 ± 0.41	4.94 ± 0.39	9.71 ± 1.10	8.57 ± 1.48	8.63 ± 0.65
1×10^8	3.53 ± 0.42	4.72 ± 0.38	4.39 ± 0.45	8.13 ± 1.01	7.46 ± 1.16	8.22 ± 0.86

Table 4: LC50 and LC90 of two *Beauveria* and one *Metarhizium* strains against larvae of *C. partellus*.

Isolate	Genus	LC50	LC90
APPRC44BC	*Beauveria* spp	3.0×10^4	7.07×10^6
S#10H	*Beauveria* spp	5.95×10^4	2.20×10^7
APPRC-34GM	*Metarhizium* spp	1.06×10^4	1.73×10^6

highest percentage of colonisation (43%) and had relatively good potential to move from the site of infection (seed) to the leaves. This may act on the newly hatched instars of insect pests such as *C. partellus*, which lay a mass of eggs on the leaves of maize. However, the general occurrence of EPF strains in roots with seed treatment was very low compared to that in leaves using the foliar spray method. S#10H had relatively good colonisation at the roots (48%) with seed treatment and had the potential to move over the plant parts. However, APPRC-34GM scored the lowest recovery potential (not more than 23%) at each sampled plant part through the seed treatment method.

3.5. Mortality of C. partellus Larvae after Feeding on Maize Inoculated with EPF. Larval mortality after feeding maize leaves inoculated with EPF ranged from 18% to 60% (Figure 3). The maximum mortality (60%) was recorded by APPRC-44BC, and the lowest mortality was caused by APPRC-34GM.

4. Discussion

In this study, viability and pathogenicity tests against *G. mellonella* larvae were used to screen 27 isolates to select the most virulent EPF strains. This study on entomopathogenic fungi proved that all the tested isolates were pathogenic to larvae of *C. partellus,* with significant variations among isolates. Such variations in virulence among isolates may be directly related to the production of insecticidal toxins, host immune systems, and screening conditions. Variations in the production of important virulence factors, such as insecticidal toxins, among strains of *M. anisopliae* have been reported by Wang et al. [26]. Tefera [3] indicated that the pathogenicity of some entomopathogens was affected by a decrease in temperature below 25°C. The infection process of entomopathogens is also affected by the insect cuticle, which is a physical barrier affecting adherence and germination of fungal conidia by having lower water activity, a shortage of readily available nutrients, and the production of antimicrobial compounds [27].

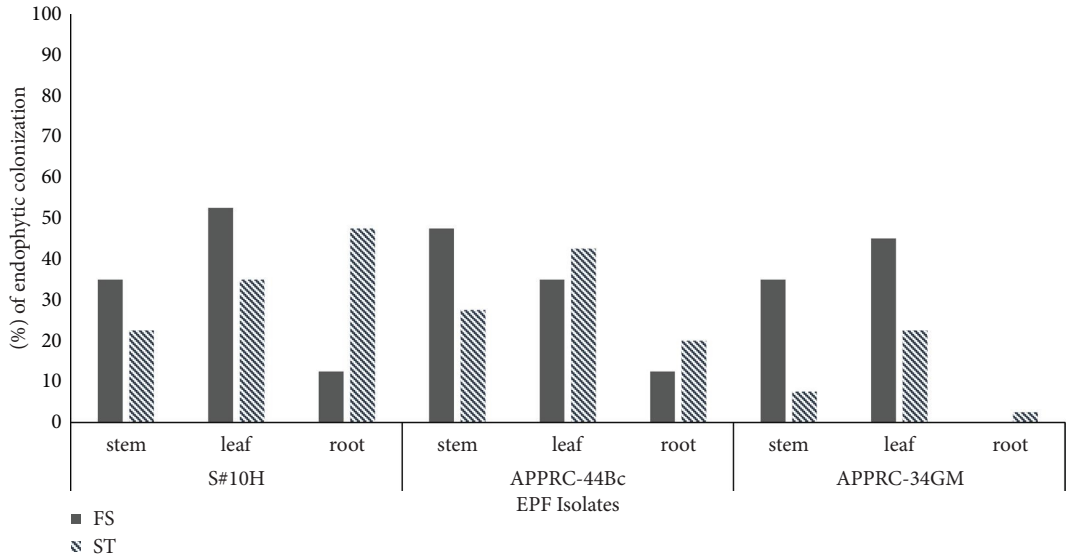

Figure 2: Percent colonisation of *e*ntomopathogenic fungi (EPF) strains using foliar spray method and seed treatment; FS: foliar spray, ST: seed treatment.

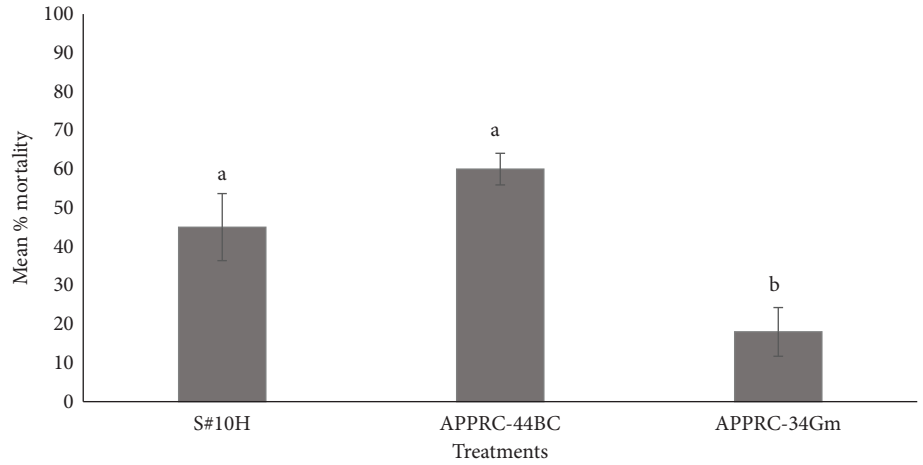

Figure 3: Mean percent mortality of *C. partellus* larvae after feeding on maize leave inoculated with entomopathogenic fungi (EPF).

The results of this study also indicated that there was a time difference between killing 50% and 90% of the given larval population. This might directly correlate with the conidial concentrations applied to the host immune system. The differences in LT_{50} and LT_{90} values may also reflect genetic and physiological variations among the tested isolates. In agreement with this, Teshome and Tefera [28] indicated the increased time required to kill the maize weevil population by *Beauveria* and *Metarhizium* with a decreased conidial concentration. Various LT_{50} caused by EPF have been reported for different insect hosts [29, 30]. Any number of infectious structures of a fungus have also been reported to cause disease in insect hosts. The efficacy of EPF isolates against storage pests was also found to be affected by grain type [31]. Hence, the most virulent entomopathogenic fungi isolates S#10H, APPRC-44BC, and APPRC-34GM tested against larvae of *C. partellus* are good candidates for mycopesticide development.

The tested EPF isolates showed the potential to colonise maize tissues with variations in their abilities. However, the mean percent colonisation of EPF isolates could not exceed 53%, and the potential to move from the site of infection may provide the advantage of managing insect pests acting on different parts of maize. Similar to this study, Pourtaghi et al. [32] also reported that *B. bassiana* translocates from the site of infection (leaf) to other parts (stem and root) of the tomato. The combined use of APPRC-44BC and S#10H, which scored 43% and 53% of recovery potential, respectively, on the leaf, may have an increased opportunity to reduce the *C. partellus* population by inhibiting active feeding instars. The differences in the successful colonisation potential of microorganisms may be affected by specific characteristics of cultivars and soil conditions, such as providing an enemy-free space and delivery methods. Vidal and Jaber [11] found that the recovery potential of *B. bassiana* varied with inoculation methods in sorghum. In

agreement with our findings, they found that the colonisation potential of *B. bassiana* using leaf inoculation resulted in the highest colonisation of leaves. González-Guzmán et al. [18] recovered *B. bassiana* from tomato using three inoculation techniques: leaf sparing, seed immersion, and root dipping, where leaf spraying was the most effective method of delivery. However, the colonisation potential decreased as the number of days after inoculation increased.

This study also revealed that *C. partellus* larvae died after feeding on maize leaves because of the endophytic occurrence of entomopathogenic fungi. Various research outputs also indicate that endophytic colonisation of EPF after artificial inoculation causes mortality and feeding deterrence effects. Increased adult whitefly mortality was recorded after feeding *B. bassiana*-endophyte tomato plants [32]. The maize leaf-feeding performance of *Rachiplusia nu* larvae was affected by the endophytic presence of *B. bassiana* [17]. Field application of mycopesticides can be done via ground or aerial broadcasting as dust or granules using atomised devices [33]. However, environmental factors, including altitude, pH, soil type, vegetation type, temperature, and UV radiation, contribute to reduced field efficacy and the occurrence of entomopathogenic fungi [6, 34]. Hence, the application of entomopathogenic fungi via endophytism may have a superior advantage over direct spraying in overcoming critical environmental factors limiting the field efficacy of entomopathogens.

5. Conclusion

The present study revealed that Eetomopathogenic *Beauveria* and *Metarhizium* have the potential to colonise maize plant tissue after artificial inoculation. However, the precent of colonisation was not surpassed 53% and varied with inoculation methods. The study also investigated whether EPF could translocate from the site of infection to other parts of maize, indicating the comparative advantage of acting on the different larval stages of the pests. The endophytic occurrences of EPF can also poison the larvae of *C. partellus*. However, the mode of action of EPF to kill the larvae due to its endophytic presence is not yet understood. Based on the findings, it is concluded that the use of EPF as endophytes can be an alternative strategy for management of stem dwelling insect pests of maize. However, the endophytic mode of action on the larvae of insect pests and inoculation methods to enhance colonisation potential of EPF should be further investigated.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

All the authors conceived the idea and designed the experiment. Denberu Kebede conducted the experiment and wrote the manuscript; Tesfaye Alemu and Tadele Tefera revised the draft. Tadele Tefera obtained funding. Denberu Kebede is a graduate student from ICIPE. All authors read and approved the manuscript.

Acknowledgments

This research work was funded by the USAID Feed the Future IPM Innovation Lab, Virginia Tech, Cooperative Agreement No. AID-OAA-L-15-00001 implemented by icipe.

References

[1] N. M. Sylvain, W. Manyangarirwa, M. Tuarira, and M. K. Onesime, "Effect of lepidopterous stemborers, Busseola fusca (fuller) and Chilo partellus (swinhoe) on maize (Zea mays L) yield: a review," *International Journal of Innovative Research*.vol. 4, pp. 181–188, 2015.

[2] M. A. Mashwani, F. Ullah, S. Ahmad, K. Sohail, M. Usman, and S. F. Shah, "Infestation of maize stem borer, Chilo partellus (Swinhoe) in maize stubbles and stalks," *Asian Journal of Accounting Research*, vol. 3, pp. 94–098, 2015.

[3] T. Tefera, "Lepidopterous stem borers of sorghum and their natural enemies in eastern Ethiopia," *Tropical Science*, vol. 44, no. 3, pp. 128–130, 2004.

[4] M. Wale, F. Schulthess, E. W. Kairu, and C. O. Omwega, "Cereal yield losses caused by lepidopterous stem borers at different nitrogen fertilizer rates in Ethiopia," *Journal of Applied Entomology*, vol. 130, no. 4, pp. 220–229, 2006.

[5] S. R. Sánchez-Pena, J. S. J. Lara, and R. F. Medina, "Occurrence of entomopathogenic fungi from agricultural and natural ecosystems in Saltillo, México, and their virulence towards thrips and whiteflies," *Journal of Insect Science*, vol. 11, pp. 1–10, 2011.

[6] E. Quesada-moraga, J. A. Navas-corte´s, E. A. A. Maranhao, A. Ortiz-urquiza, and C. Santiago-Álvarez, "Factors affecting the occurrence and distribution of entomopathogenic fungi in natural and cultivated soils," *Mycological Research*, vol. 111, no. 8, pp. 947–966, 2007.

[7] F. E. Vega, F. Posada, M. Catherine Aime, M. Pava-Ripoll, F. Infante, and S. A. Rehner, "Entomopathogenic fungal endophytes," *Biological Control*, vol. 46, no. 1, pp. 72–82, 2008.

[8] T. Kasambala Donga, R. Meadow, N. V. Meyling, and I. Klingen, "Natural occurrence of entomopathogenic fungi as endophytes of sugarcane (Saccharum officinarum) and in soil of sugarcane fields," *Insects*, vol. 12, no. 2, p. 160, 2021.

[9] A. C. L. Silva, G. A. Silva, P. H. N. Abib, A. T. Carolino, and R. I. Samuels, "Endophytic colonization of tomato plants by the entomopathogenic fungus Beauveria bassiana for controlling the South American tomato pinworm, Tuta absoluta," *CABI Agriculture and Bioscience*, vol. 1, no. 3, 2020.

[10] B. L. Wagner and L. C. Lewis, "Colonization of corn, Zea mays, by the entomopathogenic fungus Beauveria bassiana," *Applied and Environmental Microbiology*, vol. 66, no. 8, pp. 3468–3473, 2000.

[11] S. Vidal and L. R. Jaber, "Entomopathogenic fungi as endophytes: plant endophyte herbivore interactions and prospects for use in biological control," *Current Science*, vol. 109, pp. 47–54, 2015.

[12] A. Hussain, M. Tian, Y. He, L. Ruan, and S. Ahmed, "In vitro and in vivo culturing impacts on the virulence characteristics

of serially passed entomopathogenic fungi," *Journal of Food Agriculture and Environment*, vol. 8, pp. 481–487, 2010.

[13] G. Resquín-Romero, I. Garrido-Jurado, C. Delso, A. Ríos-Moreno, and E. Quesada- Moraga, "Transient endophytic colonizations of plants improve the outcome of foliar applications of mycoinsecticides against chewing insects," *Journal of Invertebrate Pathology*, vol. 136, pp. 23–31, 2016.

[14] R. A. Villamizar-Gallardo, O. O. Ortíz-Rodriguez, and J. W. Escobar, "Symbiotic and endophytic fungi as biocontrols against cocoa (Theobroma cacao L.) phytopathogens," *Summa Phytopathologica*, vol. 43, no. 2, pp. 87–93, 2017.

[15] M. H. O. Rashid and Y. R. Chung, "Induction of systemic resistance against insect herbivores in plants by beneficial soil microbes," *Frontiers of Plant Science*, vol. 8, pp. 1816–1911, 2017.

[16] B. J. Lugtenberg, J. R. Caradus, and L. J. Johnson, "Fungal endophytes for sustainable crop production," *FEMS Microbiology Ecology*, vol. 92, no. 12, pp. fiw194–15, 2016.

[17] M. L. Russo, A. C. Scorsetti, M. F. Vianna, M. Cabello, N. Ferreri, and S. Pelizza, "Endophytic effects of Beauveria bassiana on corn (Zea mays) and its herbivore, Rachiplusia nu (Lepidoptera: noctuidae)," *Insects*, vol. 10, no. 4, p. 110, 2019.

[18] A. González-Guzmán, M. D. Rey, E. Froussart, and E. Quesada-Moraga, "Elucidating the effect of endophytic entomopathogenic fungi on bread wheat growth through signaling of immune response-related hormones," *Applied and Environmental Microbiology*, vol. 88, no. 18, pp. e0088222–22, 2022.

[19] C. Senthil Kumar, T. K. Jacob, S. Devasahayam, S. Thomas, and C. Geethu, "Multifarious plant growth promotion by an entomopathogenic fungus Lecanicillium psalliotae," *Microbiological Research*, vol. 207, pp. 153–160, 2018.

[20] N. Allegrucci, M. S. Velazquez, M. L. Russo, E. Perez, and A. C. Scorsetti, "Endophytic colonization of tomato by the entomopathogenic fungus Beauveria bassiana: the use of different inoculation techniques and their effects on the tomato leaf miner, Tuta absoluta (Lepidoptera: gelechiidae)," *Journal of Plant Protection Research*, vol. 57, no. 4, pp. 206–211, 2018.

[21] S. Mantzoukas and P. A. Eliopoulos, "Endophytic entomopathogenic fungi: a valuable biological control tool against plant pests," *Applied Sciences*, vol. 10, no. 1, p. 360, 2020.

[22] H. Belay, G. Emana, D. Mohamed et al., "Molecular characterization and evaluation of indigenous entomopathogenic fungal isolates against Sorghum Chafer, Pachnoda interrupta (Olivier) in Ethiopia," *Journal of Entomology and Nematology*, vol. 8, no. 5, pp. 34–45, 2016.

[23] T. Tefera and K. Pringle, "Mortality and maize leaf consumption of Chilo partellus (Lepidoptera: Pyralidae) larvae treated with Beauveria Bassiana and Metarhizium anisopliae," *International Journal of Pest Management*, vol. 50, no. 1, pp. 29–34, 2004.

[24] T. Tefera and S. Vidal, "Effect of inoculation method and plant growth medium on endophytic colonization of sorghum by the entomopathogenic fungus Beauveria bassiana," *BioControl*, vol. 54, no. 5, pp. 663–669, 2009.

[25] W. S. Abbott, "A method of computing the effectiveness of an insecticide," *Journal of Economic Entomology*, vol. 18, no. 2, pp. 265–267, 1925.

[26] C. Wang, A. Skrobek, and T. M. Butt, "Investigations on the destruxin production of the entomopathogenic fungus Metarhizium anisopliae," *Journal of Invertebrate Pathology*, vol. 85, no. 3, pp. 168–174, 2004.

[27] H. L. Lu and R. J. Leger, *Insect Immunity to Entomopathogenic Fungi*, University of Maryland, College Park, MD, USA, 2016.

[28] A. Teshome and T. Tefera, "Susceptibility of Sitophilus zeamais (mostch.) (Coleoptera: curculionidae) to Beauveria bassiana and Metarhizium anisopliae," *Sinet: Ethiopian Journal of Science*, vol. 32, no. 1, pp. 21–28, 2011.

[29] M. Kovač, N. Lacković, and M. Pernek, "Effect of Beauveria bassiana fungal infection on survival and feeding behavior of pine-tree lappet moth (Dendrolimus pini L.)," *Forests*, vol. 11, no. 9, p. 974, 2020.

[30] B. Ramanujam, B. Poornesha, and A. N. Shylesha, "Effect of entomopathogenic fungi against invasive pest Spodoptera frugiperda (J. E. Smith) (Lepidoptera: noctuidae) in maize," *Egypt J Biol Pest Control*, vol. 30, no. 1, p. 100, 2020.

[31] S. Mantzoukas, I. Lagogiannis, K. Karmakolia, A. Rodi, M. Gazepi, and P. A. Eliopoulos, "The effect of grain type on virulence of entomopathogenic fungi against stored product pests," *Applied Sciences*, vol. 10, no. 8, Article ID 1008, 2020.

[32] E. Pourtaghi, R. Talaei-Hassanloui, F. Nasibi, and K. B. Fotouhifar, "Endophytic colonization of tomato by Beauveria bassiana for control of the greenhouse whitefly, Trialeurodes vaporariorum (Hemiptera: aleyrodidae)," *Acta Biologica*, vol. 27, pp. 149–160, 2020.

[33] S. T. Jaronski, "Ecological factors in the inundative use of fungal Entomopathogens," *BioControl*, vol. 55, no. 1, pp. 159–185, 2010.

[34] J. Medo and L. Cagan, "Factors affecting the occurrence of entomopathogenic fungi in soils of Slovakia as revealed using two methods," *Biological Control*, vol. 59, no. 2, pp. 200–208, 2011.