Türk. Biyo. Mücadele Derg. 2022, 13 (2): 103-117
DOI:10.31019/tbmd.1112324 ISSN 2146-0035-E-ISSN 2548-1002

Orijinal araştırma (*Original article*)

Evaluation of the pathogenicity of some entomopathogenic fungi against Tomato leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) larvae

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Bazı entomopatojen fungus izolatlarının Domates güvesi *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) larvalarına karşı etkinliğinin araştırılması

Öz: Bu çalışmada, *Tuta absoluta*'ya karşı entomopatojenik fungusların patojenitesi test edilmiştir. Deneme 2020/2021 yılında Bursa Uludağ Üniversitesi Bahçe Bitkileri Bölümü seralarında yürütülmüştür. Araziden toplanan *T. absoluta* erginleri, iklim kabinleri içerisindeki domates fideleri üzerinde üretilmiştir. Daha sonra, tesadüf blokları deneme deseninde faktöryel düzende her izolat için beş farklı konsantarasyonda her tekerrürde on birey olmak üzere üç tekerrürlü denemeler kurulmuştur. İzolatların öldürücü etkisi için yapılan varyans analizlerinde, izolatlar ve konsantrasyonlar arasında önemli farklılıklar ($p<0.05$) tespit edilmiştir. En düşük ölüm oranı (% 80.77), en yüksek LC50 ($2.3x10^8$) ve LT (LT_{50}, 4.9 ve LT_{90}, 9.9 gün) değerleri ile *Metarhizium anisopliae* Ak-12 izolatı en etkisiz olarak bulunmuştur. Yüksek ölüm oranı (%91) ve düşük LT_{50} ve LT_{90}, (4 ve 7.6 gün) değerleri ile 1×10^{10} konsantrasyonunda *Beauveria bassiana* Ak-10 en etkili izolat olmuştur. Sonuçlar, $1x10^9$ ve $1x10^{10}$ konidia/ml konsantrasyonlarının en etkili, $1x10^6$ konidia/ml konsantrasyonunun ise etkisiz olduğunu göstermiştir. Çalışma, izolatlar ve konsantrasyonlar arasındaki potansiyel değişimin *T. absoluta* larvalarının ölüm oranlarındaki varyasyonuna olan etkisini göstermiştir.

Anahtar kelimeler: Biyolojik mücadele, Domates, *Beauveria bassiana*, *Metarhizium anisopliae*, *Tuta absoluta*

Abstract: The current study was initiated to test the pathogenicity of entomopathogenic fungi against *Tuta absoluta*. The experiment was conducted at Bursa Uludag University, Horticulture Department glasshouse in 2020/2021. *Tuta absoluta* adults were collected and larvae were reared on tomatoe seedlings in a growth chamber. Then, ten larvae were treated with each isolate at five inoculum suspension concentrations in a factorial experiment arranged in a completely randomized block design with three replications. The analysis of variance for mortality revealed significant variations ($p<0.05$) among isolates and concentrations. *Metarhizium anisopliae* Ak-12 caused the lowest mortality of 80.77% but had the highest LC_{50}

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Alınış (Received): 3 Mayıs 2022 Kabul ediliş (Accepted): 26 Eylül 2022

($2.3x10^8$) and the longest incubation period LT_{50} 4.9 and LT_{90}, 9.9 days and considered to be less pathogenic. Whereas *Beauveria bassiana* Ak-10 showed the highest mortality 91% and the lowest LT_{50}, 4 and LT_{90}, 7.6 days at 1×10^{10} conidia /ml, followed by *Beauveria bassiana* Ak-14 and is considered the most aggressive. Conidia concentrations of $1x10^9$ and $1x10^{10}$ conidia/ml were the most effective while $1x10^6$ conidia/ml was the least effective. Overall, the current work revealed the potential variation among isolates and concentrations on the mortality of *T. absoluta* larvae.

Keywords: *Beauveria bassiana*, Biological control, *Metarhizium anisopliae*, Tomato, *Tuta absoluta*

Introduction

The tomato (*Solanum lycopersicum*) is one of the most economically important, popular and widely grown crops globally (Nicola et al. 2009). Despite the importance of tomato as a food crop, its production and productivity are threatened by various biotic and abiotic factors (Sora 2018). Mainly insect pests and pathogens are the biotic factors responsible for the loss of tomato production (Veres et al. 2020). Among insect pests, the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is the most economically important pest in tropical and subtropical regions of tomato cultivation (Rwomushana et al. 2019). It was first reported in Turkey in 2009 and in Ethiopia in 2012 (Kılıç 2010; Gashawbeza & Abiy 2012).

This insect pest can cause high total yield losses wherever tomato is grown (FAO 2017). After eggs are laid on the underside of leaves, stems or fruits, they hatch and develop into larvae. Then the larvae invade tomato fruits and leaves, feeding insidethe mesophyll tissue and creating galleries that cause necrosis resulting inreduced yield (Guedes & Picanço 2012; Biondi et al. 2018). Under extensive attacks, plants show signs and symptoms such as abnormal leaf shape, puncture marks, exit holes and necrosis (Rwomushana et al. 2019). Under non-control conditions, tomato yield losses due to *T. absoluta* have been estimated at 80 to 100% of annual production and increased the price of tomatoes by 23% (Desneux et al. 2010). Due to this pest, Ethiopia loses 60% to 82% of tomato production (Shiberu & Getu 2017).

To date, there are no effective measures for sustainable control of *T. absoluta* due to its high reproduction capacity; short generation cycle; and limited opportunity for control with insecticides, as larvae feed on mesophyll within the host plant's tissues and are hidden inside of leaves, (Siqueira et al. 2001). However, early detection by sex pheromone traps; application of various cultural and mechanical control methods; biological control by the parasitoid wasp, *Trichogramma cacoeciae* Marchal, mirid predator, *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) (Biondi et al. 2013; CABI 2021), and *Bacillus thuringiensis* (Sabbour & Nayera 2012), have been suggested as management strategies for this pest. Despite the use of these control methods, tomato leaf miner infestation has become increasingly difficult to control due to recommended control options not being effective and/or not being implemented in a timely manner.

Among biological control agents, entomopathogenic fungi have been reported to be very effective agents of infection of insect pests, particularly the lepidopterans (Kaya

& Vega 2012; Ruiu 2015). Based on their efficacy against *T. absoluta*, the entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana* species, were reported to be pathogenic to the tomato leaf miner in different countries (Youssef 2015; Erler & Ozgur 2015; Shiberu & Getu 2017). Ndereyimana et al. (2020) determined the efficacies of *M. anisopliae* and *B. bassiana* against third instar larvae of *T. absoluta* and reported highest mortality rates of 82.8% and 60.8% at $1x10^8$ conidia/ml, respectively. This result indicated that *M. anisopliae* is more virulent and pathogenic than *B. bassiana* against third instar larvae. Contradicting that result, Youssef (2015) reported that *B. bassiana* (86.7%) was more effective than *M. anisopliae* (76.7%) against *T. absoluta* larvae at $1x10^8$ spores/ml. Similarly, Shiberu & Getu (2017) tested the efficacy of *M. anisopliae* and *B. bassiana* isolates against third instar larvae and found larval mortality of 87.5% and 95.83%, respectively, at a dosage of $2.5x10^9$ conidia/ml under laboratory conditions.

These mixed results indicate that there is a research gap in the efficacy testing of entomopathogenic fungal species which should be resolved by further evaluation to select the best potential candidate for effective *T. absoluta* control. The relative efficacy testing of endemic entomopathogenic isolates occurring in different areas is not widely done (Desneux et al. 2010). This indicates that there has been no conclusive selection of the best entomopathogenic fungal candidates for use against *T. absoluta* due to insufficient comparative efficacy measurements. In addition, the differences in pathogenicity and virulence of entomopathogen fungal isolates and their concentrations have not been widely investigated. Therefore, the main objective of this study was to evaluate the pathogenicity of some local Turkish entomopathogenic fungal isolates against *T. absoluta*.

Materials and Methods

Rearing of *Tuta absoluta*

Tuta absoluta adults were collected from infested tomatoes in the glasshouse of the Horticulture Department of the Faculty of Agriculture, Uludag University, and 10 adults were released on young tomato (cultivar H-2274) seedlings planted in plastic pots (8 x 9 cm) and kept in insect-proof rearing cages in a growth chamber at 25 °C and 65% RH and 16:8 hours light: dark photoperiod. For two days, adults were fed with 10% sugar solution and then aspirated with a mechanical aspirator. After egg hatching, the larvae were allowed to feed on the potted tomato plants until the targeted third larval instar stage was reached. After 4-5 days, the eggs hatched and the larvae started feeding in galleries. After two generations, they were used for the bioassay. The third instar larvae, based on their age, colour and size (8 to 12 days old and 3-6 mm in length), were harvested by opening the mines (Rahtna & Bhat 2019).

Preparation of inoculum entomopathogenic fungal isolates

Pathogenicity testing was performed on third instar larvae of *T. absoluta* using four different entomopathogenic fungal **(**EPF) isolates - *M. anisopliae* Ak-11, *M. anisopliae*

Ak-12, *B. bassiana* Ak-14 and *B. bassiana* Ak-10 (Table 1)**.** The EPF isolates, which were provided by Prof. Dr Ali Sevim from Kırşehir Ahi Evran University, were grown on potato dextrose agar (PDA) medium and incubated at 26°C darkness for 14 days. Then the plates were placed in a biosafety cabinet at room temperature for 48hrs to allow the fungi conidial toair dry. After two days of incubation, the dry, powdered conidia were harvested from plates by gently scraping them from the surface of the media with a sterile metal spatula onto aluminium foil under laminar flow. Finally, the harvested conidia were stored at 4°C until use (Jaronski & Mascarin 2013). For inoculation purposes, 0.00002, 0.0002, 0.002, 0.02 and 0.2 g of powdered conidia were suspended in 1 ml of sterilized, distilled water in 15 ml test tubes, with 0.01% aqueous Tween 20% (two drops) used as a wetting agent. The suspensions were vortexed for one minute and then filtered through a cheesecloth to separate the conidia from the mycelia and then homogenized by vortexing for 3 minutes, as described in Shiberu & Getu (2017).

The concentration of the conidia suspensions of each isolate were adjusted to $1x10^6$, $1x10^7$, $1x10^8$, $1x10^9$ and $1x10^{10}$ conidia /ml by using a hemocytometer, in preparation for inoculation (Kushiyev et al. 2018). Before the bioassays, the viability of the conidia was determined by spreading 0.1 ml of conidial suspension on PDA plates. A sterile microscopic coverslip was placed on each plate and the plates were incubated at 26±2°C and examined after 15 hours. The percentage germination of the conidia was determined by counting 100 spores of each isolate. The viability testing for each isolate was replicated three times. Over 90% of the conidia of each isolate germinated.

Table 1. Source and detail information of entomopatogenic fungal isolates

	Entomopathogenic fungual species			
	Beauveria bassiana AK-10	*Beauveria bassiana* AK-14	*Metarhizium anisopliae* AK-11	*Metarhizium anisopliae* AK-12
Source	Soil	Soil	Soil	Soil
Location	Konya, Beyşehir	Konya, Beyşehir	Konya, Beyşehir	Konya, Beyşehir
Coordinates	37°43'04.5"N 31°43'16.9"E	37°42'31.0"N 31°43'38.4"E	37°42'56.1"N 31°43'19.4"E	37°42'29.0"N 31°43'35.7"E
	GenBank accession numbers			
ITS	MW689267	MW689268	MW689278	MW689279
Bloc	ON089022	ON089023	-	-
EF1-α	ON093091	ON09392	-	-
Rpb1	ON093085	ON093086	ON125481	ON125482
β-tubulin	-	-	ON125494	ON125495

Experimental design

A glasshouse experiment was carried out to determine the pathogenicity of four entomopathogenic fungal isolates at six suspension concentrations (0,$1x10^6$, $1x10^7$, 1×

10^8, 1×10^9 and 1×10^{10} conidia /ml) against third larval stages of *T. absoluta* in a completely randomized block design with three replicates. The pathogenicity of these isolates was tested by using a factorial experimental design with concentration as a subfactor treatment and selected entomopathogenic fungal isolates as the main factor treatment.

For this purpose, tomato cultivar H-2274 was planted in plastic pots (8 cm in diameter x 9 cm in height) filled with a mixture of peat soil and perlite at a 2:1 ratio and grown in wooden boxes (150 x120 x 90 cm) lined with netting and allowed to grow for two months. Ten third instar larvae were released onto the upper side of leaves of each tomato seedling (20cm in height). The pots were covered with muslin cloth and incubated in screened cages for 24 h to allow the formation of mines and galleries. After 24 h, three tomato seedlings infested with third stage larvae (n=30) for each entomopathogen fungal isolate were sprayed with 2000 µl of the appropriate conidial concentration as a foliar spray using a micropipette (Eppendorf pipette, 5000µl) whereas the control seedlings were sprayed with the same volume of distilled sterile water amended with 0.02% Tween 20. After application, observations were performed on days 3, 5 and 7 as described in Youssef (2015), and Shiberu & Getu (2017).

Data on disease measurement parameters included larval mortality; larvae were counted as dead when they were not moving, and they were removed from the sample once the data were collected. In subsequent days counting, data for each assessment day were summed and calculated as cumulative larval mortality.

$$\text{Mortality (\%)} = \frac{\text{Dead larvae x 100}}{\text{Total larvae}}$$

In the case of death occurring in control treatment larvae as natural mortality, the percentage mortality of larvae killed by the entomopathogenic fungal isolates alone was corrected by using Abbott's formula (Abbott, 1925):

$$\text{Corrected mortality (\%)}\frac{\text{Observed mortality (\%)} - \text{Control mortality (\%)} * 100}{100 - \text{Control mortality (\%)}}$$

However, when the observed mortality in the treatment was the same as or less than that in the control, the formula was not applied (Singh & Zahra 2017). Furthermore, dead larvae were surface-sterilized in 1% sodium hypochlorite solution, washed 3 times with sterile water, and then taken to another Petri-dish covered with moistened filter paper and kept in darkness at 25±2 °C for one week to allow fungal growth and confirm whether larval death was due to fungal infection or not. The lethal times (LT_{50} and LT_{90}) for each entomopathogenic fungal isolate against *T. absoluta,* ie., the number of days required to reach 50% and 90% cumulative mortality, were determined. Lethal concentrations (LC_{50} and LC_{90}) were also calculated.

Statistical Analysis

A two-way analysis of variance was performed on mortality percentage data using the General Linear Model of JMP @7.0 software package (SAS, Institute 1989–2021). The interaction effect between entomopathogenic fungal isolates and different concentrations was also analyzed with the ANOVA. Differences in mortality means were determined by using Tukey's honestly significant difference test ($P \leq 0.05$). Lethal time and concentration values (LC50, LT50, LC90 and LT90) were determined by probit analysis using SAS statistical analysis software (Version 9.4) (Finney 1971).

Results and discussion

Typical symptoms of fungal infection of *Tuta absoluta*

Observation of disease symptoms was started three days after spraying. Visible disease symptoms were observed on *T. absoluta* third instar larvae which had been infected with the entomopathogenic fungi, *M. anisopliae* and *B. bassiana* isolates. After infection, typical disease symptoms were observed in the tomato leaf galleries such as the immobility of the larvae and their colouration which turned to brown, red and finally black at death (Figure 1). Further, mycosis had emerged from the dead larvae which were removed and placed onmoistened filter paper in a Petri dish. Typical mycosis symptoms on the dead larvae treated with *B. bassiana* isolates including whitish mycelia and conidial growth, whereas the dead larvae treated with *M. anisopliae* isolates had a dull green appearance. Similarly, Fergani & Yehia (2020), described such types of symptoms

Figure 1. Third larval stage of *Tuta absoluta* showing typical disease symptoms after infection. (a) Green muscardine on cadavers (b) White muscardine on cadavers.

Pathogenicity of entomopathogenic fungi against *Tuta absoluta* third instar larvae in aglasshouse

Analysis of variance for percentage mortality of larvae revealed significant differences ($P<0.05$) between the isolates and their conidia concentrations on the third, fifth and seventh days after application (Tables 2 and 3). There was also a significant difference in the interaction five and seven days after application ($P<0.05$) (Table 2 and 3).

Pathogenicity testing showed that the isolates caused cumulative mortality ranging from 8% to 91% at different conidial concentrations, while 0% to 13.33% mortalities were recorded for sterilized, distilled water-treated controls (Table 2). Seven days after inoculation, the *B. bassiana* isolates AK-10 and AK-14 had caused the highest cumulative mortality (91.35% and 90.39%) whereas the *M. anisopliae* isolates AK-11 and AK-12 isolates had caused 80.07% mortality at $1x10^{10}$ conidia/ml compared to the control (13.33%), respectively (Table 2). In addition, the highest corrected cumulative mortalities were 59.30%, 59.26%, 51.85% and 40.74% for *B. bassiana* AK-10, *B. bassiana* AK-14, *M. anisopliae* AK-11 and *M. anisopliae* AK-12 isolate at $1x10^{10}$ conidia/ml, respectively, whereas the least corrected cumulative mortalities of 37.03%, 15.65%, 14.81% and 8.24% mortality were recorded for *B. bassiana* AK-10, *B. bassiana* AK-14, *M. anisopliae* AK-11 and *M. anisopliae* AK-12 , respectively, at the lowest concentration ($1x10^6$ conidia/ml) five days after inoculation. Also, compared to the control, *B. bassiana* AK-10 and *B. bassiana* AK-14 caused the highest mortality rates of 33.33% and 33.67%, respectively, followed by *M. anisopliae* AK-11 (26.67%) and *M. anisopliae* AK-12 (23.33%), respectively, three days after treatment (Table 2).

High mortality was recorded in the larvae inoculated with the *B. bassiana* isolate AK-10, followed by *B. bassiana* isolate AK-14, while the other two isolates caused lower mortality at both $1x10^{10}$ and $lx10^9$ conidia/ml. Moreover, the *B. bassiana* AK-10 and AK-14 isolates were significantly superior at all conidial concentrations in their ability to cause larval mortality. In addition, the lowest concentration (1×10^6) conidia ml^{-1} of all the tested EPF isolates, except for *B. bassiana* AK-10, had caused less than 50% larvae mortality at the termination of the experiment. This result indicates that *B. bassiana* was more effective than *M. anisopliae* as it caused the highest mortality percentage to third instar larvae at higher concentrations. Furthermore, the highest mortality rates were obtained with the highest concentrations ($1x10^9$ and $1x10^{10}$ conidia/ml). Hence, the concentrations of 10^9 conidia/ml and 10^{10} conidia/ml were the most effective on the third larval instars of *T. absoluta*, followed by 10^8 conidia/ml. The most likely reasons for the pathogenicity and virulence differences among the four isolates may be to differences in their enzyme activities and/or a virulence factor which enables them to penetrate the host and cause infection.

The current results were in accordance with the findings of Abdel-Raheem et al. (2015) who reported that the higher the concentration of *B. bassiana* and *M. anisopliae*, the higher the mortality of *T. absoluta* larvae. Tsoulnara & Gordon (2016) also tested the efficacy of *B. bassiana* against third instar larvae and reported 90 % mortality at a high concentration. Likewise, the result was in agreement with the

finding of Rodriguez et al. (2006) who stated that more than 90% larval mortality after larvae were treated with both *M. anisopliae* and *B. bassaina* in a pot experiment. In addition, Ndereyimana et al. (2019) recorded a highest larval mortality of 82.8% after applying *B. bassiana* against third instar *T. absoluta* larvae, and, *M. anisopliae* (77%) caused lower mortality than *B. bassaina* (87%) at 10^8 spores/ml (Youssef, 2015).

Table 1. Corrected mortality percentages for *Beauveria* and *Metaharizium* isolates used at different conidial concentrations against the third larval stage of the Tomato leaf miner, *Tuta absoluta*, under glasshouse conditions.

EPF isolates	Concentration (Conidia/ml)	% Corrected mortality larval days after inoculation		
		3DAT	5DAT	7DAT
Beauveria bassiana AK-10	10^{10}	33.33^{a*}	59.30^{a}	91.35^{a}
	10^{9}	26.67^{ab}	53.707^{ab}	88.46^{ab}
	10^{8}	26.6^{ab}	48.15^{ab}	84.61^{ab}
	10^{7}	23.33^{abc}	40.74^{abc}	69.23^{cde}
	10^{6}	20.00^{abc}	37.03^{abcd}	50.0^{defgh}
Beauveria bassiana AK-14	10^{10}	33.67^{a}	59.26^{a}	90.39^{a}
	10^{9}	26.67^{ab}	51.85^{ab}	87.5^{ab}
	10^{8}	23.33^{abc}	48.15^{ab}	84.61^{ab}
	10^{7}	20.00^{abc}	37.03^{abcd}	61.54^{def}
	10^{6}	16.67^{abcd}	15.65^{def}	39.32^{efghi}
Metarhizium anisopliae AK11	10^{10}	26.67^{ab}	51.85^{ab}	80.77^{abc}
	10^{9}	23.33^{abc}	40.74^{abc}	80.77^{abc}
	10^{8}	20.00^{abc}	33.33^{bcd}	57.70^{defg}
	10^{7}	13.33^{bcd}	14.81^{def}	30.77^{fghi}
	10^{6}	10.00^{abc}	14.81^{def}	27.18^{ghi}
Metarhizium anisopliae AK12	10^{10}	23.33^{abc}	40.74^{abc}	80.77^{abc}
	10^{9}	20.00^{abc}	29.63^{bcde}	73.08^{bcd}
	10^{8}	13.33^{bcd}	18.52^{cdef}	42.31^{defghi}
	10^{7}	10.83^{bcd}	18.52^{cdef}	30.77^{fghi}
	10^{6}	7.5^{cd}	8.24^{ef}	19.23^{hi}
Control	0.0	0.00^{d}	3.33^{f}	13.33^{i}
	LSD at %5	9.09	12.48	15.44
	CV (%)	18.52	25	17.11
	SE-+	5.55	7.63	9.43

*Means with the same letter(s) in columns are not significantly different from each other.
DAT -Days after treatment

Concentration–Mortality Bioassay (LC_{50} and LC_{90})

The response of third instar larvae of *T. absoluta* to different concentrations **of** EPF isolates fitted the probit model very well and showed a non-significant chi-square value, but not for for *M. anisopliae* AK-11 and AK-12, seven days after application (P > 0.05; Table 3). The lethal concentrations needed to induce 50% and 90% mortality (LC50 and LC90) ranged from 7.47E+06 to 4.32E+19 conidia/ml on the 3rd, 5th and 7th after application. The lowest LC_{50} and LC_{90} values obtained against third instar larvae were for the *B. bassiana* isolates AK-10 AK-14 whereas *M. anisopliae* AK-11 and *M. anisopliae* AK-12 showed the highest LC_{50} and LC_{90} values on all days after treatment. The two *B. bassiana* isolates are therefore better prospects for the control of *T. absoluta*.

In this study, *B. bassiana* AK-10 had the lowest LC_{50} and LC_{90} values at all days after inoculation, a steeper slope (b=0.26) and the least standard error (SE=0.038) for 50% and 90% larval mortality. Hence, based on these lethal concentration values and the regression slope, *B. bassiana* AK-10 is deemed the most toxic and virulent isolate, followed by *B. bassiana* AK-14, whereas *M. anisopliae* AK-12 had the highest LC_{50} and LC_{90} values and the lowest slope and is therefore considered the least toxic isolate. Similar results were reported by different authors who conducted concentration-dependent mortality experiments with different conidial suspensions in glasshouses. The current results approximate those of Sabbour (2014) who reported a LC_{50} value at $1.02x10^6$ spores/ml for *B. bassiana*, and $1.00\ x10^6$ spores/ml for *M. anisopliae* at $8.25\ x\ 10^8$ conidia/ml under glasshouse conditions. Also, the LC_{50} values for *B. bassiana* and *M. anisopliae* were 0.7×10^7 conidia ml^{-1} and 2.5×10^7 conidia ml^{-1}, respectively (Wekesa et al. 2006) and Aynalem et al. (2020) determined LC_{50} values ranging from 1.3×10^3 to 7×10^4 conidia/ml for *M. anisopliae* against third instars of *T. absoluta*.

Time–Mortality Bioassays (LT_{50} and LT_{90})

The period for inducing 50% and 90% mortalities of third instar larvae of *T. absoluta* inoculated with four EPF isolates at different concentrations was determined using a probit analysis program. The response of larvae to isolate concentrations over time (Log10 day) fitted the probit model as the χ^2- Chi-square values were not significant (P>0.05) (Table 4). The time taken in days to kill 50% and 90% of the larvae treated with the four isolates at varying conidial concentrations differed among the isolates at all concentrations.

Probit analysis produced LT_{50} values that ranged from 4.0 days to 13.1 days and LT_{90} values from 7.5 days to 19.5 days for all isolates across all conidial concentrations (Table 4). The lowest LT_{50} and LT_{90} values for *B. bassiana* AK-10were 4 and 7.6 days, respectively, followed by *B. bassiana* AK-14 at 4.1 and 7.8 days, respectively, at $1x10^{10}$ conidia/ml. The second lowest values were 4.3 and 8.2 days for *B. bassiana* AK-10, respectively, and 4.4 and 8.3 days for *B. bassiana* AK-14, respectively, at $1x10^9$ conidia/ml.

Table 2. Lethal concentrations (LC 50 and LC 90) for *Beauveria bassiana* and *Metarhizium anisopliae* isolates from Turkey against third instar larvae of *Tuta absoluta* on the 3rd, 5th and 7th days after treatment

7 Days after treatment						
EPF isolates	LC50 value	LC90 value	Slope±SE	$\chi 2$ (df=4)	95% FL for LC50	P-value
Metarhizium anisopliae AK-12	6.62E+09	9.83E+12	0.21±0.07	14.9	1.65E+06-5.68E+12	0.005[a*]
Metarhizium anisopliae AK-11	1.20E+09	6.38E+12	0.22±0.07	12.7	1.48E+06-2.5E+11	0.013[a]
Beauveria bassiana AK-14	2.13E+07	6.50E+10	0.25±0.038	6.97	1.66E+04-1.45E+06	0.14
Beauveria bassiana AK-10	7.47E+06	1.88E+10	0.26±0.038	2.75	4.83E+04-5.53E+05	0.6
5 Days after treatment						
Metarhizium anisopliae AK-12	1.39E+12	1.95E+14	0.18±0.053	2.22	1.97E+10-1.57E+13	0.7
Metarhizium anisopliae AK-11	2.08E+10	2.75E+13	0.22±0.053	2.84	1.48E+09-7.96E+12	0.59
Beauveria bassiana AK-14	6.96E+08	6.36E+11	0.23±0.05	2.4	9.15E+07-1.51E+10	0.66
Beauveria bassiana AK-10	2.27E+08	5.89E+11	0.24±0.042	0.7	2.25E+07-5.34E+09	0.95
3 Days after treatment						
Metarhizium anisopliae AK-12	2.12E+13	4.32E+19	0.20 ±0.08	0.22	9.18E+10-4.26E+27	0.99
Metarhizium anisopliae AK-11	9.20E+11	1.42E+19	0.20±0.068	0.45	1.48E+10-2.92E+18	0.98
Beauveria bassiana AK-14	4.91E+12	7.02E+18	0.19±0.058	0.92	4.25E+10-1.87E+22	0.92
Beauveria bassiana AK-10	9.38E+11	3.10E+12	0.21±0.053	1.77	1.28E+10-4.32E+18	0.78

*In the P value column, 'a' means the Chi-square value is significant; FL = fiducial limits; df = degrees of freedom of a number of concentrations (n: 4)

The *M. anisopliae* isolates AK-11 and AK-12 had higher LT_{50} values of 4.5, 4. 9, 4.8 and 5.5 days and LT_{90} values of 9.5, 9.9, 9.9 and 12 days at $1x10^9$ and $1x10^{10}$ conidia/ml, in the same order. On the other hand, at the lowest conidia concentration of $1x10^6$ conidia/ml, the LT_{50} values were 7, 8.5, 11.7 and 13.1 days for *B. bassiana* AK-10, *B. bassiana* AK-14, *M. anisopliae* AK-11 and *M. anisopliae* 12, and the LT_{90} values were 13.8, 16, 18.7 and 19.5 days. As shown in Table 4, larval mortality increased with increasing isolate concentrations whereas thelethal time values decreased.

Each isolate of the two tested EPF species showed a positive pathogenicity against *T. absoluta* but differed in their pathogenicity levels. *Beauveria bassiana* Ak-10 had an LT_{50} of 4 days, followed by *B. bassiana* AK-14 with an LT_{50} of 4.1 days and can be considered more virulent than the other tested EPF isolates. Furthermore, *B. bassiana* AK-10 had the lowest LT_{90} of 9.5 days, followed by *B. bassiana* AK-14 with an LT_{50} of 9.9 days. By having the lowest LT_{50} and LT_{90} values, *B. bassiana* (AK-10) can be considered the most pathogenic isolate, followed by *B. bassiana* AK-14 , while *M. anisopliae* Ak-12 had the third highest LT values and was moderately lethal, followed by *M. anisopliae* Ak-11 isolates.

The results of the present study approximate those of Ozdemir et al. (2020) who reported LT_{50} and LT_{90} values of 4.45 days and 5.34 days, respectively, for *M. anisopliae*, and 4.07 days and 5.11 days, respectively, for *B. bassiana*, at 1×10^8 conidia/ml. Similarly, Shiberu & Getu (2017) reported LT_{50} and LT_{90} values of 5.01 days and 8.06 days, respectively, for *B. bassiana*, and 4.82 days and 8.14 days, respectively, for *M. anisopliae*, at $2.5x\ 10^9$ conidia/ml. In contrast to the results of the current study and some other studies cited herein, Nyalal et al. (2019) reported lowest LT_{50} values of 3.9 days and 5.2 days for commercial *M. anisopliae* Metatech® WP and *B. bassiana* Beauvitech, respectively, andAynalem et al. (2020) reported an LT_{50} value of 3.9 days for *M. anisopliae* was LT50.

Conclusions and recommendations

In the current experiments, the pathogenicity results showed that the evaluated EPF isolates caused different third instar larval mortalities. *Beauveria bassiana* caused the highest mortality (91%), had the shortest lethal time (LT50: 4 days), and the lowest lethal concentration (LC_{50}, $7.47x10^6$conidia/ml) and therefore showed superior virulence compared to the other three EPF isolates tested against third instar *T. absoluta* larvae, while *Metarhizium anisopliae* caused the lowest mortality (20% to 82%), and had the long incubation period before causing death (4.9 to 13 days), and the highest LC_{50} ($6.62E+10^9$ -$2.12x10^{13}$), and hence it was considered to have low to moderate virulence. Overall, *B. bassiana* was more effective in killing *T. absoluta* larvae. It was also found that increasing conidial concentrations resulted in increased larval mortality. Among the applied EPF isolates, concentrations of $1x10^9$ and $1x10^{10}$ conidia/ml were the most effective in causing larval mortality and can be recommended for application, subject to further laboratory testing and then field

testing. Hence, means that the entomopathogenic fungal isolates were more effective at higher concentrations against the third larval instars of *T. absoluta*. As the current work revealed potential pathogenicity variation among the four EPF isolates and different mortalities at different concentrations, further research is needed to better understand why these differences are occurring. Moreover, research should be focused on identifying the mechanisms of pathogenicity of *B. bassiana* and *M. anisopliae* isolates.

Table 3. The mean LT_{50} and LT_{90} values of third instar *Tuta absoluta* larvae after the application of four entomopatogen fungi (EPF) isolates at a range of different concentrations

EPF isolates	**Conidia/ml**	**LT50**	**LT90**	**Slope ±SE**	**95% FL LT50**	**χ2**
Beauveria bassiana AK-10	10^{10}	4.0	7.6	4.66±1.00	3.19-4.56	1.73[a*]
	10^{9}	4.3	8.2	4.34±0.980	3.6-4.9	1.55[a]
	10^{8}	4.5	9.0	4.22±0.98	3.7-5.3	1.89[a]
	10^{7}	5.3	11.0	3.28±0.95	4.8-16	0.73[a]
	10^{6}	7.0	13.8	2.29 ±0.94	8.31-nf	0.01[a]
Beauveria bassiana AK-14	10^{10}	4.1	7.8	4.33±0.99	3.2-4.6	1.51[a]
	10^{9}	4.4	8.3	4.53±0.99	3.6-5	1.72[a]
	10^{8}	4.6	8.8	4.54±0.99	3.9-5.3	1.53[a]
	10^{7}	5.9	12.8	3.06 ±0.95	4.8-9.00	0.35[a]
	10^{6}	8.6	16.0	1.87 ±0.99	-	1.94[a]
Metarhizium anisopliae AK-11	10^{10}	4.5	9.5	3.94±0.96	3.7-5.3	0.61[a]
	10^{9}	4.8	9.9	4.14±0.98	4.2-5.8	2.49[a]
	10^{8}	6.4	11.1	2.79±0.95	5.1-12.2	0.53[a]
	10^{7}	9.4	16.2	1.65±1.04	-	0.79[a]
	10^{6}	11.7	18.7	1.85±1.096		0.25[a]
Metarhizium anisopliae AK-12	10^{10}	4.9	9.9	4.14±0.98	4.1-5.8	2.49[a]
	10^{9}	5.5	12.0	3.38±1.86	-	3.58[a]
	10^{8}	9.2	13.2	2.51±1.03	6.5-70	1.09[a]
	10^{7}	11.2	17.4	1.99±1.06	-	0.097[a]
	10^{6}	13.1	19.5	1.58±1.2	-	0.56[a]

*χ^2- Chi-square values marked with "a" were not significant at the level of α=0.05, indicating a good fit of the probit model; DF:degrees freedom of a number of days (n:2); FL: fiducial limits; LT_{50}: Time taken to cause 50% mortality; LT_{90}: Time taken to cause 90% mortality.

Acknowledgements

The authors acknowledge the support of the Bursa Uludağ University, Faculty of Agriculture, Crop protection, Mycology and Horticulture Departments for providing all the necessary experimental materials including reagents, chemicals, laboratory, greenhouse and other materials for this research work. We also sincerely thank Prof. Dr Ali Sevim (Kırşehir Ahi Evran University, Faculty of Agriculture, Department of Plant Protection) for the provision of different Turkish entomopathogenic fungal isolates. Sincere thanks are also extended to the Plant Protection Department staff and greenhouse workers for providing the experimental site and other support during our experiments.

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