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Susceptibility of different life stages of *Tenebrio molitor* (Coleoptera: Tenebrionidae) to indigenous entomopathogenic fungi

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ABSTRACT

Tenebrio molitor L, known as the yellow mealworm, is a polyphagous insect that infests cereals, flour, bran, and pasta worldwide. Both larvae and adults of the pest cause significant damage to stored products. In this study, entomopathogenic fungi were isolated from soil samples using the Tenebrio-bait method and the susceptibility of different life stages of *T. molitor* to these indigenous isolates was investigated. Based on morphology and nuclear ribosomal internal transcribed spacer (ITS) sequence, a total of seven fungal isolates, one *Beauveria bassiana* and six *Metarhizium anisopliae*, were identified. The screening test showed that the virulence of entomopathogenic fungi varies according to fungal species and strain, and that different life stages differ in their susceptibility to fungal infection. *B. bassiana* BL8 was the most effective isolate for all life stages tested. While adults were the most resistant life stage ($LC_{50} = 5.6 \times 10^6$ conidia/ml) to *B. bassiana* infection, pupae were the most susceptible life stage ($LC_{50} = 3.9 \times 10^2$ conidia/ml). The results show that *B. bassiana* BL8 is a promising microbial control agent that can support the control of *T. molitor*.

1. Introduction

Total global food production needs to increase by 56% to feed the estimated world population in 2050 (van Dijk et al., 2021). To meet this demand and ensure future global food security, in addition to increasing production, we should focus on reducing post-harvest food losses, i.e., food losses throughout the food supply chain from harvest to consumption. Most post-harvest losses occur due to infestation by insect pests (Boxall, 2001; Hodges et al., 2011; Kumar and Kalita, 2017). Avoiding losses due to pests in stored products is therefore an important cornerstone.

Tenebrio molitor Linnaeus (Coleoptera: Tenebrionidae), known as the mealworm, causes losses of up to 15% of grain and flour production worldwide (Neethirajan et al., 2007). It not only eats the product directly, but also contaminates the feed with corpse fragments and faeces, leading to a decline in market value (Plata-Rueda et al., 2017). The pest is mainly controlled with chemical insecticides such as organophosphates, synergised pyrethrins, and pyrethroid compounds (Athanassiou et al., 2015). Due to the development of insect resistance and increasing concern about chemical residues, more environmentally friendly means of controlling storage pests are needed instead of chemical insecticides.

Entomopathogenic microorganisms could be an alternative strategy for the management of *T. molitor*. In particular, the insect-pathogenic fungi *Beauveria bassiana* (Vuill.) Balsam (Hypocreales: Cordycipitaceae) and *Metarhizium anisopliae* (Metch.) Sorokin (Hypocreales: Clavicipitaceae) are the most important species for pest control. Insecticidal activity does not require the insect to ingest fungi such as bacteria and viruses, because fungal spores adhere to the insect cuticle and enter the body cavity when they germinate (Mannino et al., 2019; Mantzoukas et al., 2022). Effective control with fungi has been reported for *T. molitor* (Shah et al., 2005; Adatia et al., 2010; Maistrou et al., 2018; Altahawi et al., 2020). The pathogenicity of one of 23 *B. bassiana* strains isolated from different habitats showed rapid and effective insecticidal activity against the larval stage of *T. molitor* (Oreste et al., 2012). The virulence of an indigenous isolate of *B. bassiana* was similar against both larval and adult stages (Rodríguez-Gómez et al., 2009). However, there is little knowledge about pathogens' toxicity to different larval instars of *T. molitor*. In fact, the pest should always be controlled at the most sensitive life stage to increase the effect of pathogen application (Ansari and Butt, 2012; Idrees et al., 2021).

Thus, the objective of the current study was to characterise new

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indigenous entomopathogenic fungi morphologically and molecularly from agricultural and forestry soils using the *Tenebrio*-bait method and to investigate the susceptibility of the developmental stages of *Tenebrio molitor* to these isolates in order to determine the most susceptible life stage to enhance the effect of pathogen application.

2. Materials and methods

2.1. Soil sampling

Soil samples were collected from eight different locations in the city of Bilecik, Turkey (40°06′24 "N latitude, 30°07′33 ″E longitude). A total of forty samples of agricultural and forestry soils were collected in October and November 2020. The surface of the soil was scraped and about 500 g of soil was collected from a depth of 15 cm using a soil core borer. The sample was then placed in a sterile, autoclaved glass jar (500 mL). To avoid possible contamination between successive samplings, the soil core borer was washed with water, 5% sodium hypochlorite, and water. Sampling was completed within one day and the soil samples were taken to the laboratory and stored at 20 °C before further processing (Mantzoukas et al., 2020).

2.2. Insect rearing

The larvae of *Tenebrio molitor* to be used for the isolation of entomopathogenic fungi were purchased from a local pet shop. To confirm that the insect was *Tenebrio molitor*, larvae and adults were examined under a stereomicroscope to identify characteristic features such as the evenly divided linear grooves extending the entire length of the abdomen and four tarsal segments on the hind legs (Brendell, 1975). Insects were reared in plastic boxes (40 × 25 × 10 cm) disinfected with 0.5% sodium hypochlorite solution. Larvae were fed a diet consisting of whole grain flour (90%) and instant dry yeast (10%) (Rumbos et al., 2021). In addition, potato slices were placed in the boxes to provide the necessary humidity for rearing *T. molitor* (Baek et al., 2015). Rearing took place at 25 ± 2 °C, 60 ± 5% relative humidity (RH) and continuous darkness (Ribeiro et al., 2018).

2.3. Entomopathogenic fungus isolation

Entomopathogenic fungi were isolated from soil samples using the insect baiting method described by Zimmermann (1986) with minor modifications. Ten larvae of *Tenebrio molitor* (4th instar stage, ≈5–7 mm body length) were placed in the glass jars containing the soil samples and incubated at 25±2 °C for three weeks. Each jar was inverted every three days. After five days, the samples were examined daily for dead larvae. The cadavers were removed and surface sterilised in a 5% sodium hypochlorite solution for 2 min and then rinsed with sterile distilled water. They were then placed in a humid chamber for mycosis. Conidia from all mycosed *T. molitor* cadavers were transferred to Sabouraud dextrose agar (SDA) plates and incubated at 25 ± 2 °C until fungal growth was observed. The cultured fungi were subcultured onto SDA plates in multiple passages to obtain pure cultures.

2.4. Morphologic characterization

The fungal isolates were morphologically characterised based on macroscopic and microscopic features. First, the colony characteristics such as color, shape, surface and margin were observed. Then the microscopic features such as hyphae, conidiophores and conidia were observed using the slide culture technique (Humber, 2012). The fungal isolates were grown on SDA plates at 25 ± 2 °C until sporulation. An agar block (10 × 10 mm) was cut with a sterile scalpel and placed on a slide. After the lactophenol-cotton blue stain was drained, a coverslip was placed on the agar block and examined under a light microscope (Woo et al., 2010). The morphological characteristics of the isolates were evaluated according to the identification key described by (Humber, 2012).

2.5. Molecular characterization

Genomic DNA (gDNA) was extracted from approximately 100 mg of fresh mycelium of each isolate using the Quick-DNA Fungal/Bacterial MiniPrep Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions. Molecular identification of the fungal strains was performed by amplification and sequencing of the ITS1-5.8SITS2 region of the gDNA. The universal primer sets ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and ITS5 (5′- GGAAGTAAAAGTCG-TAAC AAGG -3′) were used for PCR. PCR reactions (25 μL) contained 2.5 μL of 10 × standard Taq reaction buffer, 0.5 μL of each 10 μM primer, 0.5 μL of 10 mM dNTPs, 1.5 μL of MgCl2, 50 ng of template DNA, 0.125 μL of 5U/μL Taq DNA polymerase (New England BioLabs, Massachusetts, USA). PCR was performed using the following programme: 95 °C for 30 s; 30 cycles at 95 °C for 30 s, 53 °C for 30 s and 68 °C for 30 min, and a final extension at 68 °C for 5 min. Amplicons were analysed by electrophoresis on 1.0% agarose gel and visualised under UV light. Fragments were purified using the Zymoclean Gel DNA Recovery Kit and sent to Ficus Biotechnology (Ankara, Turkey) for sequencing. The sequences were compared with those stored in the Basic Local Alignment Search Tool (http://blast.ncbi.nlm.nih.gov). All sequences were submitted to the GenBank database.

Phylogenetic relationships among fungi were inferred using the Neighbour-Joining method (Saitou and Nei, 1987). Evolutionary distances were calculated using the number of differences method (Nei and Kumar, 2000) and evolutionary analyses were performed in MEGA X (Kumar et al., 2018).

2.6. Virulence assay

2.6.1. Production of conidia

The conidia used for the assay were produced by solid phase fermentation on rice. First, each fungus was grown in 50 ml of Sabouraud dextrose broth for 3 days at 25 ± 2 °C with stirring at 150 rpm. Then, 20 ml (10% v:w) fungal suspension was added to sterile polyethylene bags containing 200 g of parboiled rice and the bags were mixed gently to distribute the inoculum homogeneously. The bags were incubated for 3 weeks and shaken by hand every 12 h during fermentation (Pham et al., 2018). The conidia on the rice were harvested by adding 200 ml of a 0.1% Tween 80 solution and collected with a centrifuge. The pellet containing the conidia was resuspended in 50 ml Falcon tube in a 0.1% Tween 80 solution and homogenised with a vortex for 3 min. Finally, the conidia concentrations were counted with a hemocytometer under a light microscope and adjusted to 1×10^7 conidia/ml for use in the virulence assay.

2.6.2. Screening assays

Considering that the insect can reach twenty larval stages, it is quite difficult to accurately distinguish each larval stage and to perform a screening test for all larval stages. Therefore, the virulence of fungal isolates for early (4th instar), mid (10th instar), late (16th instar) larval stages, adults and pupae of *T. molitor* was determined by screening tests. Larval stages were determined by measuring larval body weight and length (Graham et al., 2000; Park et al., 2014). Thirty 4th instar larvae were placed in sterile Petri dishes (15 cm diameter × 1.5 cm height) covered with filter paper. 3 ml fungal suspensions (1×10^7 conidia/ml) were sprayed from a distance of 25 cm using a 10-ml mini mist sprayer. Control larvae were treated with a 0.1% Tween 80 solution. After drying, the treated insects were transferred to new sterile Petri dishes containing food and potato slices. Experiments were conducted at 25 °C, 60% relative humidity for 14 days. All experiments were performed with 30 larvae, included three replicates and were repeated 3 times on different days. The experiments were also conducted with 10th and 16th

instar larvae, adults (newly hatched pupae) and pupae (newly entered pupae), separately.

2.6.3. Concentration response assays

Based on the preliminary screening results, the most virulent fungal isolate (*B. bassiana* BL8) for different life stages of *T. molitor* was used in concentration reaction tests. The six different concentrations (from 1×10^8 to 1×10^3) of BL8 isolate were prepared by serial dilution and tested on different life stages (4th, 10th, 16th larval stages, adults and pupae) of the insect. Virulence tests were performed as in the screening tests.

2.7. Data analysis

Mortality data obtained from the study were corrected using Abbott's (1925) formula and normalised using the arc transformation before analysis of variance. Means were compared with Duncan's multiple range test ($P < 0.01$). Data from the concentration response tests were subjected to probit regression analysis and the concentration of BL8 isolate required to kill 50 and 90% (LC_{50} and LC_{90}) of the pests was calculated. Survival analysis was also performed using the Kaplan-Meier method, and survival characteristics were evaluated using log-rank tests with the Holm-Sidak method for multiple comparisons to compare different survival curves. The SPSS Statistics 25 software package was used for the analyses.

3. Results

3.1. Morphological characterization

Seven fungal isolates (BL1-BL8) were obtained from forty soil samples using the *T. molitor*-bait method. The isolates, belonging to the genera *Beauveria* and *Metarhizium*, were identified by morphological characteristics. BL8 showed white to yellowish-white colonies with irregular margins and powdery appearance, typical macroscopic features of the genus *Beauveria*. Microscopic observation of the isolates showed spherical conidia with hyaline hyphae and branched conidiophores. The other isolates (BL1, BL5, BL19, BL21, BL23 and BL24) showed cottony and powdery consistency, green to dark green colonies. These isolates had branched conidiophores with cylindrical phialides, the conidia were ovoid and had septate hyphae.

3.2. Molecular characterization

After amplification of ITS1-5.8S-ITS4 rDNA region of the isolates, 600 bp amplicons were visualized in agarose gel. Sequence analysis obtained for BL8 morphologically identified as *Beauveria* sp. confirmed that it belonged to *B. bassiana* with similarity indexes of 99%. Also, the other fungal isolates, morphologically identified as *Metarhizium* sp., showed 99% sequence similarity with *M. anisopliae* deposited in NCBI/Genebank database. The sequence data were deposited in the GenBank database under the accession numbers MW832538 to MW832544. In addition, phylogenetic analysis revealed that the isolates BL1, BL5, BL19, BL21, BL23199 and BL24 clustered with reference *M. anisopliae* strains, BL8 isolate clustered with reference *B. bassiana* strains (Fig. 1).

3.3. Screening experiments

The results showed significant differences between all tested fungal isolates against different developmental stages of *T. molitor* 14 days after treatment. For all larval stages tested, isolates of mortality for *B. bassiana* (BL8) and *M. anisopliae* (BL23 and BL24) achieved 100% mortality at an exposure of 10^7 conidia/ml. However, for *M. anisopliae* BL1 isolate, larval mortality was less than 20% at the same concentration. Adult mortality was significantly lower compared to larval stages. At a concentration of 10^7 conidia/ml, the *B. bassiana* BL8 isolate outperformed the other isolates and caused the highest adult mortality of 65% ($F = 83.18$; $df = 6$, $P < 0.01$). Exposure to this isolate also caused a significant reduction (96.6%) in the percentage of adults ($F = 472.8$; $df = 6$, $P < 0.01$). In addition, a reduction in hatched adults was observed in 86% and 80% of pupae treated with *M. anisopliae* BL19 and BL24, respectively (Table 1).

3.4. Concentration response assays

Different life stages of the pest exposed to different concentrations of BL8, the most effective isolate in the screening test, had survival probabilities over the 10 days. Except for the highest concentration, pupal mortality was significantly different from that of other life stages. Even the lowest concentration (10^3 conidia/ml) of BL8 caused 60% mortality in the pupal stage. However, the survival rate of all life stages tested decreased when the application concentration was increased. Survival analysis showed that there was no significant difference between life stages except in the adult stage when *T. molitor* was treated with 10^9 conidia/ml (Fig. 2). The concentration-response experiment showed that BL8 had different median lethal concentration (LC_{50}) values for each life stage (Table 2). BL8 had relatively lower LC_{50} values at the 16th larval instar and pupal stages than at the other life stages. It had the lowest LC_{50} value in the pupal stage with 3.9×10^2 conidia/ml.

4. Discussion

This is the first detailed study to show that different developmental stages of *Tenebrio molitor* are susceptible to entomopathogenic fungi and that some isolates are clearly more pathogenic than others. The entomopathogenic fungi used in this study were isolated from soil samples using the *Tenebrio*-bait method. Although the use of selective media allows the isolation of putative entomopathogenic fungi, the use of insects as bait is a sensitive method that allows the direct isolation of

Table 1
Virulence of local entomopathogenic fungi on different life stages of *Tenebrio molitor* 14 days after treatment.

Fungal isolates	Corrected mortality (%) ± SD					*F*	*df*	*P*
	4th instar	10th instar	16th instar	Adult	Pupae			
BL1	12.5 ± 2.3 D*b*	17.5 ± 2.0 C*a*	15 ± 2.6 Cb	12.5 ± 3.0 Eb	10 ± 3.0 D*b*	3.57	4	<0.01
BL5	87.5 ± 3.5 B*a*	90 ± 4.0 B*a*	67.5 ± 3.7 Bb	12.5 ± 3.0 Ed	26.6 ± 2.8 C*c*	321.1	4	<0.01
BL8	100 ± 0 A*a*	100 ± 0 A*a*	100 ± 0 A*a*	65 ± 5.8 Ab	96.6 ± 3.4 A*a*	140.3	4	<0.01
BL19	67.5 ± 3.0 C*c*	90 ± 2.3 Bb	100 ± 0 A*a*	20 ± 4.1 D*d*	86.6 ± 4.0 Bb	321.8	4	<0.01
BL21	87.5 ± 5.5 B*a*	92.5 ± 3.2 B*a*	71.5 ± 3.5 Bb	15 ± 3.6 E*c*	20 ± 3.4 C*c*	268.5	4	<0.01
BL23	100 ± 0 A*a*	100 ± 0 A*a*	100 ± 0 A*a*	45 ± 3.6 Bb	23.3 ± 2.5 C*c*	1061.7	4	<0.01
BL24	100 ± 0 A*a*	100 ± 0 A*a*	100 ± 0 A*a*	30 ± 4.1 C*c*	80 ± 3.6 Bb	139.5	4	<0.01
F	371.1	525.4	647.3	74.7	380.1			
df	6	6	6	6	6			
P	<0.01	<0.01	<0.01	<0.01	<0.01			

Values are averages of three replicates. Means within a column followed by different uppercase letters, and within a row followed by different italic lowercase letters, are significantly different (Duncan's multiple range test: $P < 0.01$). SD: standard deviation.

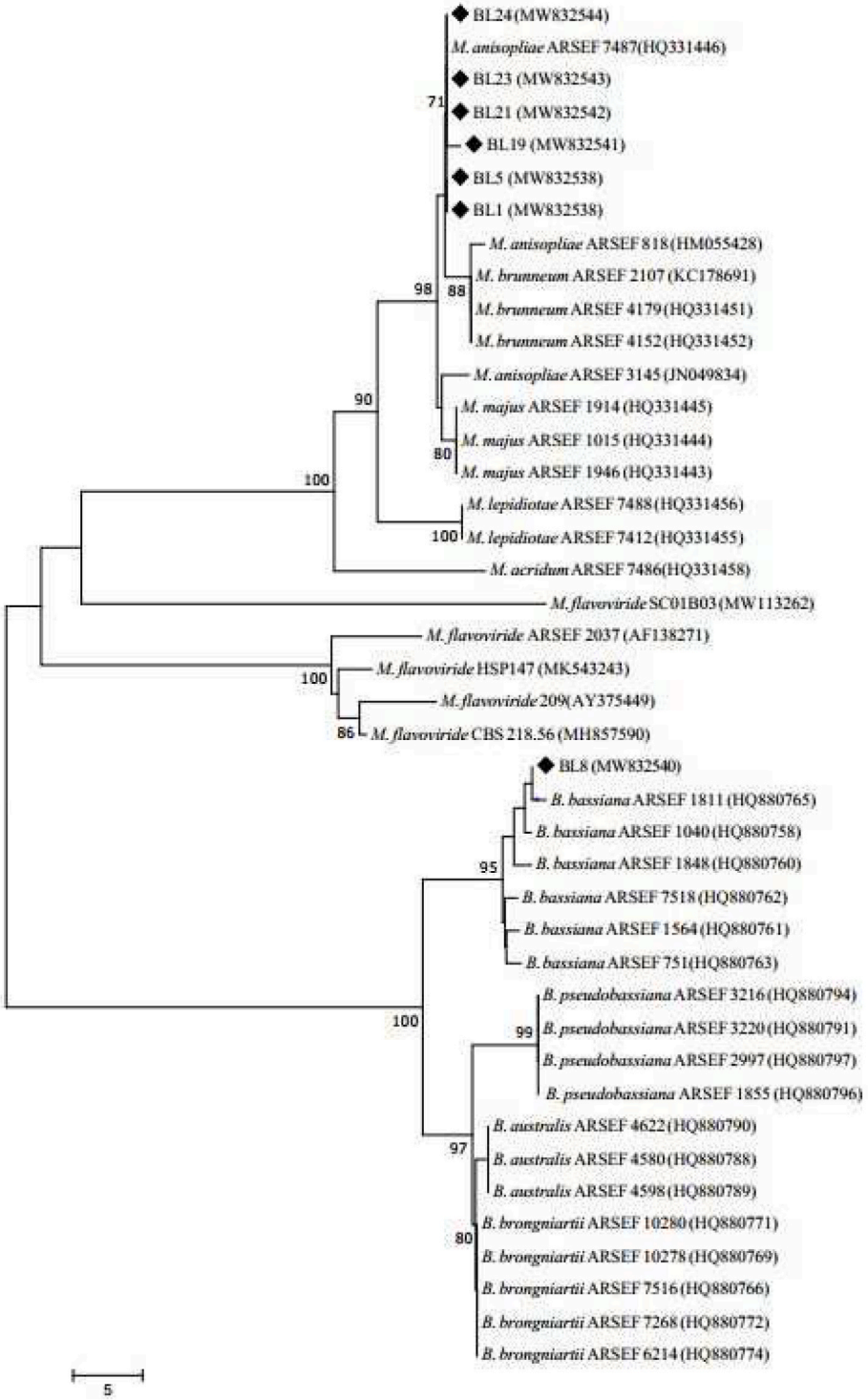

Fig. 1. Phylogenetic analysis of entomopathogenic fungal isolates inferred from neighbourhood analysis of ITS sequences. The reliability of the tree was assessed by bootstrap analysis with 1000 replicates. Bootstrap values greater than 70% are indicated in the branches. GenBank accession numbers are indicated in parentheses.

entomopathogenic fungi (Sánchez-Peña et al., 2011; Steinwender et al., 2021; Aguilera-Sammaritano et al., 2016). Seven fungal strains with typical characteristics of EPF were isolated. The macroscopic and microscopic observations allowed the identification of the isolates down to the genus level. Of these fungi, six isolates belonged to *Metarhizium* (BL1, BL5, BL19, BL21, BL23, and BL24) and only one isolate belonged to *Beauveria* (BL8). The fungal isolates had similar conidial shape and size to those described by Humber (2012). However, identification of these entomopathogenic fungi based on morphological characteristics alone is very difficult, which may lead to misidentification. Therefore, ITS regions of nrDNA, approximately 600 bp, were amplified and sequenced to identify the fungal isolates to species level. The ITS -nrDNA region has been used extensively for molecular identification because it has a low level of intraspecific variation and a high level of interspecific variation (Horton and Bruns, 2001; Irinyi et al., 2016; Bueno-Pallero et al., 2020). The phylogenetic tree confirmed that the BL8 isolate was a strongly supported clade with a bootstrap value of 95%, which split into a subclade with *B. bassiana* isolates. The other five isolates belonged to

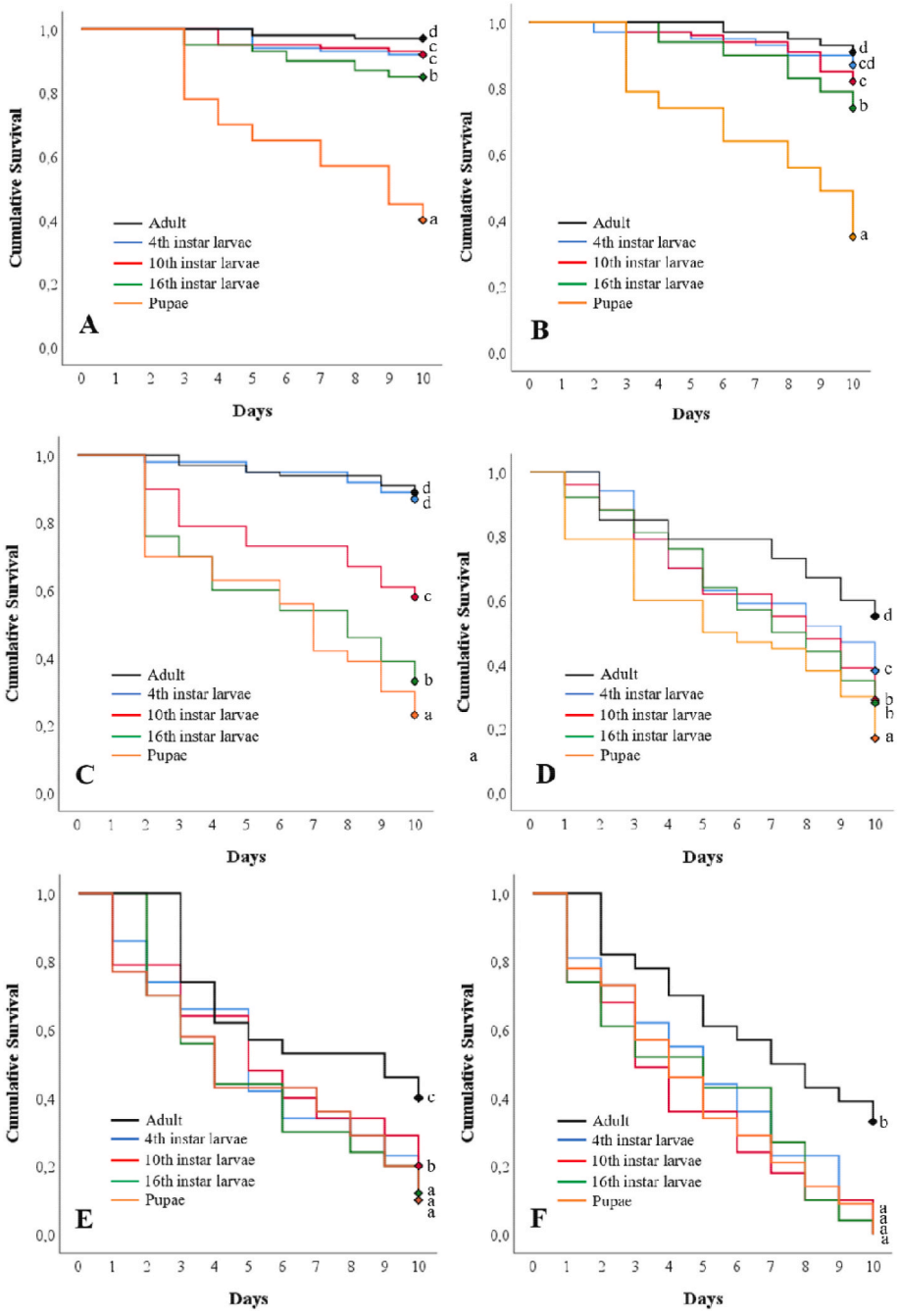

Fig. 2. Kaplan-Meier survival diagram for different life stages of *Tenebrio molitor* exposed to isolate *B. bassiana* BL8 at six different concentrations (A: 10^3 conidia/ml, B: 10^4 conidia/ml, C: 10^5 conidia/ml, D: 10^6 conidia/ml, E: 10^7 conidia/ml, F: 10^8 conidia/ml). Holm-Sidak estimates of survival data show significant differences between treatment groups represented by lowercase letters.

M. anisopliae with a bootstrap support of 98%, which split into a subclade with *M. anisopliae* isolates. After morphological and molecular examination, the isolates were identified as *B. bassiana* (BL8) and *M. anisopliae* (BL1, BL5, BL19, BL21, BL23, and BL24). The reason that most of the isolated fungi are *Metarhizium* is that *Metarhizium* species tolerate a wide range of temperature and soil moisture conditions (Lingg and Donaldson, 1981; Raid and Cherry, 1992).

Previous studies tested the pathogenicity and virulence of EPF isolated from different habitats such as agricultural and forest soils or insects for *T. molitor* (Batta et al., 2010; Mora et al., 2016; Altahawi et al., 2020). In this study, of six isolates of *M. anisopliae* tested, only two were highly pathogenic to larvae. Similarly, *B. bassiana* BL8 was highly pathogenic to larval stages. Kiliç et al. (2019) reported that different strains of *B. bassiana* at a concentration of 2×10^7 conidia/ml caused 66–81.5% mortality in larval stages of *T. molitor*. Oreste et al. (2012) reported that *M. anisopliae* isolates were more effective on *T. molitor* larvae than *B. bassiana* isolates. It can be hypothesised that the differences in virulence are related to fungal virulence factors such as lipases, proteases, and chitinases, as their overexpression in engineered strains resulted in higher mortality in insects (Fan et al., 2007; Fang et al., 2009). However, these studies focused on the pathogenicity of EPFs in a single larval or adult stage and did not consider the possible differences in susceptibility between different life stages. Considering that 20 larval stages can be reached depending on environmental conditions, it is important to determine life stage most susceptible to pesticides to increase the efficacy of pesticide application (Ansari and Butt, 2012).

Table 2
Median lethal concentration (LC_{50}) of isolate of *Beauveria bassiana* BL8 on different life stages of *Tenebrio molitor*.

Life stage	LC_{50} (FL, 95%)	Slope ± SE	*df*	X^2	*P*-value
4th instar larvae	4.3×10^5 (6.8×10^4 - 3.3 $\times 10^6$)	0.75 ± 0.28	4	35.5	0.01
10th instar larvae	1.9×10^5 (8.4×10^4–4.2 $\times 10^5$)	0.62 ± 0.25	4	6.9	0.01
16th instar larvae	6×10^4 (1.4×10^4 - 2 $\times 10^5$)	0.75 ± 0.23	4	13.9	0.01
Adult	5.6×10^6 (1.5×10^6-3.6 $\times 10^7$)	0.5 ± 0.25	4	7.1	0.01
Pupae	3.9×10^2 (2 - 3.3 $\times 10^2$)	0.3 ± 0.20	4	7.1	0.01

FL: fiducial limit, SE: standard error, *df*: degree of freedom, X^2: chi-square.

Rohde et al. (2006) and Alexandre et al. (2006) reported that larvae of *T. molitor* are more susceptible to attack by *B. bassiana* isolates than adult insects. These results are consistent with our study. Pupal and 16th instar larval stages were also highly susceptible to infection by *B. bassiana* at all insect developmental stages tested. The differences in susceptibility between life stages of *T. molitor* can be explained by the fact that adults have a thick and highly sclerotized cuticle, whereas larvae and pupae have a softer, more flexible cuticle. In contrast, the least susceptible stage was the adult stage. This is due to the fact that adults are able to upregulate the activity of GST, an antioxidant enzyme, when exposed to toxic compounds. Therefore, the metabolic system provides faster clearance of toxic compounds and increases the insect's chances of survival (Pedersen et al., 2020). In contrast, Rodríguez-Gómez et al. (2009) reported that adults of *T. molitor* are highly susceptible to *B. bassiana* infection compared to larvae. Similarly, recent studies have shown that *T. molitor* adults are sensitive to essential oils. In a previous study, Kavallieratos et al. (2021a) pointed out that even at the highest concentration (1000 ppm) of essential oil-based nanoemulsion of *Hazomalania voyronii*, larval mortality was only 10.3%, whereas adult mortality at the same concentration reached 100% after 7 days of exposure. They also showed that 12 commercial essential oils, *Coriandrum sativum* L. (Apiaceae), *Boswellia carteri* Birdw. (Burseraceae), *Pogostemon cablin* Benth. (Lamiaceae), *Thymus vulgaris* L. (Lamiaceae), *Copaifera offcinalis* L. (Fabaceae), *Corymbia citriodora* Hook. (Myrtaceae), *Melaleuca cajuputi* Powell (Myrtaceae), *Syzygium aromaticum* L. (Myrtaceae) and *Elettaria cardamomum* L. (Zingiberaceae), killed 21.1–50.0% of *T. molitor* adults on wheat at 1000 ppm after 7 days of exposure (Kavallieratos et al., 2021b). Similar efficacy was reported for *Tanacetum vulgare* L. (Asteraceae) essential oil, which caused moderate adult mortality (52.2%) and low larval mortality (8.9%) at 1000 ppm (Kavallieratos et al., 2021c). The low larval mortality is an expected result because larvae of this species are tolerant to chemical and botanical insecticides (Kavallieratos et al., 2022). Therefore, the use of entomopathogenic fungi with essential oils may represent a new approach that can be effective at all stages of the pest.

Published data on the efficacy of entomopathogenic fungi against pupae are conflicting. Some studies suggest that pupae are resistant to fungal infection because of their thick and sclerotized cuticle, which provides an effective barrier (Shelton et al., 2007). In contrast, mortality of pupae treated with *B. bassiana* reached up to 96% in this study. Moreover, of the life stages tested, the lowest LC_{50} value of BL8 was observed in the pupal stage (Table 2). In addition, reduced wing size and malformations were observed in adults hatching from pupae treated with BL8. These results are consistent with Ansari and Butt (2012) who reported that *M. robertsii* ARSEF 4556 completely kills the pupal stage of *H. abietis*. The susceptibility of pupal stage to entomopathogenic fungi has also been observed in many insects from different orders (Nguyen et al., 2007; Anand et al., 2009; Ángel-Sahagún et al., 2010; Beris et al., 2013).

5. Conclusion

The results indicate that testing differences in susceptibility between life stages seems to be of great importance for biological control of *Tenebrio molitor*. Most of the entomopathogenic fungi used in this study were pathogenic to all life stages of *T. molitor*. However, the isolate *B. bassiana* BL8 was more effective for late instar larvae and pupae than for other stages. *B. bassiana* BL8 could be considered as a microbial control agent for integrated pest management against *T. molitor*. Further studies should be conducted to validate these results under field conditions.

Declaration of interest

The authors report no conflict of interest.

Author statement

Ardahan ESKİ: Conceptualization, methodology, writing original draft, writing review and editing, and supervision, **Muhammed Murat Gezgin:** Investigation, validation, and data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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