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EFFICACY OF *BEAUVERIA BASSIANA* AND *METARHIZIUM ANISOPLIAE* AGAINST TOMATO LEAF MINER *PHTHORIMAEA ABSOLUTA* (MEYRICK)

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ABSTRACT

The tomato leaf miner *Phthorimaea absoluta* (Meyrick), earlier known by the scientific name *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is one of the most devastating global pests of tomatoes. Using indigenous entomopathogenic fungi can be a promising solution to manage it. Efficacy of EPF isolates on third-instar larvae was carried out in Ethiopia during 2021-2022. Ten larvae were inoculated with conidiaa suspensions with three replications. The result showed that larval mortality was significantly different ($p<0.05$) among concentrations and isolates. *Beauveria bassiana* isolates caused 70 to 81% mortality, the lowest LC_{50} was $1.87x10^4$ to $9.79x10^4$ conidia/ ml; and LC_{95} was $5.68x10^8$ to $1.10x10^{11}$ conidia/ml and shortest LT_{50} was 3.6 to 5.7 days. These were considered highly virulent while *Metarhizium anisopliae* determined as moderately virulent having 46 to 63% mortality (highest LC_{50} was $1.36x10^6$ to $4.94x10^7$ and LT_{50} was 4.3 to 8 days). Results also revealed $1x10^{10}$ conidia/ ml is the most effective dose while $1x10^6$ is the least effective.

Key words: *Beauveria bassiana*, Biological control, efficacy, Entomopathogenic fungi isolates, *Metarhizium anisopliae, Phthorimaea absoluta*, Tomato, Pathogenicity, control

Despite the economic importance of tomato (*Solanum lycopersicum*), its production is threatened by major insect pests and pathogens (Veres et al., 2020). Among common insect pests, tomato leaf miner (*Phthorimaea absoluta (Meyrick)* is the most economically devastating and seriously affects yield (Varela et al., 2003). For the first time, it was reported in Peru, in South America, in the tropical and subtropical regions. Since then it has spread globally wherever tomatoes are grown, causing significant yield reduction (Rwomushana et al., 2019). Since that time it has spread to Europe, Africa and Asia continents (Desneux et al., 2010). Further, it was detected in Turkiye and Ethiopia around eleven years ago (Kılıç 2010, Gashawbeza and Abiy, 2012). Today it is considered the most devastating key pest of tomato worldwide. Furthermore, it has a devastating lifecycle that severely damages tomato. Eggs are laid on the undersides of leaves, stems, and fruits. Upon hatching, larvae penetrate fruits and leaves, creating mines and galleries that cause necrosis (Biondi et al., 2018). By feeding within the mesophyll tissue of tomato leaves, larvae reduce tomato yields (Guedes and Picanço 2012). Then, the plant starts showing signs and symptoms including necrosis, deformation and drying of leaves, ruptured marks and exit holes under devastative attack (Rwomushana et al., 2019). Desneux et al. (2010) found that without intervention, *P. absoluta* can cause 80-100% annual yield losses in tomato crops and increase tomato market prices by 23%.

In spite of the implementation of various management strategies, such as early detection through sex pheromone traps, insecticide application, and the use of parasitoids and predators (Roditakis et al., 2012; Biondi et al., 2013; Sabbour and Nayera, 2012), the pest remains difficult to control. Furthermore, the challenges in managing *P. absoluta* include its short generation life, development of insecticide resistance, and cryptic behavior (Siqueira et al., 2001; Silva et al., 2015; Lietti et al., 2005). Consequently, there is a growing need for a paradigm shift from insecticides to more efficient, safer and eco-friendly pest management options. Biological control, particularly entomopathogenic fungi (EPF), has emerged as a promising alternative to insecticides for managing insect pests (Keçeci and Öztop, 2017; Vega and Kaya, 2012; CABI, 2021). Moreover, entomopathogenic species have virulence factors to cause disease and kill insect pests (Vega and Kaya, 2012). Entomopathogenic fungi (EPF) are one of the types of biological control agents to combat *P. absoluta* (CABI, 2021). Using naturally existing indigenous or exotic entomopathogen fungi species such as *Metarhizium anisopliae* and *Beauveria bassiana* are effective bioagents and have been recommended and

reported as they are pathogenic to tomato leaf miners (Youssef, 2015; Shiberu and Getu, 2017).

Previous studies have reported the effectiveness of indigenous and exotic EPF species, such as *Metarhizium anisopliae* and *Beauveria bassiana* against *P. absoluta* (Youssef, 2015; Shiberu and Getu, 2017). However, inconsistencies in the reported efficacy of these fungi have been observed (Inanli et al., 2012; Ndereyimana et al., 2019; Youssef, 2015; Getu and Shiberu, 2017; Tsoulnara and Port, 2016). Inanli et al. (2012) and Ndereyimana et al. (2019) conducted the efficacy test and found that *M. anisopliae* caused more mortality rate than B. *bassania* against larvae of *P. absoluta*. Contradicting these results Youssef (2015) and Getu and Shiberu (2017) reported that *B. bassiana* is more effective than *M. anisopliae*. This highlights a research gap in the efficacy testing of EPF strains and the need for further investigation to identify the most effective candidates for *P. absoluta* management. The main objective of this study was to evaluate the efficacy of isolated virulent Ethiopian and Turkish entomopathogen fungi isolates against third-instar larvae of *P. absoluta* under laboratory conditions.

MATERIALS AND METHODS

The laboratory experiments were conducted at the Crop protection department laboratory at Kulumsa Agriculture Research Center in Asella (8º.2' N, 39º.10'E, 2200 masl). During the experiments, the mean daily temperature in the laboratory was maintained at 24°C± 2. During experiments tomato plants, specifically cultivars Chali, was used as the primary hosts for rearing *P. absoluta* larvae. The tomato seedlings were initially planted in foam seedling trays and then transplanted into plastic pots filled with a soil peat and perlite mixture. The pots were placed in a climate-controlled room (25°C, 65% RH). The larvae were reared on the three-leaf stage seedlings. The larvae were provided with potted plants placed inside insect-rearing cages, with each cage containing ten adult *P. absoluta* collected from infested fields. The cages were covered with 32-mesh cloth to prevent other pests. The adults were fed a 10% sugar solution for two days and then removed. After the eggs hatched, the larvae were allowed to feed on the potted tomato plants until they reached the targeted third instar larvae stage. After two generations of larvae rearing, the larvae were used for the bioassay. The third instar larvae, aged 8 to 12 days old and measuring 3-6 mm in length, were harvested by opening the mines according to a provided key by Binu and Ajaya (2019).

The pathogenicity test was conducted on these larvae using eight different entomopathogenic fungi (EPF) isolates from Turkey (*M. anisopliae*: Ak-11, AK-12; *B. bassiana*: Ak-14, Ak-10) and Ethiopia (*B. bassiana*: B1, PPRC-56; *M. anisopliae*: M1, M2). The isolates were propagated by placing a single colony from each on a potato dextrose agar (PDA) medium and incubating it at 26°C in the dark. Once fully sporulated, the conidia were harvested and stored. The conidia suspensions were prepared by suspending dry powder conidia into 1ml of distilled sterilized water with two drops of 0.01% aqueous tween 20% in a 15 ml test tube, followed by vortexing for 1 min. The conidia suspensions were filtered through cheesecloth to discard agar particles and mycelial, and homogenized by vortexing as described in Getu and Shiberu (2017). Finally, the concentration of each isolate conidia suspension was adjusted to $1x10^6$, $1x10^7$, $1x10^8$, $1x10^9$ and $1x10^{10}$ conidia/ ml using a hemocytometer ready for inoculation (Kushiyev et al., 2018). Before inoculation, conidia viability was determined by spread-plating 0.1 ml of conidial suspension on PDA plates. The sterile microscopic cover slip was placed on each plate and plates were incubated at 26± 2^0C and examined after 15 hrs. Germination was determined from 100-spore counts, with each plate replicated four times. The effectiveness of virulent four *B. bassiana* and four *M. anisopliae* isolates with five conidian concentrations against *P. absoluta* larvae was tested using a randomized complete plot design with three replications.

The detached leaf method, as proposed by Ndereyimana et al. (2019) was used. Two-piece of fresh tomato leaf discs (5 cm^2) were cut and surface sterilized using 80% ethanol for 2 minutes and rinsed 3 times in sterile distilled water. Then leaves were dried and placed in a petri dish (10 cm dia) containing tissue paper and then the upper side of tomato leaves was placed with 10 third instar larvae of *P. absoluta* (3-6 mm) which were randomly selected from the stock colony using a fine paintbrush in each petri dish. Larvae were topically inoculated with 100 µl of each isolate's conidia suspension at concentrations ranging from 10^6 to 10^{10} conidia/ ml, while control treatments received distilled water with 0.02% Tween 20 (Getu and Shiberu, 2017). The petri dishes were sealed and placed in a humid incubator for 24 hrs. The larvae were then provided with fresh leaves daily. The experiments were repeated two times, and data on larval mortality and lethal concentrations were collected. The percentage mortality was calculated using the number of dead larvae compared to the total larvae. Abbott's formula

(1925) was used to correct the percentage mortality if necessary, except when the number of dead larvae in the treatment was the same as or less than that in the control (Singh and Zahra, 2017). Data normality was checked using arcsine transformation. The mortality percentage data was analyzed using a two-way ANOVA in the JMP software package, with a focus on the interaction effect between different isolates of entomopathogenic fungi and various concentrations. Tukey's honestly significant difference test ($P \leq 0.05$) was used to separate treatment mean values. Lethal time and concentration values for each isolate were obtained through probit analysis in SAS statistical analysis software (Version 9.4) based on Finney's method (1971).

RESULTS AND DISCUSSION

The susceptibility of third instar larvae to *B. bassiana* and *M. anisopliae* isolates and their conidia concentrations were determined. Analysis of variance showed significant differences ($p<0.05$) in virulence among *B. bassiana* and *M. anisopliae* isolates and their concentrations for causing third-instar larvae mortality (Table 1). After infection, larvae infected with isolates of *B. bassiana* and *M. anisopliae* exhibited visible disease symptoms and mycosis onset, such as folding down, morphological body color changes from brown to red, and eventually turning black and dead. Additionally, whitish mycelium growth and green sporulation were observed on the dead larvae infected with *B. bassiana* and *M. anisopliae* were observed respectively. Such typical symptoms and mycosis developed after larvae treatment with entomopathogenic fungi were described by Dabsu and Kovanci (2022) and Fergani and Yehia (2020). EPF isolates induced disease symptoms, and mycosis and result in mortality in larvae. Each isolates and conidia concentration differed in their ability to cause larvae mortality rate. It was observed that the mortality rate increased as the day after inoculation increased. The maximum larvae mortality was achieved by *B. bassiana* isolates, ranging from 65% to 81%, while 46 to 60% mortality was caused by *M. anisopliae* isolates at the end of post-inoculation. Most larvae inoculated with *B. bassiana* isolates were completely dead seven days after inoculation. On average, 80.86% mortality was recorded for larvae inoculated with B. *bassiana* B1, followed by *Beauveria bassiana* AK-10 which caused 77.43%, while M. *anisopliae* AK-12 caused 46.12% larvae mortality (Table 1). In all cases, *B. bassiana* showed higher mortality rates than *M. anisopliae* isolates. Thus, according to the current finding, *B. bassiana* is higher pathogenic, virulent, and effective than *M. anisopliae*. Similarly, some studies have been conducted and reported the pathogenicity and virulent difference between *B.bassiana* and *M.anisopliae* against *P.*absoluta. The current findings align with Shiberu and Getu (2017), who suggested that *B. bassiana* was more pathogenic than *M.anisopliae* against third-instar larvae. In accordance with the findings of Dabsu and Kovanci (2022), *B. bassiana* exhibited higher larvae mortality than *M. anisopliae* isolates against third-instar larvae. Similarly, Fite et al. (2019) pointed out that *B. bassiana* is more virulent than *M. anisopliae*. However, the current findings contradict Ndereyimana et al. (2020) finding who reported *M. anisopliae* was more virulent and pathogenic than *B. bassiana*.

As well as it was found that as conidia concentrations of EPF isolates increased, larvae mortality increased correspondingly. The highest concentrations (1×10^{10} and 1×10^{9} spores ml−1) exhibited mortality percentages were 86.38 and 82.21%, while the least concentration ($1x10^{6}$ spores/ ml) showed mortality percentages between 46 and 65% after seven days of inoculation. The maximum mean mortality of 86.38% was caused by the highest concentration being $1x10^{10}$ conidia/ml, while the lowest concentration of $1x10^{6}$ conidia/ ml resulted in 46% larvae mortality post of inoculation (Table 1). Mortality of *P. absoluta* third instar larvae exposed to *B. bassiana* and *M. anisopliae* increased with both increasing concentration and exposure time. According to the current result, larval mortality increased gradually with dosage and time. In all exposure duration, the mortality rates were maximum at the highest concentration ($1x10^{10}$ conidia/ ml) compared to the lower concentration ($1x10^{7}$ and $1x10^{6}$ conidia/ ml) and control. This indicates that higher concentrations led to higher mortality rates, making the mortality rate directly proportional to spore concentration and exposure times. It was reported by different authors that a significantly higher mortality rate was recorded at higher concentrations than at lower concentrations. In agreement with the current experiment.

Youssef (2015) reported that *B. bassiana* was more effective than *M. anisopliae* against *P. absoluta* larvae at $1x10^{8}$ spores/ ml. Similarly, Fite et al. (2019) pointed out that *B. bassiana* is more virulent than *M. anisopliae*. Likewise, Dabsu and Kovanci (2022) conducted the efficacy of EPF and found that *B. bassiana* caused more larval mortality than *M. anisopliae* against the third instar larvae at various conidia concentrations. The analysis of lethal concentration values revealed that *B. bassiana* isolates were the most toxic and virulent

Table 1. Mean efficacy of *Beauveria* and *Metaharizium* species isolates in different conidial concentrations against third-instar larvae of *P. absoluta* under laboratory conditions

Treatment	% larval mortality in Ethiopia		
	3 DAT	5 DAT	7 DAT
Entomopathogen fungi isolates			
Beauveriabassiana B1	22.22[a]	54.68 [a]	80.86[a]
Beauveriabassiana PPRC-56	18.47[a]	48.77[ab]	70.34[b]
Beauveriabassiana AK-10	21.25[a]	48.73[ab]	69.51[b]
Beauveriabassiana AK-14	18.06[a]	45.16[b]	64.48[bc]
Metarhzium anisopliae M1	7.80[b]	38.01[c]	59.43[c]
Metarhziumanisopliae M2	7.64[b]	37.78[c]	50.43[d]
Metarhziumanisopliae AK-11	7.50[b]	35.63[cd]	48.58[d]
Metarhziumanisopliae AK-12	7.22[b]	30.83[d]	46.12[d]
LSD (%5)	3. 13	4. 12	5.07
Concentrations (Conidia/ ml)			
$1x10^{10}$	22.50[a]	60.53[a]	86.38a
$1x10^{9}$	18.75[ab]	51.14[a]	82.21[a]
$1x10^{8}$	17. 19[b]	51.58[b]	74.78b
$1x10^{7}$	13. 13[c]	45.09[c]	64.67c
$1x10^{6}$	11.04[c]	33.51[d]	46.776[d]
0 (Water)	0.00[d]	6.67[e]	10.00[e]
LSD (%5)	2.71	5.48	4.39
CV (%)	24.38	22. 52	12.58

Note: Means values followed by the different letters are significantly different at α=0.05. DAT- days after treatment.

for controlling *P. absoluta* larvae. The lowest LC_{50} and LC_{95} values of 1.87E+04 and 5.68E+08 conidia/ ml were obtained against third-instar larvae with B-1, followed by PPRC-56 (LC50, 2.75E+04, LC95, 9.10E+09 conidia/ ml), AK-10 (LC50, 7.96E+04, LC95, 4.86E+10conidia/ ml) and AK-14 (LC50, 9.79E+04, LC95, 1.10E+11conidia/ml) after seven days of inoculation respectively (Table 2). In contrast, the highest LC_{50} and LC_{95} values were recorded for *M. anisopliae* AK-12 and AK-11 were found to be 4.94E+07, 1.17E+07, 6.99E+15, and 2.17E+14 conidia/ ml in Ethiopia respectively. These findings align with previous research on concentration-dependent mortality experiments using various conidia suspensions in laboratory settings. For instance, Sabbour (2014) reported LC_{50} values of $1.02x10^{6}$ spores/ml for *B. bassiana* and $1.00x10^{6}$ spores/ ml for *M. anisopliae* at 8.25×10^{8} conidia. Similarly, Wekesa et al. (2006) found LC_{50} values of 0.7×10^{7} and 2.5×10^{7} conidia ml^{-1} for *B. bassiana* and *M. anisopliae*, respectively. Consistent with Dabsu and Kovanci's (2022) findings, it was demonstrated that *B. bassiana* isolates had the lowest LC_{50} and LC_{90} values against third-instar larvae, while $_{M}$. *anisopliae* exhibited the highest lethal concentration values.

In the probit analysis, the LT_{50} values ranged from 3.6 to 8 days, while LT_{95} values were between 7 and 25 days for all isolates at each conidia concentration (Table 3). The lowest LT_{50} and LT_{95} values for *B. bassiana* B-1 isolate were found to be 3.6 and 7 days, followed by AK-10 isolate with 3.8 and 8 days at $1x10^{10}$ conidia/ ml respectively while *M. anisopliae* AK-12, AK-11, M1 and M-2 had longer incubation periods (LT_{50}, 5.4, 4. 9, 4.8 and 4.6 days) to kill 50% of tested third instar larvae population. The corresponding LT_{95} values for these isolates were 12, 10, 10, and 8 days at the same conidia concentration to induce 95% larval mortality in the same order. Consequently, considering the shortest incubation period for both LT_{50} and LT_{95} values, *B. bassiana* B-1 isolate could be considered the most pathogenic isolate, followed by *B. bassiana* AK-10 and PPRC-56. On the other hand, *M. anisopliae* Ak-12 showed moderately aggressive and had the highest lethal time followed by *M. anisopliae* Ak-11 isolate. These finding align with a study by Ozdemir et al. (2020), who reported the lethal time causes of 50%

Table 2. The lethal concentration effect of *Beauveria bassiana* and *Metarhizium anisopliae* isolates against *T. absoluta* third instar larvae after 7th days of inoculation under laboratory conditions

EPF isolates	LC50 values	LC95 values	95% FL for LC50	Slope± SE	$\chi2$ (df=4)[a]
Metarhziumanisopliae AK-12	4.94E+07	6.99E+15	4.94E+06-6.55E+08	0.202± 0.039	4.12
Metarhziumanisopliae AK-11	1.17E+07	2.17E+14	1.27E+06-9.50E+07	0.20± 0.037	4.68
Metarhziumanisopliae M-2	5.37E+06	1.09E+15	3.90E+05-5.50E+06	0.20± 0.037	0.73
Metarhziumanisopliae M-1	6.05E+05	5.98E+11	5.89E+04-3.62E+06	0.27± 0.041	5.15
Beauveriabassiana AK-14	9.79E+04	1.10E+11	5.06E+03-5.62E+05	0.27± 0.039	2.06
Beauveriabassiana AK-10	7.96E+04	4.86E+10	7.93E+03-6.39E+05	0.29± 0.041	2.37
Beauveriabassiana PPRC-56	2.75E+04	9.10E+09	1.89E+03-1.92E+05	0.298± 0.041	3.89
Beauveriabassiana B-1	1.87E+04	5.68E+08	1.34E+03-1.28E+05	0.31± 0.042	0.40

Notes: X2a-Chi-square is insignificant; FL = fiducial limits; df = degree freedom of a number of concentrations (n-2=4)

Table 3. The mean values of lethal time in days to kill 50% and 95% of third instar larvae inoculated with four EPF isolates at different concentrations

EPF isolates	Conidia/ml	Lethal time (days)				
		LT50	LT95	Slope± SE	95% FL for LT50	$\chi 2$ (dfa)
	1.00E+10	3.6	7.0	5.20± 1.1	3.0-4.0	1.47
	1.00E+09	3.8	7.0	5.67± 1.1	3.33-4.5	1.62
Beauveria bassiana B1	1.00E+08	4.1	8.0	5.01± 1.03	3.0-5.0	0.34
	1.00E+07	4.8	10.0	4.28± 0.97	4.0-6.0	0.01
	1.00E+06	4.9	14.5	3.52± 0.95	4.05-6.20	0.75
	1.00E+10	3.8	8.0	4.90± 1.04	3.0-4.0	0.84
	1.00E+09	4.1	8.0	4.94± 1.02	3.5-4.7	0.75
Beauveria bassiana AK-10	1.00E+08	4.5	10.0	4.38± 1.64	-	2.79
	1.00E+07	5.0	16.0	3.4± 0.96	4.0-7.0	0.38
	1.00E+06	5.0	16.0	3.3± 0.95	4.0-6.0	0.54
	1.00E+10	3.9	7.0	5.7± 1.08	3.0-4.0	0.82
	1.00E+09	4.0	7.9	5.51± 1.07	3.3-4.5	0.46
Beauveria bassiana PPRC-56	1.00E+08	4.0	8.0	5.5± 1.1	3.0-5.0	0.86
	1.00E+07	4.2	8.0	5.09± 1.03	3.4-4.6	0.09
	1.00E+06	5.7	18.0	3.3± 0.97	4.7-8.0	2.19
	1.00E+10	4.0	9.0	4.63± 1.01	3.33-6.7	0.99
	1.00E+09	4.3	-	4.53± 0.99	3.5-4.9	0.70
Beauveria bassiana AK-14	1.00E+08	4.6	11.0	4.17± 0.98	3.0-6.0	0.55
	1.00E+07	5.0	10.0	5.2± 1.07	4.0-6.0	2.63
	1.00E+06	5.6	16.6	3.5± 0.98	4.7-7.5	1.07
	1.00E+10	4.8	10.0	4.62± 1.01	4.0-5.7	0.010
	1.00E+09	5.6	13.0	4.38± 1.04	4.8-6.8	0.55
Metarhizium anisopliae M1	1.00E+08	5.8	12.0	4.95± 1.12	5.0-7.0	0.20
	1.00E+07	6.0	16.0	4.35± 1.14	5.0-6.0	0.06
	1.00E+06	6.0	15.0	4.2± 1.09	5.0-8.0	0.93
	1.00E+10	4.6	8.0	6.35± 1.13	4.0-5.0	0.607
	1.00E+09	4.9	8.0	6.81± 1.2	4.5-5.6	2.57
Metarhizium anisopliae M2	1.00E+08	5.3	10.0	5.17± 1.08	4.0-6.0	0.03
	1.00E+07	6.0	14.0	4.6± 1.14	5.0-8.0	0.01
	1.00E+06	6.0	15.0	4.2± 1.09	5.0-8.0	0.93
	1.00E+10	4.9	10.0	5.28± 1.06	4.4-5.7	0.25
	1.00E+09	5.3	11.0	5.18± 1.08	4.7-6.2	0.01
Metarhizium anisopliae AK-11	1.00E+08	6.0	16.0	3.91± 1.04	5.0-8.0	0.27
	1.00E+07	7.0	19.0	3.9± 1.14	6.0-12.0	0.06
	1.00E+06	7.0	19.0	3.8± 1.1	6.0-12.0	0.01
	1.00E+10	5.4	12.0	4.67± 1.07	4.6-6.5	0.02
	1.00E+09	5.8	12.0	4.67± 1.07	4.9-7.0	0.09
Metarhizium anisopliae AK-12	1.00E+08	6.0	16.0	3.94± 1.045	5.0-8.0	0.01
	1.00E+07	8.0	25.0	3.4± 1.14	6.0-22.0	0.08
	1.00E+06	8.0	25.0	3.4± 1.14	6.0-22.0	0.08

Notes: [a]χ^2- Chi-square value not significant at α=0.05 level, indicating a good fit of the probit model, DF-degree freedom of a number of days (n-2=1), FL = fiducial limits, LT50- Lethal time that caused 50% mortality, LT95- Lethal time that caused 95% mortality.

(LT_{50}) and 90% (LT_{90}) of *M. anisopliae* were 4.45 and 5.34 and *B. bassiana* were 4.07 and 5.11 days at 1×10^8 conidia/ml respectively. It is also nearly consistent with the study by Shiberu and Getu (2017), who found the lowest LT_{50} values of 4.8 and 5.2 days and LT_{95} values of 8.14 and 8.06 for *M. anisopliae* and *B. bassiana* at $2.5x10^9$ conidia/ ml respectively. Similarly, Aynalem et al., (2020) reported a similar result that the toxicity of *M. anisopliae* was LT_{50}=3.9 days. In contrast to our findings, Ndereyimana et al. (2019) reported that the LT_{50} values for *M. anisopliae* and *B. bassiana* were 3.9 and 5.2 days respectively.

In general, based on the current pathogenicity test and generated data, significant differences in the pathogenicity levels of *B. bassiana* and *M. anisopliae* isolates were found ($P<0.05$) among isolates and concentrations in terms of larvae mortality rates. All tested isolates at different conidia concentrations caused compatible reactions and showed degree aggressiveness variations to third instar larvae. *B. bassiana* isolates were found to cause the highest mortality rate, the shortest lethal duration and the lowest lethal dose, and were substantially more effective in all the tested larvae in their ability to induce larval mortality, On the other hand, *M. anisopliae* isolates exhibited the least larval mortality, the longest incubation period and the highest lethal concentrations, indicating relatively lower pathogenicity and moderate virulence. Considering the present investigation indicated the possible pathogenicity difference between EPF isolates and concentrations, more research should be undertaken on the response to *P. absoluta* infection. More research is required to test the efficiency of highly virulent isolates against different *P. absoluta* larval instars in the field.

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AUTHOR CONTRIBUTION STATEMENT

All authors collaboratively contributed to the conception, design and execution of the research project, ultimately leading to significant findings.

CONFLICT OF INTEREST

There are no conflict of interests.

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