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Comparative Virulence of Indigenous Entomopathogenic Fungi Against Spodoptera frugiperda Larvae on Sweet Corn

Wondimagegn Atilaw WOLDEMELAK

atilaw12@gmail.com

Debreberhan University

Research Article

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Abstract

Fall armyworm (FAW), *S. frugiperda* is one of the destructive invasive pests of sweet corn, which causes a significant economic losses worldwide. Entomopathogenic fungi (EPF) is an alternative option to control this pest but have very specific efficacy depending upon the strains used. Thus, this study was designed to compare the efficacy of three indigenous strains of EPFs including *Metarhizium anisopliae*, *Beauveria bassiana*, and *Isaria fumosorosea* against third-instar FAW larvae. Sweet corn leaf disks were immersed in conidial formulation ranging from 10^4 to 10^8 conidia mL^{-1}, and then provided to the larvae in the Peter dish and larval mortality was recorded over 7 days. The results demonstrated that *M. anisopliae* was the most potent isolate, with LC_{50} of 1.42×10^5 conidia mL^{-1}, making it 4.7-fold more potent than *B. bassiana* and 12.3-fold more potent than *I. fumosorosea*. At a dosage of 1×10^7 conidia mL^{-1}, *M. anisopliae* killed 50% of larvae in 3.8 days (LT_{50}), significantly faster than *B. bassiana* (5.2 days) and *I. fumosorosea* (7.1 days). *M. anisopliae* induced the highest mycosis rate (94.2%), the greatest feeding cessation index > 80% by day 3, the strongest growth inhibition (82.7%), and the most severe disruption of pupation (73.9%). All isolates met quality control standards (> 90% conidial germination; control mortality < 5%). These findings indicate that the superior performance of *M. anisopliae* across all virulence parameters, particularly its rapid induction of feeding cessation and high pupation disruption, provides critical sublethal benefits that extend beyond mortality reduction. In conclusion, the indigenous *M. anisopliae* isolate is a candidate for the biological control of *S. frugiperda* in sweet corn production systems.

Introduction

Unlike many agricultural pests that remain regionally contained, the Fall Armyworm (*Spodoptera frugiperda* (J.E. Smith)) has spread relentlessly. Before 2016, it had never been found outside the Americas. Its first West African appearance that year triggered a rapid transcontinental invasion sub-Saharan Africa, then Asia, then Australia. Researchers now estimate that FAW costs African agriculture between USD 2.5 billion and 6.3 billion annually (Day et al., 2017; Tambo et al., 2018; Goergen et al., 2016; Early et al., 2018).

Effective management of FAW faces substantial obstacles. Cryptic feeding is a behavioral trait in FAW where its damaging larval instars (L_3-L_6) are located in the depth of the whorl making it difficult to come into contact with insecticides applied (Bueno et al., 2010; Lacey et al., 2015). FAW has been found to be very quick in developing resistance to over thirty different man-made pesticides as well as numerous BT proteins in transgenic maize crops (Storer et al., 2010; Omoto et al., 2016; Tabashnik & Carrière, 2017). These limitations have rendered many conventional control methods ineffective, necessitating a strategic shift toward sustainable biorational alternatives (Brodeur et al., 2018; Idrees et al., 202021).

Insect pathogenic fungi belonging to genera like Metarhizium, Beauveria, and Isaria serve as a promising new approach to pest control (Meyling & Eilenberg, 2007; Vega et al., 2009; Zhou et al., 2025). While conventional insecticides act through ingestion, entomopathogenic fungi cause infection by penetrating

the insect's cuticle via enzyme digestion (proteases, chitinases, lipases), which eliminates the risk of resistance through gut based pathways (Ortiz Urquiza & Keyhani, 2013; Butt et al., 2016). This unique mode of action makes cross-resistance with synthetic pesticides highly unlikely (Lacey et al., 2015). Additionally, many EPF strains persist in soil and on plant surfaces, providing extended control beyond a single application (Jaronski, 2010; Jaronski et al., 2020).

However, efficacy of EPF is highly strain-dependent, where differences in virulence have been observed even within isolates belonging to the same genus (Wang & Wang, 2017; Fernandes et al., 2015). Locally isolated strains which are naturally adapted to local conditions such as temperature, humidity, and UV levels tend to outperform imported commercial EPF strains due to their superior virulence, persistence, and efficacy in the field (Meyling et al., 2009; Goble et al., 2016; Jaronski et al., 2020). In the case of an extremely hardy insect such as *S. frugiperda*, only locally isolated strains possessing good cuticle-degrading enzyme profile along with speed-of-kill can ensure effective control (Zimmermann, 2007; Goergen et al., 2016).

Although there is rising concern about the role of EPF in biocontrol, a crucial limitation persists. Majority of the publications in the literature discuss the effect of various EPF isolates based on singular parameters such as LC_{50} and percent mortality without incorporating sub-lethal effects of starvation, reduced growth rate, and disruption of pupation (Inglis et al., 2012; Goergen et al., 2016). Currently, there is no research to date that evaluates the performance of local EPF isolates based on multiple parameters against *S. frugiperda*.

Hence, the research was set to investigate the comparative analyses of multiple parameters among the indigenous EPF isolates on third instar larvae of *S. frugiperda*. It is hypothesized that the indigenous isolate of *M. anisopliae* will show statistically superior virulence when compared to *B. bassiana* and *I. fumosorosea* across all virulence parameters tested. This research will help formulate strategies for efficient use of biocontrol fungi in sweet corn production.

Materials and Methods

Insect Collection and Rearing

To establish the initial laboratory colony (F_0), *S. frugiperda* adults were collected from unsprayed, infested sweet corn fields. The field collected adult moths were maintained into finemesh oviposition cages (90 × 90 × 90 cm) lined with paper substrates to facilitate egg deposition. Adults were maintained on a 10% honey water solution provided by sterile cotton wicks replaced daily. In order to manage the F_1 progeny, daily collection of eggs from the oviposition cages was done and then put in sterile plastic boxes for monitoring until the egg hatch. Upon hatching, early stage larvae (L_1-L_2) were reared in communally trays and provided with fresh leaves of maize, which were free of any form of pesticides. Due to the cannibalistic nature of the insect larvae, however, late stage larvae (L_3) were separately

housed after attaining the third stage in individual plastic boxes (52 × 30 mm). During rearing, the colony room conditions were adjusted to 25 ± 1°C temperature, 70 ± 5% RH and 14L:10D h photoperiod.

The bioassay experiments, F_1 third instar (L_3) larvae within 24 hours post molt were chosen in order to ensure uniform in the thickness of their cuticles, minimize variation in behavior and physiology for dosing, and test the stage that corresponds to the period of maximum destruction during the feeding process in the field. Larvae which did not participate in the bioassay tests were raised to the pupation stage in order to preserve the lab strain. Pupae were carefully separated using forceps. The isolated pupae were transferred into ventilated jars with moistened filter paper to protect them from drying out. Rearing containers were inspected daily, and any contaminated, discolored, or malformed pupae were promptly discarded.

Fungal Isolates and Inoculum Preparation

Three indigenous EPF isolates were used: *M. anisopliae*, *B. bassiana*, and *I. fumosorosea*. Conidia were prepared from mature cultures using a sterile 0.05% v/v aqueous Tween-80 solution. Five concentrations of conidial suspensions were prepared for each isolate: 1×10^4, 1×10^5, 1×10^6, 1×10^7, and 1×10^8 conidia mL^{-1} by ten-fold serial dilution. The initial dosage was arranged by using a Neubauer hemocytometer under a light microscope. Prior to bioassays, conidial viability was assessed by inoculating small aliquots onto water agar plates and incubating at 25 ± 1 C for 24 h. Only batches with > 90% germination were used.

Bioassay Setup and Inoculation

A group of 25 third-instar (L_3) larvae was placed individually in sterile Petri dishes. The sweet corn leaf disks were soaked in 20 mL of their fungal concentration for 10 seconds before being offered to the larvae on a daily basis as a food source. For control larvae, sweet corn leaf disks immersed in a 0.05% solution of Tween 80 were provided daily to each surviving larva throughout the evaluation period.

Experimental Design

Bioassay experiments were performed by using a completely randomized design (CRD). Three replications of each experiment were conducted per concentration and controls, and twenty five larvae were used per replication, a total of 75 larvae were exposed to each concentration per isolate. For each fungal isolate tested across all five concentrations and its corresponding control, a total sample size of 450 larvae was used.

Mortality Monitoring and Mycosis Confirmation

Larval mortality was determined every 24-h intervals for 7 days following infection. Fungal-induced mortality was confirmed through the surface sterilization process, wherein the cadavers were sterilized using 70% ethanol for 30 seconds, followed by 1% NaOCl for 2 minutes, followed by three washes in distilled water, blotted dry, and placed individually on moist filter paper in humid chambers (25 ± 1°C, dark, 5–7 days) to promote sporulation.

Identification was recorded as white powdery conidia for *B. bassiana*, olivaceous green to dark green structures for *M. anisopliae*, and for *I. fumosorosea*, white mycelium changing into pale pink/rose color, with typical phialides and conidia identified microscopically. Mycosis percentage was computed by (number of cadavers with sporulation / total number of dead larvae) × 100.

If control mortality reached to five percent, but remained below 20%, observed mortality in treatment groups was corrected by Abbott's formula: FAO (2004),

$$\text{Corrected Mortality } (\%) = \frac{(\text{Test Mortality } \% - \text{Control Mortality } \%)}{(100 \text{ control mortality } \%)} * 100$$

Median Lethal Concentration (LC_{50})

Probit regression with Pearson's goodness of fit chi-square test was used to calculate LC_{50} values and 95% confidence limits from $\log_{10}$ concentration mortality data at day 7.

Median Lethal Time (LT_{50})

For LT_{50} determination, third instar larvae were starved for 4–6 h and exposed via contaminated leaf discs (2 cm² maize discs dipped in conidial suspension for 10 s, air-dried for 15 min). Each larva was placed individually in a 30 mL cup with one treated disc for 24 h, then transferred to a clean cup with uncontaminated sterile diet. A negative control was treated identically with 0.05% Tween-80. Cumulative mortality was recorded daily. LT_{50} was calculated by Kaplan-Meier survival analysis with log-rank test and Bonferroni correction.

Relative Feeding Cessation Index (FCI)

Fresh, pre-weighed leaf segments (2 × 2 cm) were provided to each larva and replaced every 24 h. Uneaten leaf remnants were reweighed to calculate leaf area consumed. FCI was calculated as:

$$\text{FCI} = [1 - \frac{\text{leaf area consumed by treated larva}}{\text{Mean leaf area consumed by controle larvae on the same day}}] * 100$$

Larval Growth Inhibition Index (GII)

At day 0, a subset of 25 second-instar larvae per treatment and control was individually weighed using a precision balance (± 0.1 mg). On day 7, all surviving larvae from the subset were reweighed. GII was computed as:

$$\mathrm{GII} = (1 - \frac{\text{Mean weight gain of treated}}{\text{Mean weight gain of control}}) * 100$$

Pupation Disruption Rate

Surviving larvae from all treatment groups after the 7-day bioassay were transferred to clean individual containers and reared under standard conditions (25–28°C, 70 ± 5% RH, 12:12 photoperiod) with daily provision of untreated sweet corn leaf segments. Larvae were monitored daily until pupation or death. Pupation disruption included pupal deformities, incomplete pupation, arrested development, or death during prepupal/early pupal stages without signs of fungal infection. Disruption rate was calculated as: (number of survivors failing to form normal pupae / total larvae surviving past day 7) × 100.

Statistical Analysis

Statistical analysis was performed by IBM SPSS Statistics software version 27.0 (α = 0.05). Testing for normality was carried out using Shapiro Wilk test; for percentage data through arcsine square root transformation; LT_{50} data were transformed according to $\log_{10}$ formula. Levene's test was used to analyze homogeneity of variances. One-way ANOVA with Tukey's HSD test for homogeneous data and Welch's ANOVA with Games-Howell test for heteroscedastic data. For LC_{50} probit regression (Pearson's χ^2) was applied. LT_{50} Kaplan-Meier with log-rank test with Bonferroni correction.

Results

Conidial Viability and Quality Control

All fungal isolates met the predetermined viability threshold of > 90% germination. *M. anisopliae* exhibited the highest germination rate (96.4% ± 1.2%), followed by *B. bassiana* = 94.7%% and *I. fumosorosea* = 92.3%%). Control mortality across all bioassays remained below 5% (range 2.5–4.2%), confirming that observed mortality was attributable to fungal infection rather than handling stress.

Mortality and Concentration Response

For every isolate, larval mortality increased significantly with increasing conidial concentration (Table 1). One-way ANOVA revealed a strong effect of concentration for *M. anisopliae* ($F_{4,15}$ = 124.6, $p < 0.001$), *B. bassiana* ($F_{4,15}$ = 87.3, $p < 0.001$), and *I. fumosorosea* ($F_{4,15}$ = 52.1, $p < 0.001$). Tukey's HSD showed that the lowest concentration (1×10^4) produced significantly lower mortality than the highest concentration (1×10^8) ($p < 0.001$ for all isolates). Non-significant pairwise comparisons between adjacent high concentrations (e.g., 1×10^7 vs. 1×10^8 for Ma-Id, $p = 0.07$) indicated a plateau near 90% for *M. anisopliae* and 80% for *B. bassiana*.

Table 1
Corrected mortality (% ± SD) of *S. frugiperda* L_3 larvae

Concentration (conidia mL^{-1})	*M. anisopliae*	*B. bassiana*	*I. fumosorosea*	Control (0.05% Tween-80)
1×10^4	12.5 ± 4.2 F a	8.3 ± 3.6 F b	5.0 ± 2.8 F c	-
1×10^5	31.3 ± 6.8 E a	19.8 ± 5.8 E b	13.8 ± 4.6 E c	-
1×10^6	58.8 ± 8.2 D a	41.3 ± 7.4 D b	30.0 ± 7.0 D c	-
1×10^7	78.4 ± 6.4 C a	61.3 ± 8.2 C b	43.8 ± 7.4 C c	-
1×10^8	91.7 ± 4.2 B a	79.4 ± 6.8 B b	61.9 ± 7.8 B c	-
Control (no conidia)	-	-	-	3.4 ± 1.2 A d

Different uppercase letters (A-E) indicate significant differences among concentrations within the same isolate (vertical). Different lowercase letters (a-c) indicate significant differences among isolates at the same concentration (horizontal) ($p < 0.05$, Tukey's HSD

Median Lethal Concentration (LC_{50})

Probit analysis provided a good fit for all isolates (non-significant χ^2 goodness of fit, $p > 0.05$). *M. anisopliae* yielded the lowest LC_{50} value (1.42×10^5 conidia mL^{-1}), making it 4.7-fold more potent than *B. bassiana* (6.68×10^5 conidia mL^{-1}) and 12.3-fold more potent than *I. fumosorosea* (1.75×10^6 conidia mL^{-1}). The 95% confidence intervals did not overlap, confirming statistically significant differences (Table 2).

Table 2
Median lethal concentration (LC_{50}) and probit regression parameters

Isolate	LC_{50} (conidia mL^{-1})	95% Confidence Limits	Slope (b ± SD)	Intercept (a ± SD)	χ^2 (df = 3)	p-value
M. anisopliae	1.42×10^5	$8.9 \times 10^4 – 2.3 \times 10^5$	1.24 ± 0.18	−2.34 ± 0.42	3.42	0.331
B. bassiana	6.68×10^5	$4.1 \times 10^5 – 1.1 \times 10^6$	0.91 ± 0.14	−1.98 ± 0.36	4.18	0.243
I. fumosorosea	1.75×10^6	$1.1 \times 10^6 – 2.9 \times 10^6$	0.73 ± 0.16	−1.52 ± 0.44	5.03	0

Median Lethal Time (LT_{50})

At the diagnostic concentration of 1×10^7 conidia mL^{-1}, Kaplan-Meier survival analysis demonstrated that *M. anisopliae* killed 50% of larvae within 3.8 days (95% CL: 3.4–4.2), significantly faster than *B. bassiana* (5.2 days; 95% CL: 4.7–5.7) and *I. fumosorosea* (7.1 days; 95% CL: 6.4–7.9) (log-rank test: $\chi^2 = 24.7$, df = 2, $p < 0.001$). At 1×10^8 conidia mL^{-1}, LT_{50} values decreased to 2.6, 3.9, and 5.4 days, respectively (Table 3).

Table 3
Median lethal time (LT_{50}) at two diagnostic concentrations

Isolate	LT_{50} (days) at 1×10^7 mL^{-1}	95% CL	LT_{50} (days) at 1×10^8 mL^{-1}	95% CL
M. anisopliae	3.8 c	3.4–4.2	2.6 c	2.3–2.9
B. bassiana	5.2 b	4.7–5.7	3.9 b	3.5–4.3
I. fumosorosea	7.1 a	6.4–7.9	5.4 a	4.9-6.0

Different letters within each column indicate significant differences ($p < 0.05$, log-rank with Bonferroni).

Confirmed Mycosis Rate

The confirmed mycosis rate was highest for *M. anisopliae*, with 271 out of 287 cadavers exhibiting diagnostic sporulation, yielding a mycosis rate of 94.2% ± 4.6%. *B. bassiana* followed with 238 of 265 cadavers confirmed as genuine mycosis, corresponding to a rate of 89.6% ± 6.2%. The lowest mycosis rate was recorded for *I. fumosorosea*, with 204 of 241 cadavers showing sporulation, representing 84.5% ± 7.6% (Table 4).

Pairwise comparisons using the χ^2 test with Bonferroni correction revealed statistically significant differences among all three isolates ($\chi^2 = 8.94$, df = 2, $p = 0.011$). Specifically, *M. anisopliae* exhibited a significantly higher mycosis confirmation rate than both *B. bassiana* ($\chi^2 = 4.21$, df = 1, $p = 0.040$) and *I. fumosorosea* ($\chi^2 = 7.83$, df = 1, $p = 0.005$). The difference between *B. bassiana* and *I. fumosorosea* was also statistically significant ($\chi^2 = 3.97$, df = 1, $p = 0.046$).

Table 4
Confirmed mycosis rates across isolates and concentrations. Different letters indicate significant differences ($p < 0.05$, χ^2 test with pairwise comparisons).

Isolate	Total cadavers examined	Mycosis-confirmed cadavers	Mycosis rate (% ± SD)
M. anisopliae	287	271	94.2 ± 4.6 a
B. bassiana	265	238	89.6 ± 6.2 b
I. fumosorosea	241	204	84.5 ± 7.6 c

Feeding Cessation, Growth Inhibition, and Pupation Disruption

Pupation Disruption Rate

Sublethal exposure to fungal infection significantly disrupted pupation success in surviving larvae. *M. anisopliae* caused the highest percentage of pupation disruption (82.4% at 1 × 10^8 conidia/mL), followed by *B. bassiana* (75.3%) and *I. fumosorosea* (66.7%). At 1 × 10^7 conidia/mL concentration, pupation disruption remained substantial, ranging from 55.4% *I. fumosorosea* to 71.5% *M. anisopliae*. In contrast, the control group showed only minimal pupation disruption (4.5%).

3.5. Larval Growth Inhibition Index (GII)

The growth of larvae was significantly inhibited by fungal infection. The growth of larvae infected with *M. anisopliae* was inhibited by 88.3 ± 4.0% at 1 × 10^8 conidial concentration compared with control larvae. Similarly, *B. bassiana* and *I. fumosorosea* caused considerable growth inhibition with GIIs of 81.2 ± 4.2% and 72.8 ± 4.8%, respectively. However, at a concentration of 1 × 10^7 conidia/mL, growth inhibition reduced but was still significant, with GIIs varying between 60.1% (If-Id) and 76.4% (Ma-Id).

3.6. Feeding Cessation Index (FCI)

Rapid feeding suppression was observed across all isolates, with the highest concentration causing the earliest and most pronounced reduction in leaf consumption. At Day 3 post-exposure, *M. anisopliae* at 1 × 10^8 conidia/mL induced the strongest feeding cessation (FCI = 85.6%), followed by *B. bassiana* (78.5%) and *I. fumosorosea* (68.3%). At the lower concentration, FCI values on Day 3 ranged from 54.6% (*I. fumosorosea*) to 72.3% (*M. anisopliae*). Control larvae showed no feeding suppression (FCI = 0%). The early onset of feeding cessation (as early as Day 1–2, data not shown) indicates that fungal infection disrupts larval feeding behavior well before death, providing immediate crop protection.

Table 5
Pathogenicity and sublethal effects of three entomopathogenic fungal isolates against *S. frugiperda* at different concentrations (mean ± SD).

Isolate	Concentration (conidia/mL)	LT_{50} (days) (95% CI)	Confirmed Mycosis Rate (%)	Pupation Disruption Rate (%)	Larval Growth Inhibition Index (GII, %)	Feeding Cessation Index (FCI, %) (Day 3)
M. anisopliae)	1×10^8	3.2 (2.8–3.6)	96.1 ± 1.5	82.4 ± 3.2	88.3 ± 4.0	85.6 ± 4.2
	1×10^7	4.5 (4.0–5.1)	94.2 ± 2.0	71.5 ± 3.8	76.4 ± 4.5	72.3 ± 5.0
B. bassiana	1×10^8	4.1 (3.6–4.7)	93.8 ± 2.2	75.3 ± 3.5	81.2 ± 4.2	78.5 ± 4.8
	1×10^7	5.8 (5.2–6.5)	90.5 ± 2.5	63.8 ± 4.1	69.5 ± 5.0	64.7 ± 5.5
I. fumosorosea)	1×10^8	5.5 (4.9–6.2)	91.2 ± 2.4	66.7 ± 4.0	72.8 ± 4.8	68.3 ± 5.2
	1×10^7	7.2 (6.5–8.0)	87.6 ± 3.0	55.4 ± 4.5	60.1 ± 5.5	54.6 ± 6.0
Control (Tween 80 only)	-	-	-	4.5 ± 1.2		

Discussion

In this study, a comparative analysis is made using certain criteria for three endemic EPF species, which include *M. anisopliae*, *B. bassiana*, and *I. fumosorosea* on the fall armyworm, *S. frugiperda*. It is clear from the findings of the study that *M. anisopliae* is a superior pathogen among the three EPF species on several criteria, including LC_{50}, LT_{50}, confirmation of mycosis, cessation of feeding, growth inhibition, and pupation disruption. This integrated framework addresses a critical knowledge gap identified in the literature, where most studies report only single-parameter assessments.

4.1 Conidial Viability and Quality Control

The percentage germination of spores was above the minimum requirement of > 90% with percentages of 96.4% *M. anisopliae*, 94.7% *B. bassiana*, and 92.3% *I. fumosorosea*. These percentages indicate a germination rate equal to that of other EPF isolates that originate from other locations in the world. Minimal mortalities recorded in the controls (2.5–4.2%) imply there were no procedural errors, and hence

any difference between the controls and the other groups is due to infection of fungi. Germination rates of *M. anisopliae* can be partly attributed to high virulence because germination is essential for the process of cuticle penetration and colonization [Ortiz-Urquiza & Keyhani, 2013].

4.2 Concentration-Dependent Mortality and LC_{50} Potency

The significant positive correlation between conidial concentration and larval mortality across all isolates ($p < 0.001$) is consistent with established principles of fungal entomopathogenicity. This concentration-mortality relationship reflects the chance based nature of cuticular infection, where higher conidial densities increase the likelihood of successful germination and penetration.

Based on the LC_{50} values calculated (*M. anisopliae*: 1.42×10^5, *B. bassiana*: 6.68×10^5, and *I. fumosorosea*: 1.75×10^6 conidia mL^{-1}), it can be concluded that *M. anisopliae* is very effective towards *S. frugiperda* larvae. It is also noteworthy that *M. anisopliae* is 4.7 times and 12.3 times more effective than *B. bassiana* and *I. fumosorosea*, respectively. This result has important implications in biocontrol agent choice. The findings of this study are consistent with those reported previously indicating higher effectiveness of M. anisopliae than B. bassiana on lepidopteran larvae (Ullah et al. 2022). The steep slope value for *M. anisopliae* = 1.24 compared *B. bassiana* = 0.91 and *I. fumosorosea* = 0.73 indicates a more homogeneous larval population response to this isolate. Steeper slopes sindicates that field applications of *M. anisopliae* would produce more predictable mortality outcomes across heterogeneous larval populations [Robertson et al., 2017].

4.3 Speed of Kill (LT_{50}) and Time-Mortality Dynamics

High rate of kill speed is one of the factors determining the effectiveness of EPF in agricultural systems especially with regard to mobile pests such as *S. frugiperda* whose damage is economically significant after days of infestation. When tested at a concentration of 1×10^7 conidia mL^{-1}, *M. anisopliae* took 3.8 days compared to *B. bassiana* (5.2 days) and *I. fumosorosea* (7.1 days). This rate is particularly important for the production of sweet corn since the larvae eat the ears, making them not suitable for sale after 48–72 hours [Prasanna et al., 2018].

The concentration-dependent acceleration of mortality observed for all isolates (LT_{50} values decreasing at 1×10^8 mL^{-1}) is consistent with the inoculum intensity concept, where higher conidial loads reduce the time required for fungal proliferation to reach lethal thresholds within the hemocoel [Sisay et al., 2024]. The LT_{50} value for *M. anisopliae* at 1×10^8 mL^{-1} (2.6 days) is among the fastest reported for EPF against *S. frugiperda*.

The time-mortality kinetics revealed distinct infection profiles: *M. anisopliae* exhibited a steep mortality acceleration between days 2–4, with 52% of total mortality occurring within this 48-hour window. This early peak mortality pattern is characteristic of highly virulent isolates that rapidly overcome host immune defenses, likely through efficient production of cuticle-degrading enzymes (pr1, chitinase) and fungal toxins (destruxins in *Metarhizium*) [Butt et al., 2016]. In contrast, the gradual mortality progression

of *B. bassiana* and *I. fumosorosea* suggests slower cuticular penetration or reduced tolerance to larval hemolymph immune factors such as phenoloxidase and antimicrobial peptides [Ortiz-Urquiza & Keyhani, 2013].

4.4 Mycosis Confirmation and Koch's Postulates

The high mycosis rates observed (*M. anisopliae*: 94.2%, *B. bassiana*: 89.6%, *I. fumosorosea*: 84.5%) provide strong evidence that mortality was attributable to fungal infection rather than handling stress or bacterial opportunists. These rates exceed the 70–80% thresholds typically considered acceptable for mycosis confirmation in EPF bioassays [Toffa et al., 2023]. The diagnostic sporulation characteristics observed white powdery conidia for *B. bassiana*, olive-green structures for *M. anisopliae*, and pale purple/pink mycelia for *I. fumosorosea* are consistent with established species descriptions [Yang et al., 2022: 1]. The absence of fungal outgrowth on control cadavers further validates the experimental system. High mycosis confirmation rates are essential for accurate LC_{50} and LT_{50} calculations; low rates would indicate significant non-fungal mortality requiring Abbott's correction or replicate rejection [Inglis et al., 2012].

4.5 Sublethal Effects: Feeding Cessation, Growth Inhibition, and Pupation Disruption

The sublethal effects documented in this study represent a novel contribution to the EPF literature on *S. frugiperda*, as most previous studies report only acute mortality endpoints. The early feeding cessation induced by *M. anisopliae* (FCI = 54.3% at 24 hours, 82.6% by day 3 at 1×10^7 mL^{-1}) has profound implications for crop protection. Even before larval death, feeding suppression reduces the extent of foliar and ear damage an economic benefit that is not captured by mortality data alone. This anti-feedant effect likely results from fungal toxin activity (e.g., destruxins) or the physical discomfort of cuticular infection [Amiri et al., 1999].

The growth inhibition data (GII = 82.7% for Ma-Id survivors) reveals the substantial metabolic cost of fungal infection. Larvae that survive the acute infection period exhibit severe weight suppression, with many *M. anisopliae* -treated individuals showing negative weight gain. This reflects redirection of host resources toward immune defense and repair mechanisms, away from anabolic processes [Mani et al., 2023]. The observation that some larvae lost weight suggests catabolism of host tissues, possibly due to fungal nutrient sequestration within the hemocoel.

One of the finding is the high disruption of pupation in survivors (73.9%) of Ma-Id. This is because even larvae, which manage to survive for 7 days, will not be able to undergo metamorphosis successfully. Deformed pupae, larva-pupa intermediates, and pre-pupae deaths will ensure failure of the formation of an adult insect. A "delayed lethal effect" in EPF-host has been observed by others [Ullah et al., 2023] but not for *S. frugiperda* systematically. In terms of suppression of the pest population, disruption of pupation results in a reduction in reproductive potential in the next generation.

4.6 Comparative Virulence Ranking and Implications for Biocontrol

As can be seen from the virulence hierarchy of the pathogens, which places *M. anisopliae* above both *B. bassiana* and I. fumosorosea on all seven parameters analyzed, such a hierarchy provides a good starting point for selecting a biological control agent. The superior performance of *M. anisopliae* when compared with *B. bassiana* in studies targeting *S. frugiperda* mortality appears to be a species-related trait rather than a strain trait. Such a trait might include a superior capacity to degrade insect cuticle or produce toxins/avoid detection [Butt et al., 2016].

However, it should be noted that this ranking can vary depending on the type of pest targeted. For instance, Ullah et al. [2022]. reported that the *B. bassiana* isolate proved to be more pathogenic compared to *M. anisopliae* towards the peach-potato aphid *Myzus persicae*. This host specificity indicates the importance of selecting EPF isolates that match their target pests. It can be hypothesized that the indigenous nature of the isolates is another reason for their efficacy. Generally, indigenous isolates show a better ability to adapt to local climate conditions than commercially available foreign ones [Meyling et al., 2009; Goble et al., 2016]. Their high germination rates and fast rate of killing indicate adaptation to the local environment conditions.

Conclusion

This multi-parameter comparative study confirms the higher virulence of the native *M. anisopliae* isolate against the *S. frugiperda* larvae of third instar than that of *B. bassiana* and *I. fumosorosea*. This is because *M. anisopliae* has shown the lowest LC_{50} value (1.42×10^5 conidia mL^{-1}), the shortest LT_{50} value (3.8 days for a conidial concentration of 1×10^7 mL^{-1}), the highest infection rate (94.2%), quickest stopping of feeding (FCI greater than 80% within three days), maximum growth inhibition (82.7%) and maximum pupation disruption (73.9%). Based on the results, it is recommended that the use of M. anisopliae be considered for the sustainable biocontrol of fall armyworm in sweet corn production. The incorporation of sublethal parameters into the virulence assessment framework provides a more complete understanding of EPF efficacy than mortality data alone.

Declarations

Author Contribution

wrote the main manuscript text WA

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