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# Pathogenicity of indigenous *Beauveria* bassiana (Balsamo) against *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) under laboratory conditions



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#### **Abstract**

**Background:** The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is the major insect pest of fruit production worldwide. Chemical control of this pest has induced the selection of resistant medflies populations and negative environmental impact. In addition, the use of pesticides has become an obstacle to the fresh agricultural products exported to many countries that restrict pesticides residues. The aim of this study was to propose alternatives based on the use of microbiological products for the control of *C. capitata*.

**Main body:** The pathogenicity of the Turkish isolate *Beauveria bassiana* BMAUM M6-4 was evaluated against immature stages and adult of *C. capitata* under laboratory conditions at a concentration of 10<sup>7</sup> conidia/ml via different routes of exposure. Conidial treatment was applied by spraying soil and filter paper against 3rd instar larvae and pupae. In contrast, the treatment was administered to adult males and females by contact and ingestion. The percentage of mortality was recorded on the 3rd, 7th, 12th and 15th day after inoculation. Obtained results showed that *B. bassiana* was very effective against the adult's fly, where males were more susceptible than females in both treatments (contact and ingestion). This isolate was tested against 3rd instar larvae. It achieved an infection rate of 33.33 and 43.5% of the larvae treated on filter paper and in the soil, respectively. Application of conidial preparations to soil reduced insect emergence and CAN represent a promising strategy for fruit fly integrated management (57.33 and 56.66% emergence from the treatments in soil and filter paper).

**Conclusion:** Entomopathogenic fungi are promising as a biocontrol agent that can be used under different modes of ground application against larvae, prepupae and pupae, and/or as a bait spraying or contact against adults.

Keywords: Entomopathogenic fungi, Ceratitis capitata, Pathogenicity, Bioassay, Native isolate

#### **Background**

The Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae), is the main pest in the Mediterranean region attacking more than 300 hosts. It presents the greatest threat to the production and marketing of many fruit crops, mainly fruit crops. The pest overwinters as a pupa in the soil where it is a target for

many soil-dwelling organisms, including entomopathogenic fungi (EPF), particularly Ascomycetes, *Beauveria* spp. (Hypocreales: Cordycipitacea), and *Metarhizium* spp. (Hypocreales: Clavicipitaceae) (Vega et al. 2009; Rudeen et al. 2013). EPF can be isolated from plant tissues and soil. They use the soil as a habitat for long-term persistence when crops are not present in the field (Rudeen et al. 2013). Some of EPF species have a limited number of hosts, while the others have a wide range of hosts, for example, *Beauveria bassiana* (Bals.) Vuill. (Hypocreales: Cordycipitaceae) is an EPF that can infect

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a wide host range of over 700 insect species (De Faria and Wraight 2007).

EPF have received a considerable interest from scientists for their efficacy against crop pests and their biological control potential. The laboratory work serves as an initial indicator for the selection of fungal isolates as effective biocontrol agents in terms of their pathogenicity, specialization and ease of production and adaptation to environmental conditions (El-Husseini et al. 2018; Sayed et al. 2019).

The efficacy of EPF such as Metarhizium anisopliae and Beauveria bassiana on pupae and adults of C. capitata has been reported by several authors (Ekesi et al. 2005; Konstantopoulou and Mazomenos 2005). Despite the demonstrated virulence of these fungi against C. capitata, significant intraspecific variations in pathogen capacity among various strains and isolates of the same species have been observed, depending on the origin and initial host of the isolates (Castillo et al. 2000; Quesada-Moraga et al. 2006). Thus, selection of EPF isolates is one of the most important steps in a microbial control program, as the process determines which isolates are most virulent for the pest as well as their behavior with respect to relates to mortality, sporulation and the production of harmful organisms on an artificial culture medium (Rohde et al. 2006).

The indigenous Turkish isolate of *B. bassiana* BMAUM M6-4 was determined to cause infection to early and late instar larvae of the pine processionary *Thaumetopoea wilkinsoni*, (Lepidoptera: Notodontidae) (Gök et al. 2018) and larvae of Spodoptera littoralis (Lepidoptera: Noctuidae) (Cırbın et al. 2017). Therefore, this study aimed to evaluate the virulence of this isolate against larvae, pupae (newly formed and older) and adults of *Ceratitis capitata* in the laboratory via different routes of exposure.

#### Materials and methods

#### Insect rearing

The rearing of *C. capitata* was carried out at the Department of Plant Protection, Suleyman Demirel University, at  $25 \pm 1$  °C, 65% RH, under 14:10 (L:D) photoperiod. Larvae were reared in sterile Petri dishes containing artificial diet: water (56 ml), sugar (12 g), Hcl (0.3 ml), wheat germ (4 g), yeast (3 g), benzoic acid (0.3 g), bran (23 g). Adults were provided by water and a solid diet consisting of sucrose and yeast.

#### **Fungal** isolate

The EPF *Beauveria bassiana* BAUM M6-4, used in the following bioassays, was previously isolated from a soil sample obtained from an agricultural land in Isparta Province, Turkey (Baydar et al. 2016).

#### Pathogenicity bioassays

The experiments were conducted under the controlled conditions of  $25 \pm 1$  °C,  $62 \pm 5\%$  RH and a photoperiod of 16:8 (L:D). Adults of *C. capitata* were maintained in cages and provided with diet for 5 days before using them for bioassays. The pupae and prepupae (3rd instar larvae) used in the experiments were obtained from the artificial rearing. One- and 6-day-old pupae were used for the bioassay. Fungal suspension at a concentration of  $10^7$  conidia/ml was tested and mortality rates were assessed at 3, 7, 12, and 15 days after treatment. Each treatment was performed in 4 replicates (15 individuals each), with a total of 60 individuals.

The soil tested, was obtained from a peach orchard in Isparta. The soil was sieved and sterilized before use. Soil's choice was close to the natural control conditions of this pest in agricultural areas. The soil was used as an incubation substrate or as a self-inoculation device of prepupae and pupae.

#### Exposure of 3rd larval instar to conidial suspension Essay on filter paper

A group of 15 3rd instar larvae was placed on a filter paper in a sterile Petri dish (9 cm in diameter) after spraying it with a volume of 3 ml of conidial suspension at concentration of  $10^7$  conidia/ml. The filter paper of the control group was sprayed with 3 ml of distilled water and 0.1% Tween80.

#### Soil bioassay

A group of 15 3rd instar larvae were placed in a plastic container ( $10 \times 6 \times 3$ ) containing 50 g of natural soil and sieved after spraying this soil with 3 ml of conidial suspension. For the control treatments, the same volume with 0.1% Tween 80 used distilled in water. There were four replicates of 15 larvae (N = 60/treatment).

In both tests, the number of dead larvae was counted and the pupae formed from the treated larvae were monitored until the emergence of the adult.

#### Exposure of pupae to conidial suspension Essay on filter paper

A group of 15 new formed pupae of *C. capitata*, aged 1 day old and old pupae (6 days old), was deposited on a filter paper in a Petri dish after spraying it with a volume of 3 ml of the suspension of conidia. Filter paper at the control group was sprayed with the same volume of 0.1% Tween80 was used in distilled water.

#### Soil bioassay

A group of 15 *C. capitata* pupae, aged 1 and 6 days, was placed in a box  $(10 \times 6 \times 3)$  containing 50 g of natural soil and sieved after spraying this soil with 3 ml of

suspension of conidia. For control treatments, 0.1% Tween 80 was used in distilled water.

Each treatment was replicated 4 times, i.e., 4 replicate groups of 15 pupae (total = 60). In both tests, pupae were monitored until adult emergence.

#### Exposure of adults to conidial suspension Contact bioassay

In this bioassay, 15 5-day-old male and female adults of C. capitata were placed in a box  $(11 \times 11 \times 6)$ . A filter paper was placed on the base of the box and sprayed with 3 ml of the conidia suspension before transferring C. capitata adults into the box. While walking on the treated filter paper, the flies were contacted to the spores. Each box received soaked cotton and a small amount of food. For control treatments, 0.1% Tween 80 was used in the water.

Dead adults were removed and transferred to another sterile Petri dish filled with wet filter paper. After sealing with Parafilm, the Petri dishes were stored at 25 °C and monitored daily for fungal symptoms.

#### Oral bioassay

One milliliter of *B. bassiana* suspension at a concentration of 10<sup>7</sup> conidia/ml was mixed with 1 ml of nutrient preparation for adults and placed on a piece of cotton. Flies consume this diet within 2–3 days. A diet without conidia was provided to adults and used as controls.

#### Statistical analysis

Mortalities were corrected, using Abbott formula (Abbott 1925).  $LT_{50}$  values were calculated using Probit analysis (Finney 1971). All statistical analyses were performed using GraphPad Prism version 8.0.0 at the 0.05 level of significance.

#### Results and discussion

#### Pathogenicity against 3rd larval instar

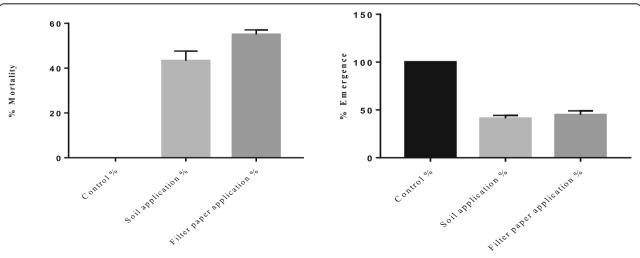
Data presented in Fig. 1 show the susceptibility of 3rd instar *C. capitata* larvae to conidial treatment. Mean mortality rate of larvae was 55.03% in filter paper treatment and 43.33% in the soil treatment. No mortality was recorded in the control. Non-significant difference was observed between the treatment of filter paper and that of the soil (T = 1.406; DF = 15; P > 0.05). Treatment with *B. bassiana* was effective on both treatments.

Some treated larvae continued to pupate but a mortality rate occurred in the pupal stage. 41.20 and 44.97% emergence rates were recorded at the treatments in soil and filter paper, respectively, compared with 100% emergence in the control groups. The results revealed a significant difference between the emergence percentages of adults obtained in the control and in the treated larvae (F = 494.3, DF = 11, P < 0.0001).

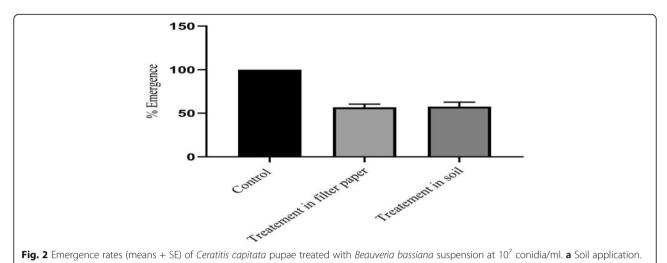
Obtained results are in contradiction with the results reported by Dimbi et al. (2003) who reported no effect for *Metarhizium anisopliae* and *B. bassiana* on larvae of *C. capitata*, *C. rosa* and *C. cosyra*, although they are highly virulent against the adult stage of these species. The results are in agreement with several studies reporting that the larval stage of the *C. capitata* was the most vulnerable to EPF (Ekesi et al. 2005; Quesada-Moraga et al. 2006).

#### Pathogenicity against pupae

Treatment of *B. bassiana* BAUM M6-4 caused mortality in *C. capitata* pupae, resulting in a decrease in the emergence rate of treated pupae than the untreated pupae (F = 41.33, DF = 11, P < 0.0001). Figure 2 clearly shows that the treated pupae had a low emergence rate than the control. Obtained results revealed non-significant difference



**Fig. 1** Percentage mortality and emergence rate (Means+SE) of *Ceratitis capitata* larvae treated with *Beauveria bassiana* BMAUM M6-4 suspension (at 10<sup>7</sup> conidia/ml)



**b** Filter paper application

between the two techniques of treatment (soil application and paper filter applications) (T = 0.9135; DF = 7; P > 0.05).

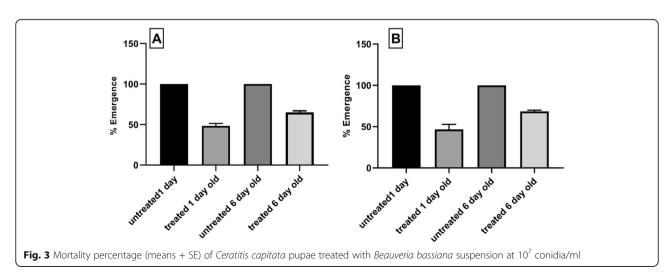
Infection with the *B. bassiana* BAUM M6-4 against *C. capitata* pupae caused a significant reduction in emergence rate and number of adults (56.66 and 57.33% emergence in filer paper and soil application, respectively, compared with 100% emergence in the control groups). This treatment contributed in reducing the population from one generation to the next. The *B. bassiana* BMAUM M6-4 isolate produced a dense condition on the treated pupae. A similar trend was reported by (Ekesi et al. 2005).

The results obtained in this study are consistent with those found by Mahmoud (2009) who tested *Bactrocera zonata* (Diptera: Tephritidae) but did not differ from those found by Quesada-Moraga et al. (2006) who reported mortality rates of *C. capitata* pupae to *B. bassiana* isolates ranging from 14 to 95.5%. Previously, Lezama-Gutiérrez et al. (2000) reported that *M.* 

anisopliae reduced adult emergence by 33 and 49% depending on soil type and about 37.9 to 98.75% mortality in *Anastrepha ludens* (Diptera: Tephritidae) larvae and puape, respectively. In another work, Alves et al. (2004) found that a high concentration of conidia ( $1 \times 10^8$  conidia/ml) of *B. bassiana* and *M. anisopliae* was necessary to prevent the emergence of *C. capitata*.

Obtained results clearly showed that newly formed pupae (1 day old) were more susceptible to infection with EPF than the 6-day-old pupae in both the filter paper (T=3.576; DF = 3; P<0.05) and soil tests (T=7.565; DF = 3; P<0.05) (Fig. 3). 46.67% emergence in larvae aged 1 day old was recorded in the soil treatment and 48.33% in the filter paper treatment against 65 and 68% emergence in the 6-day-old pupae in the 2 treatments, respectively.

Ekesi et al. (2002) evaluated the pathogenicity of *M. anisopliae* against pupae of 3 Tephritid species, including *C. capitata*, and found that pupal susceptibility



decreased with increasing pupal age. In a recent study, Oreste et al. (2015) studied the interaction between age of puparia (at 2, 4, and 6 days old) and fungal strains. They addressed both the emergence of *C. capitata* adults and the mortality of pupae. They found that the fungal treatments of different *B. bassiana* strains were high on 2-day-old puparia (49.16 and 51.33% of mycosed puparia for ATCC 74040 and AL1 strain, respectively), while the rate of mycoses was low and ranged between 39 and 27.16%, when fungal treatments were performed on 4-and 6-day-old puparia. According to Beris et al. (2013), the highest susceptibility of young pupae can be attributed to their softer cuticle.

It was recorded that adults obtained from treated pupae had a high mortality than the control. Adult mortality in the treatments was 14.29% in the soil treatment and 22.22% in the filter paper treatment, in addition uncompleted emergence was also observed. Ekesi et al. (2002) cleared that the post-adult fungal infection was associated with the penetration of the hypha through the puparia integument in the body of the adult before emergence.

#### Pathogenicity against adults

The virulence of *B. bassiana* BMAUM M6-4 against adults of *C. capitata* was estimated 4 times after treatment. The results presented in Fig. 4 show the effect of oral and contact treatment on male and female adult mortality. No mortality was observed at the control for both sexes.

The average adult mortality of *C. capitata* after contact treatment with conidial suspension of *B. bassiana* strain BMAUM M6-4 reached 51.67% of adult females and 71.67% of adult males. A highly significant difference in mean mortality between males and females of *C. capitata* treated as contact method with conidial suspension of *B. bassiana* BAUM M6-4 at 10<sup>7</sup> conidia/ml was

found (T = 2.132; DF = 30; P < 0.05). As well, similar results were estimated between males and females of C. capitata treated with oral method (T = 2.637; DF = 30; P < 0.05). The average adult mortality of C. capitata after feeding a mixture of conidial suspension and artificial food for 48 h was higher in males (86.33%) than in females (68.33%). Obtained data revealed a significant difference between the two modes of application (T = 2.158; DF = 30; P < 0.05). The mortality induced by ingestion was significantly higher than that caused by contact. 73.33% and 86.33% of mortality were recorded toward adult males and females, respectively. Indeed, treated females by ingestion were more susceptible to infection (68.33% mortality 15 days post-treatment) than those treated by contact (51.67%) (T = 2.853; DF = 30; P < 0.05). Similarly, males treated by ingestion were more susceptible to infection (68.33% mortality) than those treated by contact (73.33%) (T = 2.137; DF = 30; P < 0.05).

Dead individuals of *C. capitata*, covered with a white mycelium characteristic of the fungus *B. bassiana* (Fig. 5). The average percentage of dead treated adults, which developed mycelium was 100%.

In contact bioassay, on the 3rd day of treatment, mortality rate was 1.67% for females and 6.67% for males (T=1.567; DF = 6; P>0.05). On the 7th day, mortality became 10% for females and 33.33% for males (T=7.00; DF = 6; P<0.05). On 12th day, mortality of females was 28.33% and 58.33% of males (T=6.646; DF = 6; P<0.05). A maximum of 51.67 and 73.33% was recorded on the 15th day of treatment for males and females, respectively (T=13.01; DF = 6; P<0.05).

In oral bioassay, on the 3th day of treatment mortality was 10.33% for females and 26.67% for males (T = 8.671; DF = 3; P < 0.05). On 7th day, the fungus caused 35 and 53.33% mortality in females and males, respectively (T = 5.745; DF = 6; P < 0.05). On 12th day, the rate became 61.67% in females and 83.33% in males (T = 5.811; DF = 6;

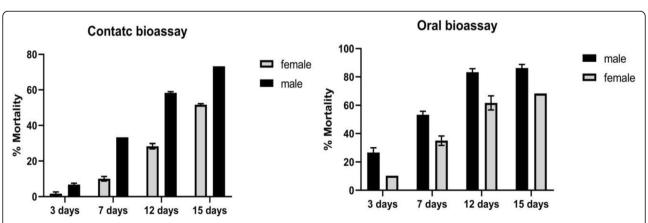


Fig. 4 Virulence and mortality responses of *Ceratitis capitata* adults treated as oral (the right figure) and contact (the left figure) bioassay with *Beauveria bassiana* BAUM M6-4



Fig. 5 Infection symptoms on adults of Ceratitis capitata induced by Beauvaria bassiana isolate BMAUM M6-4

P < 0.05). The maximum 86.33 and 68.33% were recorded on the 15th day of treatment for males and females of *C. capitata*, respectively (T = 11.01; DF = 6; P < 0.05).

In oral treatment, Probit analysis of the time mortality response (LT $_{50}$ ) for adult females was 10.87 days. Whereas, chi square was 37.61. However, LT $_{50}$  for male was 6.54 days. Whereas, chi-square was 45.59. In contact treatment, the LT $_{50}$  for adult females was 14.68 days. Whereas, chi-square was 27.28. For adult males LT $_{50}$  for male was 10.54 days. Whereas, Chi square was 47.45. The slope values of EPF toward adults are presented in Table 1. The slopes of 5.13 and 4.95 were observed in oral bioassays, while they were 3.61 and 4.04 in contact bioassays. The LT $_{50}$  values were significantly higher in contact bioassay than in contact bioassay (F = 8.569, P < 0.0001). The differences were non-significant in slopes (F = 1.598; P = 0.26). However, the differences between intercepts were significant (F = 34.34; P < 0.0001).

Obtained results agree with the findings Konstanto-poulou and Mazomenos (2005) who reported a moderate pathogenicity of B. bassiana when tested as oral bioassay against Bactrocera oleae (Diptera: Tephritidae) adults (causing 62.6% mortality) and obtained  $LT_{50}$  values of 17 days. In the same publication, authors obtained  $LT_{50}$  values of 13.4 days for adults C. capitata inoculated as oral bioassay with B. bassiana.

The fungal treatment of *B. bassiana* BMAUM M6-4 against adults of *C. capitata* caused a cumulated mortality for both sexes, whereas the male mortality was higher than of the female in both treatments (oral and contact bioassay). This result suggests that the male adults were

more susceptible than females which agree with (El-Akhdar and Ouda 2009 and Boudjelida and Soltani 2011). In addition,  $LT_{50}$  was shorter in adult males (6.54 and 10.54 days in oral and contact bioassay, respectively) than in adult females (10.87 and 14.68 days, for the same bioassays, respectively). For both tests (10.54 and 14.68 days in oral and contact bioassay, respectively), that confirms the susceptibility of males to infection with EPF than females. Mahmoud (2009) reported  $LT_{50}$  of 14.67 days for *B. oleae* treated with *B. bassiana* as oral treatment, while it was 16.6 days in contact treatment.

Fly mortality changes with time, starting 3rd day after inoculation. The effect of the fungus *M. anisopliae* against the 4th larvae and the adults of *C. capitata* showed a high toxicity with a concentration-response manner with a mortality started from day 3 to day 6 confirmed the results of Yee and Lacey (2005) and Boudjelida and Soltani (2011).

#### **Conclusions**

The present study reported that the BMAUM M6-4 strain of *B. bassiana* had a notable efficacy against different stages of *C. capitata*. The fungus could be a promising biological control agent under different modes of applications as conidial application to the soil against pupae and 3rd instar larvae. Further research is needed to determine the efficacy of this isolate in the field to assess its actual contribution as a biological control agent against the fruit flies.

Table 1 LT<sub>50</sub> of adult males and females of Ceratitis capitata treated as oral and contact bioassay with Beauveria bassiana BAUM M6-4

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Mode of treatment		TL50 ± SE (day)	Intercept	Slope ± SE	χ2 (DF = 9)
Contact bioassay	Female	14.68 ± 0.27	4.354	3.614 ± 0.42	37.61
	Male	10.54 ± 0.24	5.547	$4.041 \pm 0.53$	45.59
Oral bioassay	Male	$6.54 \pm 0.20$	5.770	$4.959 \pm 0.55$	47.45
	Female	10.87 ± 0.19	7.636	$5.130 \pm 0.7$	27.28

#### Abbreviations

EPF: Entomopathogenic fungi; LT<sub>50</sub>: Median lethal time

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#### Authors' contributions

CS: carried out the experiment and wrote the manuscript. BK: revised the manuscript, corrected language. BA: conceived and planned the experiments. KI: Analyzed data and supervised the work. All authors read and approved the final manuscript.

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#### Availability of data and materials

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#### Ethics approval and consent to participate

Not applicable

#### Consent for publication

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#### Competing interests

The authors declare that they have no competing interests.

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