Journal of
Current Opinion in Crop Science

Journal homepage: www.jcocs.com; ISSN(O):2583-0392

RESEARCH ARTICLE

Efficacy of the Key Isolated Entomopathogenic Fungi for the Biocontrol against *Tuta absoluta* in Tomato Plants

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Article history:

Received: March 01, 2023
Accepted: March 29, 2023
Published: March 31, 2023

Citation:

Magwe, M. M., Chilagane, L. A., & Mulungu, L. S. (2023). Efficacy of the Key Isolated Entomopathogenic Fungi for the Biocontrol against *Tuta absoluta* in Tomato Plants. *Journal of Current Opinion in Crop Science, 4*(1), 25-37.

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ABSTRACT

The tomato is an important vegetable crop, both domestically and commercially. Recently, the crop is facing the problem of insect pest destruction, which causes its production to drop by up to 50% if not controlled. Tomato leaf miner, *Tuta absoluta* (Meyrick) is one of the most destructive insects of tomato. The entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae are* currently used as an efficient biological control agent against *Tuta absoluta*. In this study, the efficacy of entomopathogenic fungi was tested using EPF obtained from 24 soil samples collected from tomato farms infested by *T. absoluta*. The efficacy of EPF were tested against *Tuta absoluta* larvae instars, pupa, and adults at different conidial concentrations and incubation times. The results showed that among all EPF, *Metarhizium anisopliae* had high pathogenicity and recorded a high percentage of *Tuta absoluta* larval mortality rate (98.2%), followed by *Aspergillus spp.,* which recorded 71%. Other EPF recoded lower percentages, which imply lower pathogenicity. Despite *Aspergillus spp.* being entomopathogenic fungi that have high pathogenicity for *Tuta absoluta,* it is not recommended for *Tuta absoluta* management in tomato production due to aflatoxin, which is very toxic to humans. Based on these observations, *Metarhizium anisopliae* has the potential to be used as an effective biological control of *Tuta absoluta* in tomato production.

Keywords: Biological control, Entomopathogenic fungi, *Tuta absoluta*, Tomato.

INTRODUCTION

Tomato leaf miner, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) is one of the invasive and highly destructive pests attacking tomato and other Solanaceae plants (Smith et al., 2018). Depending on the degree of the infestation, *T. absoluta* attacks tomato plants at all developmental stages and can cause considerable crop losses of up to100% in both greenhouses and open field if not controlled timely (Chidege *et al.,* 2016). The damage caused by *T. absoluta* is approximately 80% significantly when exposed tomato plants to secondary infection by facilitating the entry of pathogens (Rwomushana et al., 2019).

Tuta absoluta lifecycle has four developmental stages namely; egg, larva, pupa and adult. The pest has high reproductive rate between 10 to 12 generations per year (Chidege et al., 2016; Ramzi et al., 2018). Female moths lay eggs on different plant parts (i.e., the underside of leaves, petioles, blossom, fruit), either singly or in small batches. Each female can lay up to a maximum of 250-300 eggs during its lifetime. Female insects usually deposit eggs on the underneath of tomato leaves or stems and on immature fruits. *Tuta absoluta* larva is the most destructive stage that consumes leaves, stems and fruits of the tomato plant by hiding within mesophyll of the plant tissues (Ramzi et al., 2018). The pest has high demographical potential, which is due to a short generation time, wide host range, a good thermal adaptability and ability to develop insecticide resistance (Biondi *et al*., 2018; Ramzi et al., 2018).

Several management strategies including biological control as well as application of synthetic and botanical pesticides have been attempted against *T. absoluta*. Geraldin *et al*. (2016) reported that plant extracts from turmeric (*Curcuma longa*), lemon (*Citrus lemon*), garlic (*Allium sativum*) and ginger (*Zingiber officinale*) have insecticidal activities due to phytochemicals that exhibit varied modes of action hence reduced populations of *T. absoluta* under field conditions. Turmeric and ginger contain aromatic compounds (curcuminoids, turmerones and zingerone) that affect growth and development of *T.absoluta* while garlic contains compounds that affect the neuro-system of insects (Tabassum et al., 2013). Neem (*Azadrachtca indica*) contains alkaloids that affect the reproductive and digestive system of *T. absoluta* (Yankova et al., 2014). *Moringa oleifera* contains flavonoid which is potential by delaying digestion and disturbing the molting of the *T. absolut*a (Shah et al., 2017). Natural chemicals from plants are cheap, readily available and cost-effective in developing countries where synthetic insecticides are expensive especially for poor farmers.

Insecticides with active ingredients such as Imidacloprid, Indoxacarb, Spinosad or Deltamethrin have been recommended for *T. absoluta* management in Tanzania (Santos et al., 2011; MOA, 2020). Synthetic pesticides were previously used as a sole control method, but this method has been declining with time due to resistance created by the pest and farmers tend to use multiple combinations of active ingredients. *T. absoluta* has become less responsive and has highly developedresistance to dozens of synthetic insecticides and difficulties have been experienced when botanical pesticides and cultural practices are utilized alone (Roditakis et al., 2015; Illakwahhi and Srivastava, 2017). Common chemicals that tomato leaf miner has been reported to develop resistance are cypermethrins, pyrethroids, organophosphates, spinosad, mamectinbenzoate, bamectin, chloride channel activators, benzoylure and diamide.

Previous studies have shown that biocontrol stands out as an alternative strategy to synthetic insecticides and has more promising impact against *T. absoluta* (Tadele and Emana, 2017; Ayele et al., 2020). The application of bio-control agents like insect pathogens such as entomopathogenic fungi (EPF) have been proposed as a highly promising alternative strategy for the protection of crops against insect pests (Maina *et al*., 2018). EPF penetrates the integument, followed by multiplication in the hemocoel and relatively infect/colonize the tissues, causing insect's death (Silva et al., 2020). Parasitoids like *Trichogrammaevanesens* (Polaszek et al., 2012; Sabbour, 2014) and microbial control including fungi *Beauveriabassiana*, and *Metarhizium anisopliae* (Pires et al., 2009; Contreras et al., 2014; Ndereyimana et al., 2019) and bacteria such as *Bacillus thuriengiensis* (Sabbour, 2014) are sought to be efficient for management of *T. absoluta*. *M. anisopliae* has been reported to affect both egg and first larval stage of *T. absoluta* (Santi et al., 2011; Inanli et al., 2012) and has been reported to parasitize eggs of *T. absoluta* (Pires et al., 2009).

Attempts have been made for the search of more effective EPF species to control *T. absoluta* in Tanzania (Zekeya *et al*., 2019). However, only two fungal isolates (A-Tz1 and A-Tz2) of *Aspergillus oryzae* have been evaluated against *T. absoluta* in Tanzania so far. The fungus caused 70% larval mortality 3 days post inoculation and consequently

inhibited pupation by 84.5% and adult emergence by 74.4%. Nevertheless, information on the pathogenic effects of native EPF isolates from different *T. absoluta* hot-spot areas in Tanzania and their usage against the pest is missing. Therefore, in the present study, the key isolated EPF *Beauveria spp.,* and *Metarhizium spp.,* that were discovered from different soil types are indexed for their biological efficiency and evaluated for their pathogenicity against *T. absoluta*.

MATERIALS AND METHODS

Description of isolates collection and identification

Twenty-four (24) soil samples collected from different agro ecological zones of Tanzania were taken to Sokoine University of Agriculture for culturing, isolation of fungi, incubation, DNA extraction, and PCR analysis. EPF were isolated from soil samples collected from twenty-four (24) tomato farms infested by *T. absoluta* in Arusha and Morogoro. The soil samples were collected around the root zones of the affected plants. A stratified sampling method was used during soil sample collection. At each sampling location, a total of five soil samples were sampled, mixed to form one sample, and 10 grams of the mixture were taken to represent the five samples. The soil was taken from 0–15 cm deep using a soil auger with a 5-cm diameter. Plastic containers were used to store the collected soil samples. EPF were isolated from soil samples using a semi-selective medium as per Meyling and Eilenberg (2006). Cultures were maintained at 25^{o}C in dark and examined after four to seven days. The growths of fungi were confirmed, and conidia characteristic of EPF were transferred into PDA, where purification was done. All isolates were subsequently propagated from a single conidium. A suspension (1µl) containing a low density of conidia in distilled water was spread onto the PDA. A single germinated conidium was separated from other conidia and identified by compound microscope. A piece of medium encompassing only the target conidium was removed and transferred to the PDA, and the culture was maintained at room temperature.

DNA from the EPF mycelia was extracted using TES extraction buffer by the method described by Mahuku (2004) with some modifications. Fungal PCR amplification was completed using the ITS 1 and ITS 4 primer set to amplify the internal transcribed spacer (ITS) region (White *et al*., 1990). The PCR conditions of 94°C for 4 min for initial denaturation, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 56°C and elongation at 72°C for 1 min, followed by another cycle of final elongation at 72°C for 4 min.

PCR reaction was carried out in 25 µl reactions containing 1µl of both reverse and forward primer, 12.5 µl of 2x Taq master mix, 9 µl of PCR water, and 1.5 µl of DNA template. The expected sizes were 580 to 750 bp amplifying internal transcribed region gene. Then PCR products were sentto South Africa at the Inquaba laboratory for sequensing and isolate identification.

Establishment of *Tuta absoluta* cohort

The culture of *T. absoluta* was initiated from soil and leaves collected from farmer's fields, infected leaves were then incubated at 25±2°C, relative humidity (RH) 75±5% and a photoperiod of 12:12 (L: D) hr (Biondi *et al*., 2012). The emerged *T. absoluta* collected and reared on tomato plants in the green house. Then twenty adult pairs were introduced to the rearing cages containing tomato seedlings 40–45 days from the sowing date. At this age, the plants are suitable for egg laying. After 24 hours, all adults of *T. absoluta* were removed, and thereafter, the four larval instars from the infested plant leaves were collected and used to conduct various experiments.

Preparation of entomopathogenic fungi concentrations

Culturing and isolation were made from twenty-four (24) soil samples collected around the root zones of tomato plants from tomato farms infested by *T. absoluta* in Arusha and Morogoro. Cultures were maintained at 25^{o}C in dark and examined after four to seven days. The growths of fungi were confirmed, and conidia characteristic of EPF were transferred into PDA, where purification was done. All isolates were subsequently propagated from a single conidium. A suspension (1µl) containing a low density of conidia in distilled water was spread onto the PDA. A single germinated conidium was separated from other conidia and identified by compound microscope, A piece of medium encompassing only the target conidium was removed and transferred to the PDA, and the culture was maintained at room temperature. After 10–15 days, conidial spores were gently scraped from PDA in Petri dishes and suspended in 10 ml of distilled water with 0.1% Triton X-100 to form a stock suspension. Haemocytometer neubaeur was used to count spores from each stock suspension of isolates. (Manfield, German). After mixing the suspension in one beaker, the stock solution concentration was set at 1×10^{10} conidial/ml. in which working concentrations were prepared by the addition of

water, followed by counting spores using a haemocytometer neubaeur at a temperature range of 25.5°C under a stereo microscope to obtain 1.0 × 10^8, 1.0 × 10^7 and 1.0 × 10^6 conidia/ml for EPF test against stages of *T. absoluta.*

Pathogenicity of isolates against *T. absoluta* larva

Isolates were tested for pathogenicity against second-instar larvae at 19.5°C, 50% RH, 30.4°C, and 70% RH (the lowest and optimal, respectively). The factorial experiment used tomato leaflets from a screen house (6 × 7 cm area) as experimental units that were dipped into isolates (1.0 × 10^6, 1.0 × 10^7, and 1.0 × 10^8 conidia/ml) and control (water with 0.1% Triton X-100). The treated leaflets were blotted at room temperature to remove excess water and placed in Petri dishes (21 cm in diameter) lined with moist paper towel and cotton covering the petiole to prevent leaf dehydration. Ten *T. absoluta* larvae were put into each of the three treated leaflets and the control. Each concentration had 10 larvae that reproduced three times. The experiment started with treating leaflets only once. After 24 hours, larvae were fed new, untreated leaves. The fungus was re-isolated by placing dead larvae on a moist paper towel-lined Petri dish. The study captured data every 24 hours and observed trials until *T. absoluta* completed its life cycle and lifespan.

Data Collection

The following data were collected from the study: larval survival duration; number of dead larvae; number of active larvae (treated larvae that persisted), and number of adults emerged from total treated pupae, and number of adult emerged.

Data Analysis

The SAS General Linear Model method, version 9.1 (SAS Institute, Cary, NC, USA), was used to evaluate data for normality and variance homogeneity. Mortality rate, larval mortality, and adult life duration were converted and analysed using ANOVA and Kruskal Wallis.

RESULTS

Isolates (EPF) identification

Out of the sixteen isolates that were sent to the laboratory for sequencing, only ten isolates turned out to be identified as entomopathogenic fungi after sequence alignment and BLAST search. The other six sequences were not elated in any source as entomopathogenic fungi. The ten EPF identified are the ones used in this study to assess their efficacy in the control of *Tuta absoluta* in Tomato.

Tuta absoluta larvae mortality

The highest larvae mortality (53.3%) was recorded on *Metarhizium anisopliae* at 6th and 7th day after incubation, whereas no dead larvae (0%) was recorded on *Geotrichum candidum* at the initial incubation time (day 1), followed by the 2nd day of incubation (0.3%), (Figure 1).

Tuta absoluta active pupa following seven days of entomopathogenic fungi exposure

The results show that, significantly (p<0.001) highest active pupa (100%) was recorded on *Geotrichum candidum* at the initial incubation time (day 1), whereas the lowest active pupa (46.7%) was recorded on *Metarhizium anisopliae* at 6^{th} and 7^{th} day of incubation (Figure 3).

Interaction effect of entomopathogenic fungi and conidial concentration on *T. absoluta* active pupa

Highest active pupa (94.3%) was recorded on *Cladosporium cladosporioides s*at 1.0×10^8, conidial concentrations (Figure 4). Whereas, the lowest active pupa (4.9%) was recorded on *Metarhizium anisopliae* at 1.0×10^6conidial concentrations.

DISCUSSION

Effect of entomopathogenic fungi, conidial concentration and incubation duration on *T. absoluta* survival and mortality

Pathogenicity of the tested entomopathogenic fungi (EPFs) and conidial concentrations against motile stages of *T. absoluta* with respect to incubation time (Table 1) showed that there was a significant difference between the tested EPFs, conidial concentrations, and incubation time on percentage of dead larvae, active pupa, and active adults. Metarhizium anisopliae had the highest percentage of dead larvae (45.9%), the lowest percentageWhereas, *Geotrichum candidum* had the lowest percentage of dead larvae (1.8%), the highest percentage of active pupa (98.2%), and the most active adults (98.2%) than other tested EPFs (Maina et al., 2018).

The highest percentage of dead larvae (47.7%), the lowest percentage of active pupa (52.3%) and the highest percentage of active adults (52.3%) were recorded at 1.0×10^6 conidial concentrations compared to other tested conidial concentrations. Whereas the control (0.1 Tween solutions) recorded no dead larvae (0%), the highest percentage of active pupa (100%) and active adults (100%) than other tested conidial concentrations.

Table 1. Effect of entomopathogenic fungi, conidial concentration and incubation duration on *T. absoluta* survival and mortality

	Dead larvae	Active pupa	Active adults
	Percentage (%)	**Percentage (%)**	**Percentage (%)**
Factor A (Entomopathogenic fungi)			
Metarhizium anisopliae	45.9±2.39h	54.1±2.39a	54.1±2.39a
Aspergillus flavus	31.7±1.68g	68.3±1.68b	68.3±1.68b
Cladosporium oxysporum	29.3±1.52f	70.7±1.52c	70.7±1.52c
Aspergillus oryzae	28.6±1.48f	71.4±1.48c	71.4±1.48c
Aspergillus fimeti	23.8±1.34e	76.2±1.34d	76.2±1.34d
Aspergillus terrius	20.4±1.24d	79.6±1.24e	79.6±1.24e
Penicillium polonicum	19.8±1.20d	80.2±1.20e	80.2±1.20e
Cladosporium cladosporioides	14.1±1.04c	86.0±1.04f	86.0±1.04f
Penicillium pinophilum	8.5±0.73b	91.5±0.73g	91.5±0.73g
Geotrichum candidum	1.8±0.30a	98.2±0.30h	98.2±0.30h
Mean	22.39	77.61	77.61
P-value	<0.001	<0.001	<0.001
Factor B (Conidial concentration - ml)			
1.0×10^{6}	47.7±1.04d	52.3±1.04a	52.3±1.04a
1.0×10^{7}	28.1±0.80c	71.9±0.80b	71.9±0.80b
1.0×10^{8}	13.8±0.56b	86.2±0.56c	86.2±0.56c
Control (0.1 Tween solution)	0.0±0.00a	100.0±0.00d	100.0±0.00d
Mean	22.39	77.61	77.61
P-value	<0.001	<0.001	<0.001
Factor C (Incubation duration)			
Day 1	9.4±0.85	90.6±0.85d	90.6±0.85d
Day 2	16.3±1.17b	83.7±1.17c	83.7±1.17c
Day 3	22.9±1.34c	77.1±1.34b	77.1±1.34b
Day 4	26.4±1.38d	73.6±1.38a	73.6±1.38a
Day 5	27.1±1.38d	72.9±1.38a	72.9±1.38a
Day 6	27.3±1.38d	72.7±1.38a	72.7±1.38a
Day 7	27.3±1.38d	72.7±1.38a	72.7±1.38a
Mean	22.39	77.61	77.61
P-value	<0.001	<0.001	<0.001
Interaction			
A x B	<0.001	<0.001	<0.001
A x C	<0.001	<0.001	<0.001
B x C	<0.001	<0.001	<0.001
A x B x C	<0.001	<0.001	<0.001

Means bearing the same letter(s) within a column are not significantly different at $p = 0.05$

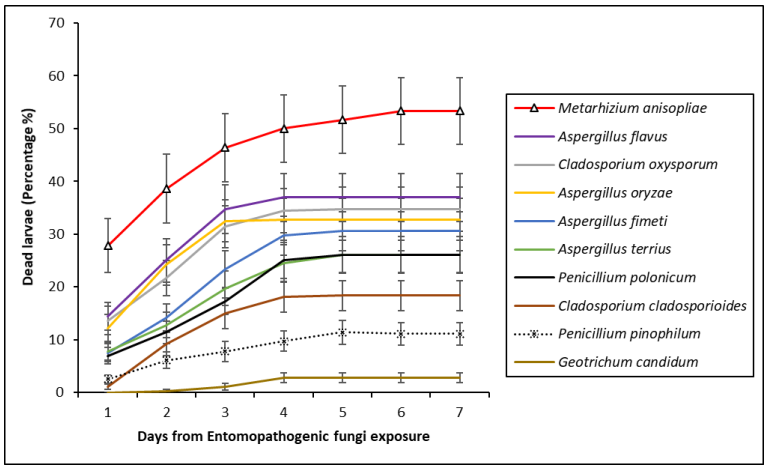

Figure 1. *T. absoluta* larvae mortality following seven days of entomopathogenic fungi exposure

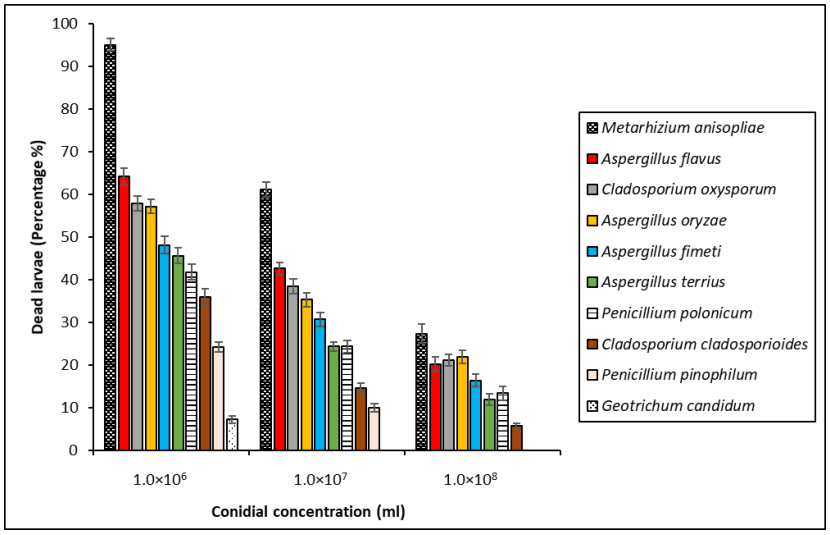

Figure 2. Interaction effect of entomopathogenic fungi and conidial concentration on T. absoluta larvae mortality

Table 2. Interaction effect of entomopathogenic fungi, conidial concentration and days of exposure on *T. absoluta* larvae mortality

	Concentrations (conidia/ml)	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Metarhizium anisopliae	1.0×10^6	71.1±3.09[a]	94.4±2.94	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	1.0×10^7	38.9±3.09	52.2±2.78	61.1±3.09	66.7±3.33	68.9±3.51	70.0±3.33	70.0±3.33
	1.0×10^8	1.1±1.11	7.8±3.24	24.4±1.76	33.3±1.67	37.8±2.78	43.3±3.33	43.3±3.33
Aspergillus flavus	1.0×10^6	33.3±3.73	55.6±2.42	71.1±2.61	72.2±2.22	72.2±2.22	72.2±2.22	72.2±2.22
	1.0×10^7	23.3±2.36	37.8±4.65	45.6±2.94	47.8±2.22	47.8±2.22	47.8±2.22	47.8±2.22
	1.0×10^8	1.1±1.11	6.7±2.89	22.2±3.24	27.8±2.22	27.8±2.22	27.8±2.22	27.8±2.22
Cladosporium oxysporum	1.0×10^6	35.6±4.44	47.8±4.34	63.3±2.36	64.4±2.42	64.4±2.42	64.4±2.42	64.4±2.42
	1.0×10^7	14.4±2.94	28.9±2.00	38.9±2.61	46.7±2.89	46.7±2.89	46.7±2.89	46.7±2.89
	1.0×10^8	4.4±1.76	10.0±2.36	23.3±2.36	26.7±1.67	27.8±1.47	27.8±1.47	27.8±1.47
Aspergillus oryzae	1.0×10^6	34.4±3.38	54.4±3.38	62.2±2.78	62.2±2.78	62.2±2.78	62.2±2.78	62.2±2.78
	1.0×10^7	13.3±2.89	33.3±3.73	40.0±2.89	40.0±2.89	40.0±2.89	40.0±2.89	40.0±2.89
	1.0×10^8	1.1±1.11	8.9±2.61	27.8±2.22	28.9±2.00	28.9±2.00	28.9±2.00	28.9±2.00
Aspergillus fimeti	1.0×10^6	21.1±3.09	33.3±3.33	51.1±2.00	57.8±2.22	57.8±2.22	57.8±2.22	57.8±2.22
	1.0×10^7	7.8±2.22	20.0±2.36	31.1±2.00	38.9±2.00	38.9±2.00	38.9±2.00	38.9±2.00
	1.0×10^8	1.1±1.11	3.3±2.36	11.1±3.89	22.2±2.22	25.6±1.76	25.6±1.76	25.6±1.76
Aspergillus terrius	1.0×10^6	21.1±2.00	31.1±3.09	44.4±3.38	55.6±1.76	55.6±1.76	55.6±1.76	55.6±1.76
	1.0×10^7	10.0±2.36	16.7±2.89	27.8±1.47	28.9±1.11	28.9±1.11	28.9±1.11	28.9±1.11
	1.0×10^8	0.0±0.00	3.3±3.33	6.7±3.73	13.3±2.36	20.0±1.67	20.0±1.67	20.0±1.67
Penicillium polonicum	1.0×10^6	20.0±2.36	27.8±2.78	35.6±2.42	52.2±2.22	52.2±2.22	52.2±2.22	52.2±2.22
	1.0×10^7	7.8±2.22	10.0±2.89	23.3±2.36	32.2±1.47	32.2±1.47	32.2±1.47	32.2±1.47
	1.0×10^8	0.0±0.00	7.8±3.24	10.0±2.89	15.6±1.76	20.0±5.27	20.0±5.27	20.0±5.27
Cladosporium cladosporioides	1.0×10^6	3.3±1.67	27.8±3.64	42.2±2.78	44.4±1.76	44.4±1.76	44.4±1.76	44.4±1.76
	1.0×10^7	1.1±1.11	7.8±2.22	13.3±2.36	20.0±1.67	20.0±1.67	20.0±1.67	20.0±1.67
	1.0×10^8	0.0±0.00	1.1±1.11	4.4±1.76	7.8±1.47	8.9±1.11	8.9±1.11	8.9±1.11
Penicillium pinophilum	1.0×10^6	6.7±2.36	21.1±2.00	25.6±1.76	26.7±2.61	31.1±1.67	28.9±1.11	28.9±1.11
	1.0×10^7	3.3±1.67	3.3±1.67	5.6±2.42	12.2±2.22	14.4±1.76	15.6±1.76	15.6±1.76
	1.0×10^8	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Geotrichum candidum	1.0×10^6	0.0±0.00	1.1±1.11	4.4±2.42	11.1±2.00	11.1±2.00	11.1±2.00	11.1±2.00
	1.0×10^7	0.0±0.00	0.0±0	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
	1.0×10^8	0.0±0.0	0.0±0	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
L.S.D	5.442							
p-value	<0.001							

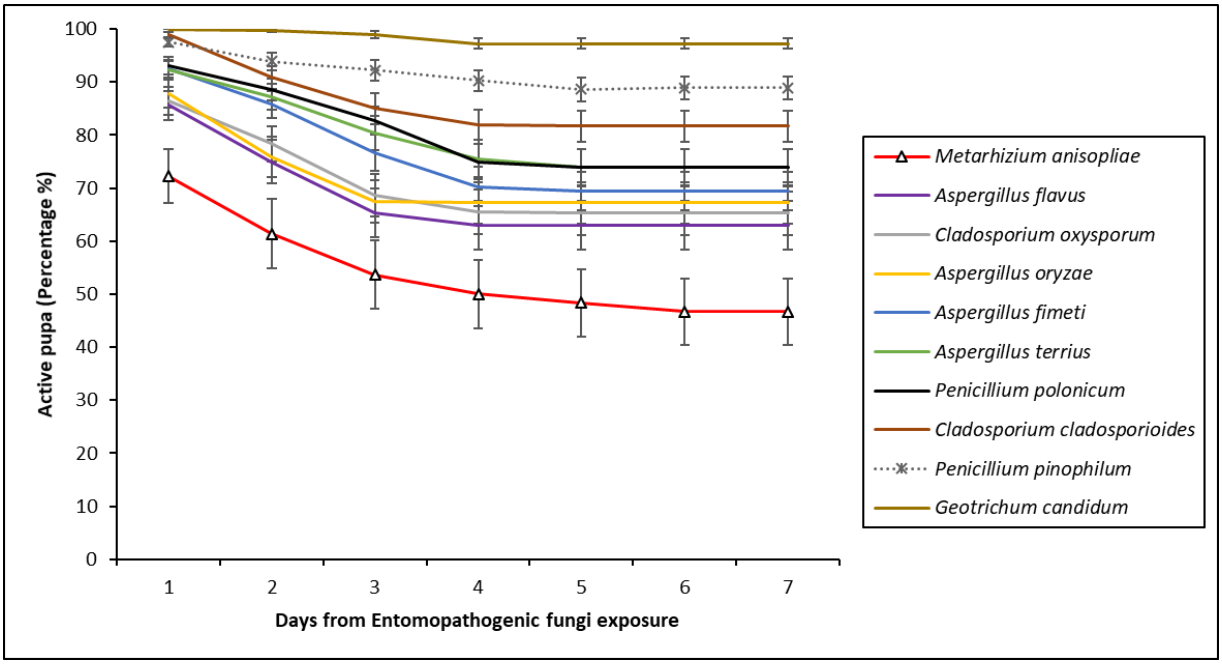

Figure 13. *T. absoluta* active pupa following seven days of entomopathogenic fungi exposure.

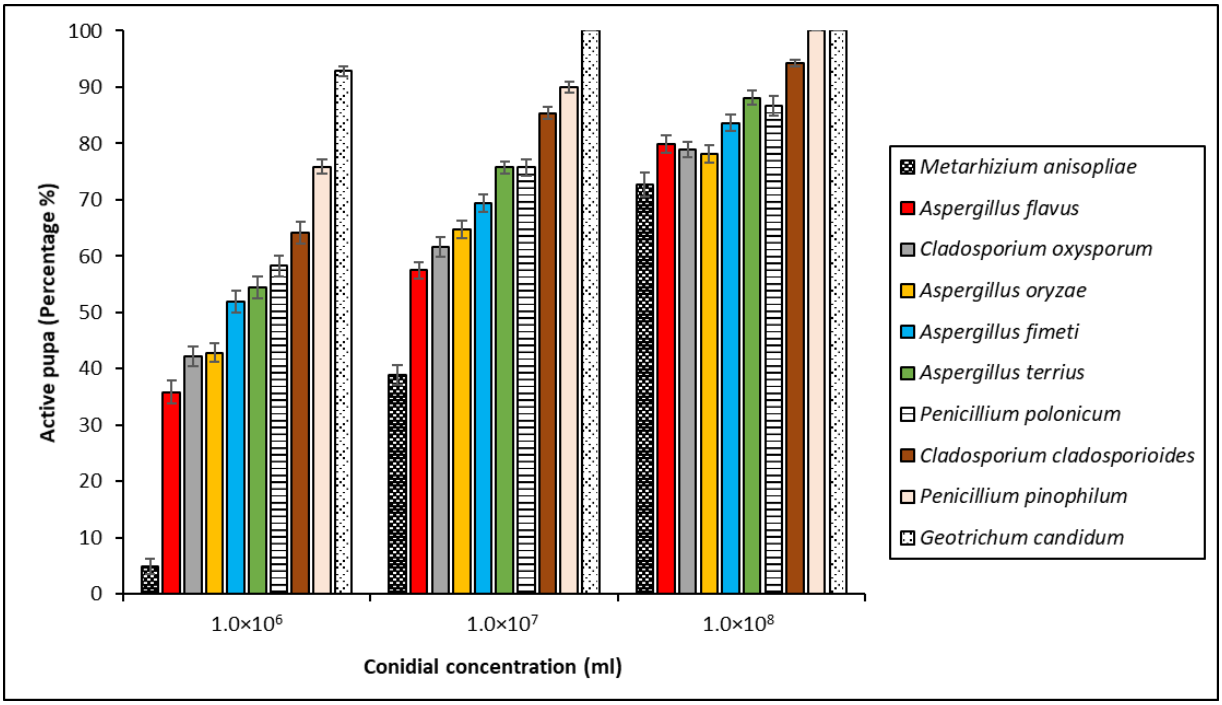

Figure 24. Interaction effect of entomopathogenic fungi and conidial concentration on *T. absoluta* active pupa

Table 3. Interaction effect of entomopathogenic fungi, conidial concentration and days of exposure on *T. absoluta* active pupa.

	Concentrations (conidia/ml)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Metarhizium anisopliae	1.0×10^{6}	28.9±3.09	5.6±2.94	0.0±0.00	0.00±0.00	0.0±0.00	0.0±0.00	0.0s±0.00
	1.0×10^{7}	61.1±3.09	47.8±2.78	38.9±3.09	33.3±3.33	31.1±3.51	30.0±3.33	30.0±3.33
	1.0×10^{8}	98.9±1.11	92.2±3.25	75.6±1.76	66.7±1.67	62.2±2.78	56.67±3.3	56.67±3.33
Aspergillus flavus	1.0×10^{6}	66.7±3.73	44.4±2.42	28.9±2.61	27.8±2.22	27.8±2.22	27.8±2.22	27.8±2.22
	1.0×10^{7}	76.7±2.36	62.2±4.65	54.4±2.94	52.2±2.22	52.2±2.22	52.2±2.22	52.2±2.22
	1.0×10^{8}	98.9±1.11	93.3±2.89	77.8±3.24	72.2±2.22	72.2±2.22	72.2±2.22	72.2±2.22
Cladosporium oxysporum	1.0×10^{6}	64.4±4.44	52.2±4.34	36.7±2.36	35.6±2.42	35.6±2.42	35.6±2.42	35.6±2.42
	1.0×10^{7}	85.6±2.94	71.1±2.00	61.1±2.61	53.3±2.89	53.3±2.89	53.3±2.89	53.3±2.89
	1.0×10^{8}	95.6±1.76	90.0±2.36	76.7±2.36	73.3±1.67	72.2±1.47	72.2±1.47	72.2±1.47
Aspergillus oryzae	1.0×10^{6}	65.6±3.38	45.6±3.38	37.8±2.78	37.8±2.78	37.8±2.78	37.8±2.78	37.8±2.78
	1.0×10^{7}	86.7±2.89	66.7±3.73	60.0±2.89	60.0±2.89	60.0±2.89	60.0±2.89	60.0±2.89
	1.0×10^{8}	98.9±1.11	91.1±2.61	72.2±2.61	71.1±2.00	71.1±2.00	71.1±2.00	71.1±2.00
Aspergillus fimeti	1.0×10^{6}	78.9±3.09	66.7±3.33	48.9±2.00	42.2±2.22	42.2±2.22	42.2±2.22	42.2±2.22
	1.0×10^{7}	92.2±2.22	80.0±2.36	68.9±2.00	61.1±2.00	61.1±2.00	61.1±2.00	61.1±2.00
	1.0×10^{8}	98.9±1.11	96.7±2.36	88.9±3.89	77.8±2.22	74.4±1.76	74.4±1.76	74.4±1.76
Aspergillus terrius	1.0×10^{6}	78.9±2.00	68.9±3.09	55.6±3.38	44.4±1.76	44.4±1.76	44.4±1.76	44.4±1.76
	1.0×10^{7}	90.0±2.36	83.3±2.89	72.2±1.47	71.1±1.11	71.1±1.11	71.1±1.11	71.1±1.11
	1.0×10^{8}	100.0±0.00	96.7±3.33	93.3±3.73	86.7±2.36	80.0±1.67	80.0±1.67	80.0±1.67
Penicillium polonicum	1.0×10^{6}	80.0±2.36	72.2±2.78	64.4±2.42	47.8±2.22	47.8±2.22	47.8±2.22	47.8±2.22
	1.0×10^{7}	92.2±2.22	90.0±2.89	76.7±2.36	67.8±1.47	67.8±1.47	67.8±1.47	67.8±1.47
	1.0×10^{8}	100.0±0.00	92.2±3.24	90.0±2.89	84.4±1.76	80.0±5.27	80.0±5.27	80.0±5.27
Cladosporium cladosporioides	1.0×10^{6}	96.7±1.67	72.2±3.64	57.8±2.78	55.6±1.76	55.6±1.76	55.6±1.76	55.6±1.76
	1.0×10^{7}	98.9±1.11	92.2±2.22	86.7±2.36	80.0±1.67	80.0±1.67	80.0±1.67	80.0±1.67
	1.0×10^{8}	100.0±0.00	98.9±1.11	95.6±1.76	92.2±1.47	91.1±1.11	91.1±1.11	91.1±1.11
Penicillium pinophilum	1.0×10^{6}	93.3±2.36	78.9±2.00	74.4±1.76	73.3±1.67	68.9±2.61	71.1±1.11	71.1±1.11
	1.0×10^{7}	96.7±1.67	96.7±1.67	94.4±2.42	87.8±1.76	85.6±1.76	84.4±1.76	84.4±2.22
	1.0×10^{8}	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00
Geotrichum candidum	1.0×10^{6}	100.0±0.00	98.9±1.11	95.6±2.42	88.9±2.00	88.9±2.00	88.9±2.00	88.9±2.00
	1.0×10^{7}	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00
	1.0×10^{8}	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00
L.S.D	5.442							
p-value	<0.001							

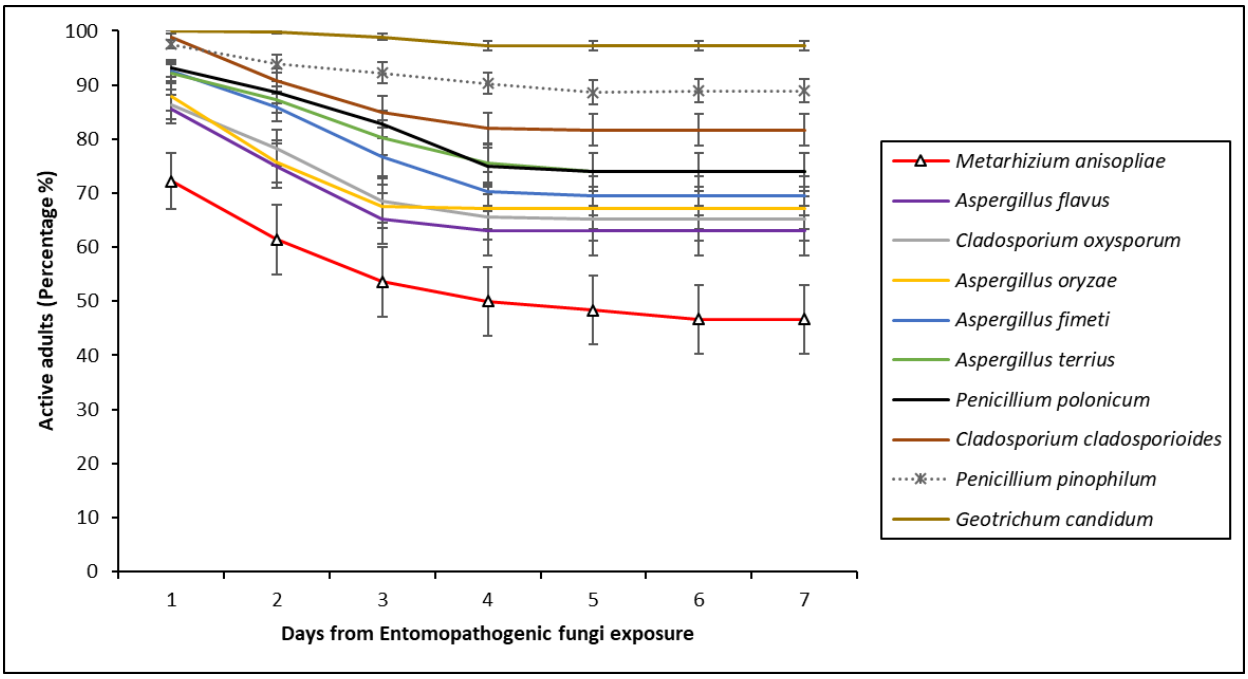

Figure 3~~5~~. *T. absoluta* adults' survivalfollowing seven days of entomopathogenic fungi exposure.

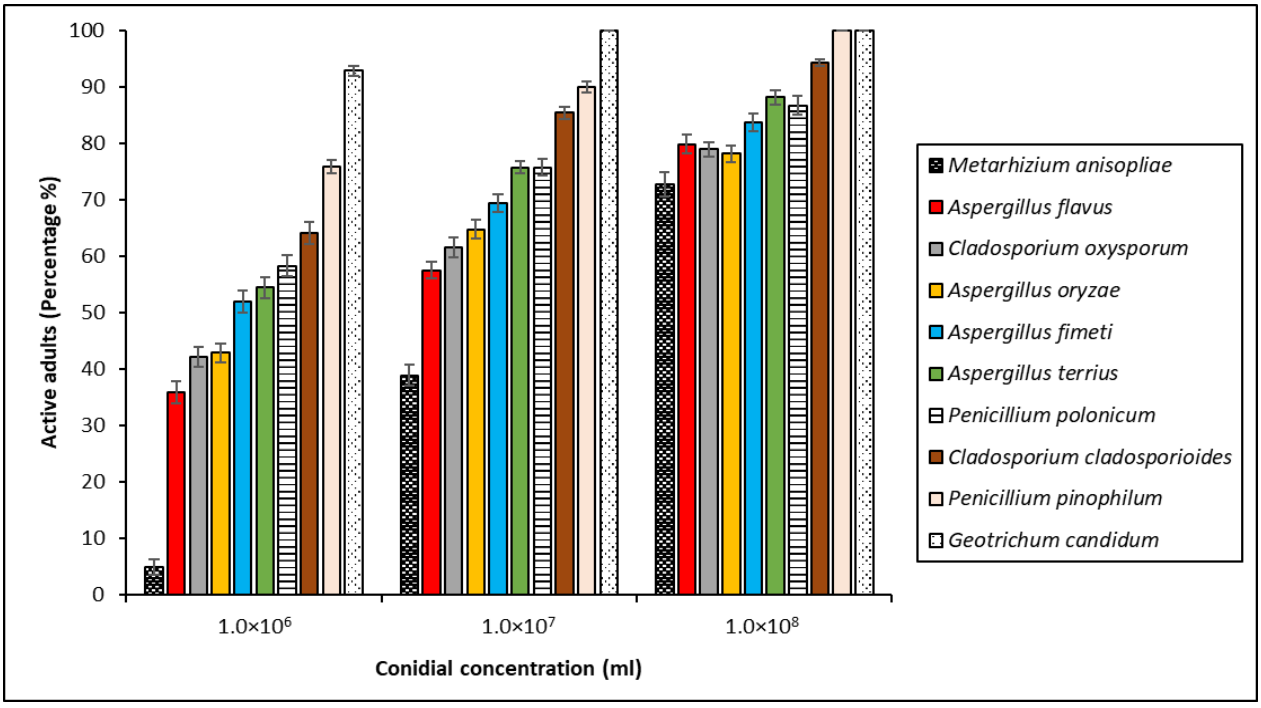

Figure 4~~6~~. Interaction effect of entomopathogenic fungi and conidial concentration on *T. absoluta* adults' survival.

Table 4. Interaction effect of entomopathogenic fungi, conidial concentration and days of exposure on *T. absoluta* adults' survival

	Concentrations (conidia/ml)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Metarhizium anisopliae	1.0×10^6	28.9±3.0	5.6±2.9	0.0±0.0	0.00±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	1.0×10^7	61.1±3.0	47.8±2.7	38.9±3.0	33.3±3.3	31.1±3.5	30.0±3.3	30.0±3.3
	1.0×10^8	98.9±1.1	92.2±3.2	75.6±1.7	66.7±1.6	62.2±2.7	56.67±3.	56.6±3.0
Aspergillus flavus	1.0×10^6	66.7±3.7	44.4±2.4	28.9±2.6	27.8±2.2	27.8±2.2	27.8±2.2	27.8±2.2
	1.0×10^7	76.7±2.3	62.2±4.6	54.4±2.	52.2±2.2	52.2±2.2	52.2±2.2	52.2±2.2
	1.0×10^8	98.9±1.1	93.3±2.8	77.8±3.2	72.2±2.2	72.2±2.2	72.2±2.2	72.2±2.2
Cladosporium oxysporum	1.0×10^6	64.4±4.4	52.2±4.3	36.7±2.3	35.6±2.4	35.6±2.4	35.6±2.4	35.6±2.4
	1.0×10^7	85.6±2.9	71.1±2.0	61.1±2.6	53.3±2.8	53.3±2.8	53.3±2.8	53.3±2.8
	1.0×10^8	95.6±1.7	90.0±2.3	76.7±2.3	73.3±1.6	72.2±1.4	72.2±1.4	72.2±1.4
Aspergillus oryzae	1.0×10^6	65.6±3.3	45.6±3.3	37.8±2.7	37.8±2.7	37.8±2.7	37.8±2.7	37.8±2.7
	1.0×10^7	86.7±2.8	66.7±3.7	60.0±2.8	60.0±2.8	60.0±2.8	60.0±2.8	60.0±2.8
	1.0×10^8	98.9±1.1	91.1±2.6	72.2±2.6	71.1±2.0	71.1±2.0	71.1±2.0	71.1±2.0
Aspergillus fimeti	1.0×10^6	78.9±3.0	66.7±3.3	48.9±2.0	42.2±2.2	42.2±2.2	42.2±2.2	42.2±2.2
	1.0×10^7	92.2±2.2	80.0±2.3	68.9±2.0	61.1±2.0	61.1±2.0	61.1±2.0	61.1±2.0
	1.0×10^8	98.9±1.1	96.7±2.3	88.9±3.8	77.8±2.2	74.4±1.7	74.4±1.7	74.4±1.7
Aspergillus terrius	1.0×10^6	78.9±2.0	68.9±3.0	55.6±3.3	44.4±1.7	44.4±1.7	44.4±1.7	44.4±1.7
	1.0×10^7	90.0±2.3	83.3±2.8	72.2±1.4	71.1±1.1	71.1±1.1	71.1±1.1	71.1±1.1
	1.0×10^8	100.0±0.0	96.7±3.3	93.3±3.7	86.7±2.3	80.0±1.6	80.0±1.6	80.0±1.6
Penicillium polonicum	1.0×10^6	80.0±2.3	72.2±2.7	64.4±2.4	47.8±2.2	47.8±2.2	47.8±2.2	47.8±2.2
	1.0×10^7	92.2±2.2	90.0±2.8	76.7±2.3	67.8±1.4	67.8±1.4	67.8±1.4	67.8±1.4
	1.0×10^8	100.0±0.0	92.2±3.2	90.0±2.8	84.4±1.7	80.0±5.2	80.0±5.2	80.0±5.2
Cladosporium cladosporioides	1.0×10^6	96.7±1.6	72.2±3.6	57.8±2.7	55.6±1.7	55.6±1.7	55.6±1.7	55.6±1.7
	1.0×10^7	98.9±1.1	92.2±2.2	86.7±2.3	80.0±1.	80.0±1.6	80.0±1.6	80.0±1.6
	1.0×10^8	100.0±0.0	98.9±1.	95.6±1.7	92.2±1.4	91.1±1.1	91.1±1.1	91.1±1.1
Penicillium pinophilum	1.0×10^6	93.3±2.3	78.9±2.0	74.4±1.7	73.3±1.6	68.9±2.6	71.1±1.1	71.1±1.1
	1.0×10^7	96.7±1.7	96.7±1.7	94.4±2.4	87.8±1.7	85.6±1.7	84.4±1.7	84.4±2.0
	1.0×10^8	100.0±0.0	100.±0.0	100.0±0.	100.0±0.	100.0±0.	100.0±0.	100±0.0
Geotrichum candidum	1.0×10^6	100.0±0.0	98.9±1.1	95.6±2.4	88.9±2.0	88.9±2.0	88.9±2.0	88.9±2.0
	1.0×10^7	100.0±0.0	100.±0.0	100.±00.	100±0.	100.0±0.0	100±0.0	100±0.0
	1.0×10^8	100.0±0.0	100±0.0	100±0.0	100±0.	100.0±0.	100±0.0	100±0.0
L.S. D	5.442							
p-value	<0.001							

The percentage of dead larvae increased with incubation time, while the percentage of active pupa and adults decreased with incubation time. The lowest percentages of dead larvae (9.4%), highest percentages of active pupa (90.6%), and adults (90.6%) were recorded at the initial time (day 1), whereas the highest percentages of dead larvae (27.3%), lowest percentages of active pupa (72.7%), and adults (72.7%) were recorded at the 7th day of incubation. Highest larval mortality was recorded on *Metarhizium anisopliae* at 1.0×10^6(95.1%), 1.0×10^7 (61.1%) and 1.0×10^8 (27.3%) conidial concentrations (Figure. 3). Whereas the lowest

larval mortality was recorded on *Geotrichum candidum* both at 1.0×10^7 and1.0×10^8 (0%) conidial concentrations, as well as on *Penicillium pinophilum* at 1.0×10^8 (0%) conidial concentrations (Ndereyimana et al., 2019).

Tuta absoluta larvae mortality differed significantly with the interaction between EPF, conidial concentration, and incubation time (Table 3). The highest percentage mortality of *T. absoluta* larvae was recorded on *Metarhizium anisopliae* at days one to seven, while the lowest mortality percentage of *T. absoluta* larvae was recorded on Geotrichum *candidum* at days one to seven (Silvia et al., 2020). The highest active pupa was recorded on *Cladosporium cladosporioides with a* conidial concentration of 1.0×10^8, (Table 4). While the lowest active pupa was recorded on *Metarhizium anisopliae* with conidial concentrations 1.0×10^6.

Interaction effect of entomopathogenic fungi, conidial concentration and days of exposure on *T. absoluta* active pupa

T. absoluta active pupa differed significantly with the interaction between EPF, conidial concentration and incubation time (Figure 4). The highest percentage of *T. absoluta* active pupa was recorded on *Geotrichum candidum* with 1.0×10^6conidia/ml, *Cladosporium cladosporioides* with 1.0×10^8conidia/ml at the initial time (day 1) as well as *Geotrichum candidum* with 1.0×10^7conidia/ml, *Aspergillus terrius*, *Geotrichum candidum*, *Penicillium pinophilum* and *Penicillium polonicum* with 1.0×10^8 at day 1-7(100%). While, no *T. absoluta* active pupa (0%) was recorded on *Metarhizium anisopliae* with 1.0×10^6 conidia/ml at 3 - 7 days.

Interaction effect of entomopathogenic fungi and conidial concentration on *T. absoluta* adults' survival

Highest adults' survival percentage (94.3%) was recorded on *Cladosporium cladosporioides* at 1.0×10^8, conidial concentrations (Table 4) whereas, the lowest adults 'survival (4.9%) was recorded on *Metarhizium anisopliae* at 1.0×10^6 conidial concentrations.

Tuta absoluta adults' survival differed significantly with the interaction between EPF, conidial concentration and incubation time (Figure 6). The highest percentage of *T. absoluta* adults' survival (100%) was recorded on *Geotrichum candidum*with 1.0×10^6conidia/ml, concentration, *Cladosporium cladosporioides* with 1.0×10^8 conidia/ml at the initial time (day 1) as well as *Geotrichum candidum* with 1.0×10^7 conidia/ml, *Aspergillus terrius*, *Geotrichum candidum*, *Penicillium pinophilum* and *Penicillium polonicum* with 1.0×10^8 at day 1-7 (100%). While, no *T. absoluta* adult's survival (0%) was recorded due to EPF *Metarhizium anisopliae* with 1.0×10^6conidia/ml at 3 - 7 day. These results suggest that entomopathogenic fungi are not equally distributed in each soil sample and location where samples were collected; this is due to a lack of environmental conditions favourable to most entomopathogenic fungi. Unfavourable environmental conditions are caused by the frequent use of pesticides in most tomato farms; the use of different pesticides can kill or harm entomopathogenic fungi or other natural enemies. Most entomopathogenic fungi are found in organic soils or in tomato farms, where a combination of organic pesticides is used.

CONCLUSION

This study revealed that entomopathogenic fungi related to *T. absoluta* management are more abundant in Morogoro, which is a hot spot area compared to Arusha, which means Morogoro weather conditions favour EPFs more than Arusha weather conditions. *Metarhizium anisopliae* strains that were isolated from soil samples collected from tomato farms in Morogoro have higher pathogenicity effects against *T. absoluta* compared to other entomopathogenic species that were identified. Other entomopathogenic species such as *Aspergillus* may be promising species for the management of *T. absoluta* at early stages, especially at the egg stage. Further field research and evaluation are required to determine the potential of entomopathogenic fungi and possibly their combinations against *T. absoluta*.

ACKNOWLEDGEMENT

The author heartfelt thanks the Ministry of Agriculture Training Institute (MATI) Horti-Tengeru for permission and financial support that enabled completion of this research work. Special thanks go to the International Centre of Insect Physiology and Ecology (*icipe*) for finding this research work.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

AUTHORS' CONTRIBUTIONS

MM performed the conception or design of the work, data collection, data analysis, interpretation and was a major contributor in writing the manuscript (drafting the article). LC and LM helped in the advisory role during the research and performed a critical revision of the article, providing

critical comments concerning the discussion of results, conclusions, and recommendations. All authors read and approved the final manuscript.

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