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RESEARCH

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Efficacy of the entomopathogenic fungi *Beauveria bassiana* and *Lecanicillium muscarium* in the control of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

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Abstract

Background: Among the phytosanitary problems, affecting tomato crops in greenhouses heated by geothermal water in southern Tunisia, the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is gaining prominence from year to year.

Results: This study determined the susceptibility of eggs, second-instar larvae of *T. absoluta* to commercial formulations of the entomopathogenic fungi (EPF) *Beauveria bassiana* and *Lecanicillium muscarium* at different doses (10^{10}, 10^{9}, 10^{8}, 10^{7} and 10^{6} spores/ml). Pathogenicity tests were carried out under controlled laboratory conditions at 27 ± 1 °C, and RH of 75 ± 5%, showed that the three commercial formulations of *B. bassiana* R444 (Bb-Protec®), *B. bassiana* ATCC 74,040 (Naturalis®) and *L. muscarium* Ve6 (Mycotal®) were effective against eggs and second instar larvae of *T. absoluta*. Mortality rates of eggs and larvae were high with increasing the concentrations of the fungi. *B. bassiana* ATCC 74,040 was the most effective one, with an egg mortality rate of (71.42%) and larval mortality was greater than (80%), at the highest concentration of 10^{10} spores/ml. The lethal concentration required to kill 50% of eggs was 1.56×10^{8}, 1.73×10^{9} and 1.09×10^{10} conidia/ml for *B. bassiana* ATCC, *B. bassiana* R444, and *L. muscarium*, respectively. After 7 days, the median lethal concentration (LC_{50}) values against larvae were the lowest for *B. bassiana* ATCC with 2.63×10^{7} and 4.76×10^{6} conidia/ml by way 1 and way 2 treatments, respectively. Thus, these three formulations affected the emergence of pest's adults. The fungi significantly reduced pupation and adult emergence. Only 50.25, 60.15 and 69.16% of *T. absoluta* adults emerged in the treatments with *B. bassiana* ATCC, *B. bassiana* R444, and *L. muscarium*, respectively.

Conclusions: Biological performances of the EPF showed the role of a biological control agent that can be played against *T. absoluta* within the framework of an Integrated Pest Management program.

Keywords: *Tuta absoluta*, Entomopathogenic fungi, *Beauveria bassiana*, *Lecanicillium muscarium*, Efficacy

Background

The tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is an insect native to South America (Desneux et al. 2011). It is considered one of the most devastating pests of tomato crop (*Solanum lycopersicum* L., Solanaceae) (Cherif et al. 2019). *T. absoluta* has a high reproductive capacity. The duration of the development cycle depends essentially on the

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environmental conditions, in particular the temperature. (Tropea Garzia et al. 2012). It soon has spread to several countries outside its original region (Han et al. 2019). At the end of 2006, it was detected for the first time outside its native area of South America in the Mediterranean basin in the east of the Iberian Peninsula; southwestern Europe, including Spain and Portugal (Urbaneja et al. 2007). In the following years, this pest quickly reached destructive levels in the main tomato-growing Mediterranean coastal areas (Desneux et al. 2010). An ineffective early surveillance network, weak quarantine efforts, and increased trade are promoting its rapid spread in tomato growing areas (Han et al. 2019). Management of this leaf miner relies heavily on applications of chemical insecticides (Roditakis et al. 2013). But the failure of chemical control clearly shows the ability of this pest to develop resistance to several classes of insecticides (Roditakis et al. 2018). This genetic resistance of populations of *T. absoluta* is a common consequence of unbalanced use of insecticides worldwide (Guedes and Picanço 2012). Biological control represents one of the most promising options with entomopathogens (fungi, bacteria, nematodes or viruses) which can effectively control this pest (Urbaneja et al. 2012). Many studies have been conducted on EPF to control a wide range of pests which have proven to be very effective (Contreras et al. 2014). Specifically, *Beauveria bassiana* (Bals.) Vuill. (Ascomycota: Hypocreales) and *Metarhizium anisopliae* (Metsch.) Sorok (Ascomycota: Hypocreales) have been used against many insect pests for over 120 years (Contreras et al. 2014). The EPF, *B. bassiana* and *M. anisopliae* have been reported to be effective against tomato leaf miner (Tadele and Emana 2017).

Faced to the demands of the consumer, who has become more sensitive to pesticide residues, this study seemed to improve Integrated Pest Management (IPM) programs by developing biological means. This study aimed to evaluate the efficacy of EPF on eggs, second-instar larvae of *T. absoluta* to commercial formulations of *B. bassiana* and *Lecanicillium muscarium* fungi at different concentrations.

Methods

Insect rearing

Leaves infested by *T. absoluta* larvae were collected from a greenhouse culture located in the Technical Center for Protected and Geothermal Cultures in the experimental plot in Chenchou (Governorate of Gabes, ElHamma delegation in southeastern Tunisia). The insect population raised on tomato leaves was placed in plastic boxes (20*10*8) at a climatic chamber, at a temperature of 27 ± 1 °C, a RH of 75 ± 5% and a 16:8 h photoperiod. The nymphs were then transferred to other boxes containing fresh tomato leaves until adults' emergence. Mating of *T. absoluta* adults was carried out on tomato plants in (9 cm diameter) pots containing peat. The pots were placed in wooden cages of dimensions ($1 \times 1 \times 1.2$ m), covered with insect-resistant fabric (20/10 threads/cm) in an experimental greenhouse. After oviposition, eggs and larvae of *T. absoluta* were used in bioassays.

Entomopathogenic fungi

Three commercial EPF (two isolates of *B. bassiana* and one of *L. muscarium*) were used in the study (Table 1). Viability of EPF conidia was confirmed on Potato Dextrose Agar (PDA) medium and incubated at 27 ± 1 °C for 7 days to confirm viability before use (Youssef 2015). Spore suspensions were obtained from Petri dishes containing EPF cultures. The concentrations of the suspensions were adjusted using the Malassez cell. Serial dilutions were carried out for each EPF product for the preparation of fungal suspensions and to reach the needed concentrations against *T. absoluta*: 10^{10}, 10^{9}, 10^{8},10^{7}et 10^{6}spores/ml, adding Tween 80 (0.01%) for each fungal suspension.

Pathogenicity test on *Tuta absoluta* eggs

On each tomato leaflet, 10 T. *absoluta* eggs, collected during rearing, were placed using a moistened brush. Five concentrations of each EPF were applied to *T. absoluta* eggs using a 0.5 l sprayer. As a control, eggs were treated with distilled water plus 80% Tween. Then, to avoid dehydration, each leaflet was placed face up on a disc of filter paper soaked in distilled water in a Petri

Table 1 Commercial entomopathogenic fungi used in the experiment

Commercial Name	Active ingrédients and concentrations	Manufacturer
Bb-Protec® WP	*Beauveria bassiana* strain R444 ($\geq 2 \times 10^{9}$spores/gram)	Andermatt Biocontrol
Mycotal® WG	*Lecanicillium muscarium* strain Ve6 (19–79) (10^{10} spores /gram)	Koppert Biological Systems, Berkel en Rodenrijs, the Netherlands
Naturalis®	*Beauveria bassiana* strain ATCC 74,040 ($2,3 \times 10^{7}$ spores/ml)	CBC (Europe) Srl–Division BIOGARD, Italie

dish 9 cm in diameter. Egg hatching and mycosis on eggs were assessed daily from the first day after treatment. Unhatched eggs were transferred to water-soaked filter papers in Petri dishes to observe the possible development of mycelium and conidia (Pires et al. 2010). The experimental protocol used was completely randomized, with five repetitions for each treatment; the experimental unit consisted of 10 eggs.

Pathogenicity test on *Tuta absoluta* larvae: the way 1

As in the previous experiment, five concentrations of each EPF were evaluated on second instar larvae of *T. absoluta*. Leaflets of tomatoes bearing active mines (stage L2) placed in 5*5 cm plastic dishes were sprayed with fungal suspensions for the two EPF (Ndereyimana et al. 2019). Control leaflets were sprayed only with distilled water and 0.01% Tween 80. In order to avoid rapid drying of the leaves, they were placed on filter paper soaked in distilled water. The experimental design was completely randomized, with five replicates, each consisting of leaflets containing 10 larvae.

Pathogenicity test on *Tuta absoluta* larvae: the way 2

Untreated *T. absoluta* larvae were brought by contact with tomato leaves treated with the same fungal suspensions and under the same conditions described above. Each batch of 10 larvae was transferred to dishes containing treated fresh tomato leaflets. Distilled water and 0.01% Tween 80 were used as a negative control. All experiments were repeated five times (Pires et al. 2010). Larval mortality was recorded daily for 7 days after inoculation. Dead larvae were incubated in Petri dishes on moistened filter papers to confirm fungal outgrowth on dead larvae.

Pathogenicity on pupation and adult emergence of *Tuta absoluta*

The reason for this bioassay was to see if these EPF formulations could have an effect on the fourth instar larvae of *T. absoluta* when they naturally drop onto silk threads to pupate in the soil (Desneux et al. 2010), therefore, whether there is an effect on adult emergence. Since these 4^{th} instar larvae fall and pupate in the soil, sand was used to simulate natural field conditions in this trial. In this experiment, the sand was sterilized at 121 °C for 1 h in an oven and then cooled to room temperature for about 72 h before processing. Ten 4^{th} instar larvae were exposed to 50 g of sterilized sand treated with 5 ml of fungal spore suspension at a concentration of 10^9/ml for each fungus with 0.01% Tween 80. Control treatments consisted of 50 g of sterilized sand treated with 5 ml of sterile distilled water containing 0.01% Tween 80. After 6 days of exposure, *T. absoluta* nymphs were transferred to tomato leaves placed in Petri dishes with droplets of honey solution to feed emerging adults. Emergence was observed and recorded up to the eleventh day (Akutse et al. 2020). The experiment was performed at 27 °C, 75 ± 5% RH at 16:8 h photoperiod and replicated five times.

Statistical analysis

Mortality data of *T. absoluta* were corrected using Abbott's formula (Abbott 1925) and checked for normality. These mortality rates were subjected to an analysis of variance and the means were compared according to Tukey's test (HSD) at a level of significance of 5%. Probit analysis (Finney 1971) was performed to estimate the median lethal concentrations (LC_{50}) was calculated for the three EPF formulations. Values were considered significantly different if their 95% confidence limits did not overlap. All analyses were performed using SPSS 22 software.

$$\%\,\text{corrected}\;\;\text{mortality} = [(A - B/A) \times 100]$$

where: A: Mean number of *T. absoluta* eggs/larvae untreated; B: Mean number of *T. absoluta* eggs/larvae treated.

Results

Pathogenicity test on *Tuta absoluta* eggs

The corrected egg mortality of *T. absoluta* was significantly affected by the three commercial formulations based on *B. bassiana* ATCC, *B. bassiana* R444 and *L. muscarium*. Egg mortality increased significantly with concentrations of *B. bassiana* R444 (Bb-Protec®) ($F = 3.957$, $P = 0.004$), *B. bassiana* ATCC (Naturalis®) ($F = 3.349$, $P = 0.011$) and *L. muscarium* (Mycotal®) ($F = 5.668$, $P = 0.0002$). All the three EPF formulations revealed a direct relationship between concentration and mortality (Fig. 1). At the peak concentration of 10^{10}

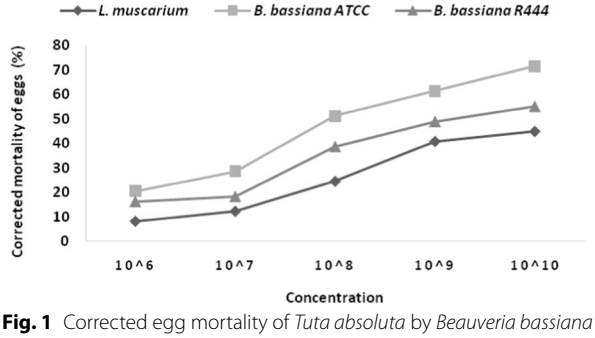

Fig. 1 Corrected egg mortality of *Tuta absoluta* by *Beauveria bassiana* ATCC, *B. bassiana* R444 and *Lecanicillium muscarium*

Table 2 Probit analysis, 7 days after treatment with *Beauveria bassiana* ATCC, *B. bassiana* R444 and *Lecanicillium muscarium* against *T. absoluta* eggs

EPF	LC_{50} (95% CL)	LC_{90} (95% CL)	Probit equation	Linear Slope (SE)	χ2 (df)
Beauveria bassiana ATCC	1.56×10^8 (5.29×10^7–5.05×10^8)	6.4×10^{11} (6.03×10^{10}–6.05×10^{13})	$Y = -2.907 + 0.355 \log_{10} C$	0.355 (0.061)	0.849 (3)
B. bassiana R444	1.73×10^9 (0.44×10^9–1.74×10^{10})	3.65×10^{13} (8.81×10^{11}–1.79×10^{17})	$Y = -2.74 + 0.297 \log_{10} C$	0.297 (0.061)	1.547 (3)
Lecanicillium muscarium	1.09×10^{10} (6.22×10^{10}–4.42×10^{13})	1.03×10^{14} (2.14×10^{12}–6.66×10^{17})	$Y = -3.237 + 0.322 \log_{10} C$	0.322 (0.065)	1.024 (3)

CL confidence limit which has been calculated with 95% confidence

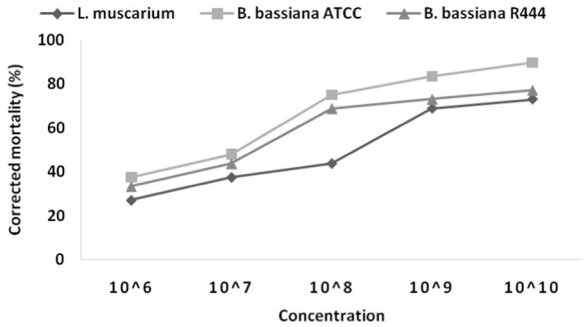

Fig. 2 Corrected mortality of second instar *Tuta absoluta* larvae by the first method with *Beauveria bassiana* ATCC, *B. bassiana* R444 and *Lecanicillium muscarium*

spores/ml, *B. bassiana* ATCC exhibited the highest ovicidal activity (71.42%), followed by *B. bassiana* R444 (55.10%) and *L. muscarium* (44.89%).

Regression analysis of *T. absoluta* egg mortality caused by *B. bassiana* ATCC, *B. bassiana* R444, and *L. muscarium* is shown in Table 2. Indeed, the median lethal concentration required to kill 50% of the eggs was (1.56×10^8 conidia/ml) for *B. bassiana* ATCC. The LD_{90} of *B. bassiana* ATCC, *B. bassiana* R444 and *L. muscarium* against *T. absoluta* eggs was obtained by probit analysis as 6.4×10^{11}, 3.65×10^{13} and 1.03×10^{14} conidia/ml (Table 2).

Pathogenicity test on *Tuta absoluta* larvae: the way 1

The evaluation of the corrected mortality rates recorded for the five concentration of *B. bassiana* ATCC, *B. bassiana* R444 and *L. muscarium* used by the first method against the second instar larvae of *T. absoluta*, followed the same pattern (Fig. 2). The concentration 10^{10} conidia/ml killed significantly more larvae than the concentration 10^9, 10^8,10^7 and 10^6 conidia/ml for the three fungi *B. bassiana* R444 (Bb-Protec®) ($F = 5.394$, $P < 0.0001$), *B. bassiana* ATCC (Naturalis®) ($F = 5.830$, $P < 0.0001$) et *L. muscarium* (Mycotal®) ($F = 3.828$, $P = 0.005$). The corrected mortality rates following the application of *B. bassiana* ATCC obtained after 7 days were the highest at 29.78, 42.55, 53.19, 61.70 and 80.85%, respectively for the concentration of 10^6, 10^7, 10^8, 10^9 and 10^{10}conidia/ml.

The median lethal concentration values (LC_{50}) after 7 days of application were significantly different at the different fungi. The lowest LC_{50} value (2.63×10^7 conidia/ml) was observed in *B. bassiana* ATCC, while the highest LC_{50} value (3.33×10^9 conidia/ml) was observed in *L. muscarium* (Table 3). For LC_{90} estimated for *L. muscarium*, *B. bassiana* R444 and *B. bassiana* ATCC had concentrations of 2.52×10^{16}, 2.64×10^{14} and 4.02×10^{11}conidia/ml, respectively (Table 3).

Pathogenicity test on *Tuta absoluta* larvae: the way 2

Mortality rates of *T. absoluta* second-instar larvae by the second method, after treatment with EPF, differed

Table 3 Probit analysis at 7 days after treatment with *Beauveria bassiana* ATCC, *B. bassiana* R444 and *Lecanicillium muscarium* against 2nd instar *Tuta absoluta* larvae by the first method

EPF	LC_{50} (95% CL)	LC_{90} (95% CL)	Probit equation	Linear Slope (SE)	χ2 (df)
Beauveria bassiana ATCC	2.63×10^7 (5.27×10^6–8.87×10^7)	4.02×10^{11} (3.07×10^{10}–9.90×10^{13})	$Y = -2.273 + 0.306 \log_{10} C$	0.306 (0.06)	0.906 (3)
B. bassiana R444	3.39×10^8 (6.19×10^7–4.38×10^9)	2.64×10^{14} (1.42×10^{12}–2.89×10^{21})	$Y = -1.856 + 0.218 \log_{10} C$	0.218 (0.58)	0.178 (3)
Lecanicillium muscarium	3.33×10^9 (4.04×10^8–1.49×10^{12})	2.52×10^{16} (1.47×10^{13}–4.28×10^{29})	$Y = -1.774 + 0.186 \log_{10} C$	0.186 (0.58)	0.289 (3)

CL confidence limit which has been calculated with 95% confidence

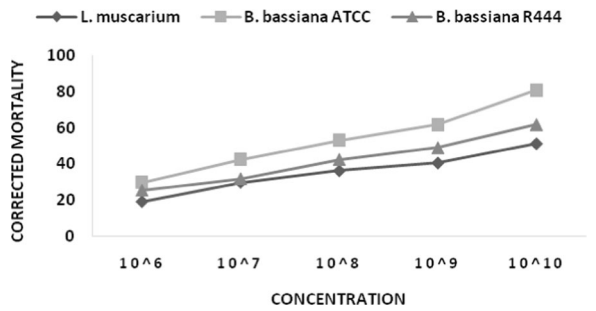

Fig. 3 Corrected mortality of 2nd instar *Tuta absoluta* larvae from by the second method with *Beauveria bassiana* ATCC, *B. bassiana* R444 and *Lecanicillium muscarium*

significantly with increasing the concentration at the end of the experiment period; *B. bassiana* R444 (Bb-Protec®) ($F=3.602$, $P=0.008$), *B. bassiana* ATCC (Naturalis®) ($F=3.278$, $P=0.012$) et *L. muscarium* (Mycotal®) ($F=4.816$, $P=0.001$). The highest corrected mortality rate for *T. absoluta* (86.81%) was caused by *B. bassiana* ATCC. *L. muscarium* EPF was found to be the least virulent with a corrected mortality rate of 46.24% (Fig. 3).

The values of the LC_{50} and LC_{90} of *B. bassiana* ATCC, *B. bassiana* R444 and *L. muscarium* were shown in (Table 4). The results showed that *B. bassiana* ATCC was the most virulent EPF on second instar of *T. absoluta* at the concentrations of 4.76×10^6 and 5.84×10^9 spores/ml to kill 50 and 90% of the population, respectively.

T. absoluta larvae infected with *B. bassiana* showed color changes to yellowish brown (Fig. 4A). Fungal hyphae began to grow from the edges of the larvae's body. The hyphae then covered most of the larvae's body (Fig. 4B).

Pathogenicity on pupation and adult emergence of *Tuta absoluta*

The three commercial EPF formulations affected pupation of *T. absoluta* compared to the untreated control, but non-significant differences for *B. bassiana* R444 and *L. muscarium* ($F=2.51$, $P=0.095$) were found. On the other hand, for *B. bassiana* ATCC, there was a significant difference than the control. In fact, only 82% of

Table 4 Probit analysis at 7 days after treatment with *Beauveria bassiana* ATCC, *B. bassiana* R444 and *Lecanicillium muscarium* against second instar *Tuta absoluta* larvae by the second method

EPF	LC_{50} (95% CL)	LC_{90} (95% CL)	Probit equation	Linear Slope (SE)	χ2 (df)
Beauveria bassiana ATCC	4.76×10^6 (1.01×10^6–1.34×10^7)	5.84×10^9 (1.41×10^9–6.73×10^{10})	$Y = -2.771 + 0.415 \log_{10} C$	0.415 (0.066)	1.428 (3)
B. bassiana R444	1.05×10^7 (1.50×10^6–3.72×10^7)	1.65×10^{11} (1.52×10^{10}–2.61×10^{13})	$Y = -2.146 + 0.306 \log_{10} C$	0.306 (0.06)	2.327 (3)
Lecanicillium muscarium	6.63×10^7 (1.72×10^7–2.27×10^8)	8.48×10^{11} (5.82×10^{10}–2.37×10^{14})	$Y = -2.441 + 0.312 \log_{10} C$	0.312 (0.059)	1.565 (3)

CL confidence limit which has been calculated with 95% confidence

Fig. 4 Effect of *Beauveria bassiana* ATCC 74,040 against *Tuta absoluta* larvae. **A**: dead larva after 4 days of treatment; **B**: Development of mushroom mycelium on the body of the larva

fourth-instar larvae exposed to soil treated with this fungus developed to the pupal stage ($P=0.021$). Adult emergence was 69.16, 60.15 and 50.25% for fungal treatments with *L. muscarium*, *B. bassiana* R444 and *B. bassiana* ATCC, respectively (Fig. 5). These fungi significantly reduced the emergence of *T. absoluta* adults than the control ($F=2.51$; $P=0.095$).

Discussion

The EPF used in this study showed pathogenicity to eggs and second instar larvae of *T. absoluta* in all bioassays performed. Corrected mortality rates increased by increasing concentration and over time. Various studies also showed the efficacy of EPFs against *T. absoluta* eggs. Rodríguez et al. (2006) showed that *B. bassiana* Qu-B912 and *M. anisopliae* Qu-M558 had pathogenicity towards *T. absoluta* eggs with mortality percentages of around 60 to 80%, respectively. Similarly, they showed that egg mortality rates were significantly high at the highest spore concentrations for *M. anisopliae*, *B. bassiana* (Abdel-Baky et al. 2021) and *Verticillium lecanii* (Abdel-Raheem et al. 2020). While *M. anisopliae* isolates tURPE-6 and URPE-19 were more pathogenic (Pires et al. 2010). On the other hand, other experiments indicated that *B. bassiana* showed superiority over *M. anisopliae* in its effect on *T. absoluta* eggs control under laboratory conditions (Abdel-Baky et al. 2021). Thus, one study suggested that *B. bassiana* was only effective at the egg stage (Inanli et al. 2012). Other results also indicated that *B. bassiana* was particularly effective against *T. absoluta* eggs, causing more than 79.7% mortality (Bayram 2019).

Several studies have been conducted on the pathogenicity of *B. bassiana* against *T. absoluta* larvae, and they have shown significant efficacy similar to this study. *B. bassiana* was virulent against 2nd or 3rd larval instars of *T. absoluta* by application of conidia to the leaf surface

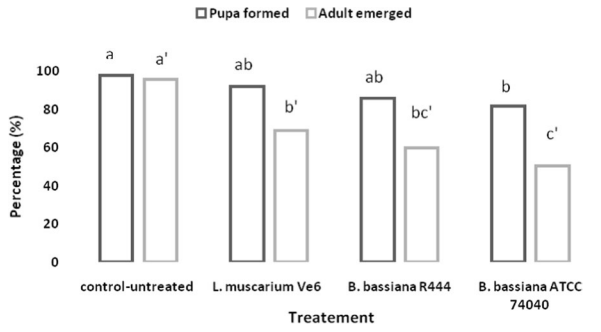

Fig. 5 Effects of commercial entomopathogenic fungi (EPF) preparations on pupation and adult emergence of *Tuta absoluta*. The means followed by the same letter are not significantly different by the Tukey test (HSD) ($p<0.05$)

(Silva et al. 2020). This fungus exhibited epiphytic and endophytic activity against *T. absoluta*. Investigation of the efficacy of a commercial mycoinsecticides based on *B. bassiana* against all stages of *T. absoluta* showed that the corrected mortality reached 30–50% (Klieber and Reineke 2016). *B. bassiana* and *M. anisopliae* were virulent against *T. absoluta* larvae under laboratory and greenhouse conditions (Tadele and Emana 2017). Ndereyimana et al. (2019) reported that the two formulations of the commercial EPFs Metatech® WP (*M. anisopliae* Strain FCM Ar 23B3) and Beauvitech® WP (*B. bassiana*, Strain J25) had a high level of virulence against *T. absoluta* larvae under laboratory conditions. The larvicidal effect was also evaluated in the laboratory and in the greenhouse by spraying two types of endophytic fungi. *Clonostachys* spp. and *B. bassiana* showed remarkable efficacy, variable depending on the strain tested (Mahmoud et al. 2021). In addition, the application of a bioinsecticide based on *M. anisopliae* to the 2nd instar larvae of *T. absoluta* by spraying was effective in reducing the population of the pest by 87% mortality on the 7th day of application (Erol et al. 2021). Four virulent isolates of *B. bassiana* MN598666, MK046654, MK046652 and *Purpureocillium lilacinum* (thom) Samson (Ascomycota: Hypocreales) MK046655 indicated promising potentials in regulating *T. absoluta* (Hammad et al. 2021). Aynalem et al. (2021) showed the efficacy of 27 strains of *Beauveria* with a mortality rate of 65.7 to 95.7% and 68.3 to 95% against 2nd and 3rd larval instars of *T. absoluta* at the concentration of 1×10^7 spores/ml, 7 days after inoculation.

Other studies have investigated the importance of this fungus when combined with other products. The combination of Azadirachtin and *B. bassiana* resulted in reductions in *T. absoluta* infestation and fruit damage up to greenhouse tomato harvest (Jallow et al. 2019). As with eggs, the highest larval mortality was also at the highest concentration (Tadele and Emana 2017) and increased over time (Ndereyimana et al. 2019).

The biological test against 4th instar larvae placed on soil treated with these fungi showed significant efficacy against pupation and adult emergence. Thus, *T. absoluta* L4 larvae were exposed to EPF spores dipped in soil, and thus this fungus grew on the cuticle of the larvae. The development of spores in the body of the larvae prevented a small percentage of the larvae from pupating and prevented a large percentage of the pupae emerging to adults. Studies have shown that soil drench applications of *B. bassiana* and *M. anisopliae* effectively controlled *T. absoluta* nymphs and affected female fecundity (Erasmus et al. 2021). Pupation and emergence of adults were significantly reduced by different isolates of *M. anisopliae* (Akutse et al. 2020). In addition, a liquid

formulation based on strains of the fungus *M. anisopliae* (MA-Prep) associated with irrigation could lead to high mortality of *T. absoluta* pupae (Contreras et al. 2014). In other study, Bali et al. (2022) indicated the efficiency of *P. lilacinum* against *T. absoluta* pupae.

Conclusion

The results showed that the EPF isolates *B. bassiana* and *L. muscarium* studied at different concentrations against *T. absoluta* under controlled conditions similar to that of geothermal greenhouses in southern Tunisia were effective, especially against the second-instar larvae of *T. absoluta*, eggs and the emergence of adults. Egg and larval mortality increased with the increase of concentration of fungal suspensions of the different fungi. The application of EPF during irrigation to the 4th instar larvae dropped into the soil to pupate can be an effective way to reduce the emergence of *T. absoluta* adults. Thus, EPF could play an important role, as a biological mean for controlling crop-associated *T. absoluta* in greenhouses as component in IPM programs.

Abbreviations

EPF: Entomopathogenic fungi; IPM: Integrated pest management; PDA: Potato dextrose agar; ANOVA: Analysis of variance; LC: Lethal concentration; CL: Confidence limit; *P*: *P*-Value; SE: Standard error; df: Degrees of freedom.

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Author contributions

Conceived and designed the experiments: SC and MSB. Performed the experiments: SC and BHA Analyzed the data: SC. Wrote the paper: SC, KLG, and MSB. All authors read and approved the final manuscript.

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Availability of data and materials

All data of the study have been presented in the manuscript, and high quality and grade materials were used in this study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not Applicable.

Competing interests

The authors declare that they have no competing interests.

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