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Effectiveness of *Beauveria bassiana* Native Isolates Against *Ceratitis capitata* [(Wiedemann, 1824) Diptera: Tephritidae)] Under Laboratory Conditions

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***Abstract*—** *The aim of this study was to investigate the effect of native entomopathogenic fungus (EPF) Beauveria bassiana (Bals.) Vuill. isolates (Bb-1, Bb-18, ET-101 and ET-10) obtained from different locations and different hosts on the last instar larvae of Ceratitis capitata (Diptera: Tephritidae) under laboratory conditions. B. bassiana isolates were applied as a single dose (10^8 conidia mL^{-1}) by spraying using a hand sprayer. Following the applications, the live individual count was documented by tallying on the 1^{st}, 3^{rd}, 5^{th}, and 7^{th} days, and the percentage mortality rate was determined. The experiments were carried out in climatic chambers with 25±1°C temperature, 65% relative humidity, 16:8 h L: D conditions in a randomized plot experimental design with five replicates. As a result of the study, the highest mortality rate in the third day counts was determined in B. bassiana ET-10 isolate (84%) and this isolate was statistically in the same group with Bb-18 and ET-101. In the fifth day counts, the highest mortality rate was detected in the B. bassiana ET-10 isolate (88%) and all applied isolates were statistically in the same group. In the seventh day counts, 100% mortality rate was determined in all applied isolates and all isolates were statistically in the same group. The LT_{50} values of the applied Bb-1, Bb-18, ET-101 and ET-10 isolates were determined as 3.28, 3.17, 2.52, and 2.26 days, respectively. Native B. bassiana isolates utilized in the research were identified as promising for the biological control of the Mediterranean fruit fly. However, the effectiveness of these isolates under field conditions also needs to be determined.*

***Keywords*—** *Entomopathogenic fungi, Beauveria bassiana, Mediterranean Fruit Fly, Native isolate, Biological control*

I. INTRODUCTION

The Mediterranean fruit fly (Medfly) [*Ceratitis capitata*, (Wiedemann, 1824); Diptera, Tephritidae] is one of the most destructive pests in the World. *C. capitata* is a quarantine pest that causes damage to more than 300 different hosts, mainly citrus fruits, including pome and stone fruits (Liquido et al., 1991; Papadopoulos et al., 1998; Satar et al., 2016). This pest is a zero-tolerance species on our country's quarantine list (Anonymous, 2013). The Medfly was first identified in the world in 1910 on the Island of Hawaii (Bergsten et al., 1999). The homeland of the Medfly is Africa, and it has been reported to be common in regions with tropical and subtropical climates, North and South Africa, South and Central America, Europe and countries with Mediterranean climates (Ricalde et al., 2012). *C. capitata* was first identified in Ankara, Turkey, in 1939 (İleri, 1961). The Medfly has been reported as one of the most important among 118 fruit fly species distributed in Turkey (Kütük et al., 2013).

In addition to damaging fruits by laying eggs, adult female Medflies also cause loss of quality and quantity as a result of larvae feeding on the fleshy part of the fruits (Bergsten et al., 1999; Elekçioğlu, 2013). The Medfly is an important pest because it is widespread in many countries around the world, is adapted to cooler climate conditions compared to other fruit flies, damages many types of fruit, and flies long distances (Meats and Smallridge 2007, Thomas et al., 2010).

Insecticides are widely used to control *C. capitata* around the World. However, due to reasons such as zero tolerance of the pest and insufficient results from insecticides, environmentally friendly and alternative methods were needed.

Environmentally friendly strategies have been developed to suppress Medfly populations, including the use of EPF, nematodes, protozoa, bacteria and viruses as biological control agents (Castillo et al., 2000; Lacey et al., 2001). EPF are one of the most common pathogens that cause diseases in agricultural and forest insect pests (Mueller and Schmit, 2007). The occurrence of fungal diseases under suitable environmental conditions leads to epizootics and, as a result, a decrease in pest populations (Lacey and Goettel, 1995). At least 90 genera and more than 700 species of fungi are insect pathogens (Roberts and Humber, 1981). *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metchnikoff) Sorokin, and *Cordyceps fumosorasea* (Wize) various isolates have appeared in the literature as promising insecticides for the control on the late-stage larvae, pupa and adult stages of *C. capitata* (Sevinç and Karaca, 2024; Quesada-Moraga et al., 2006; Almeida et al., 2007, Castillo et al., 2000; Soliman, 2020). EPF is considered as an alternative to synthetic insecticides because of their high pathogenicity and delay in biological periods. They display low mammalian toxicity, being relatively safe for the environment (Charnley, 1989; Cox and Wilkin, 1996). The aim of this study was to determine the biological activity of Bb-1, Bb-18, ET-101 and ET-10 isolates of the native entomopathogenic fungus *B. bassiana* obtained from different regions and different hosts on Medfly larvae under laboratory conditions.

II. MATERIALS AND METHODS

A. Rearing of Ceratitis capitata

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Fruit samples contaminated with *C. capitata* larvae collected from insecticide-free jujube (*Ziziphus jujuba* Mill. (Rosales: Rhamnaceae)) orchards in Çivril district of Denizli province were brought to the laboratory in plastic boxes. Mass production of *C. capitata* was carried out in plastic boxes placed inside cages in climatic chambers with 25±1ºC temperature, 60-65% relative humidity, and 16:8 h L: D conditions. A mixture of sugar, yeast and water was used to feed adults. In order to ensure continuity of production, old jujube fruits were replaced with fresh jujube fruits. Thus, different biological stages of the pest were created and the last stage larvae of the Medfly were obtained.

B. *Preparation of Beauveria bassiana isolates and spore suspensions*

In the research, the Bb-1 and Bb-18 isolates of native *B. bassiana* were obtained from the forest soil and the agricultural soil of Düzce province, respectively. ET-10 and ET-101 isolates were isolated from Coleoptera larvae and *Sphenoptera antiqua* of Erzurum province, respectively (Tozlu et al., 2017; Erdoğan and Sağlan, 2023).

All native isolates of *B. bassiana* were grown on Potato Dextrose Agar (PDA-Difco, 39 g L^{-1}) medium in sterile Petri dishes (90 mm in diameter) sealed with Parafilm. Petri dishes were incubated at 25±1°C and a photoperiod of 16:8 h (L: D) for 14 days to be completed sporulation. Spore density in stock solutions was determined using a hemocytometer. 10 ml of sterile distilled water with 0.05% (v/v) Tween 80 (Sigma-Aldrich, Munich, Germany) was poured into the Petri dishes and spores were harvested with glass spreader. The spore suspension was passed through three layers of sterile cheesecloth, and the mycelia along with agar fragments were eliminated. The suspensions that contained conidia were vortexed for 10 minutes to achieve a uniform mixture. The spore suspension was counted using a Neubauer haemocytometer under light microscope, and spor concentration was adjusted to 1×10^8 conidia mL^{-1} for each *B. bassiana* isolate (Çam et al., 2002; Fancelli et al., 2013).

C. *Effect of the Beauveria bassiana Spore Suspensions on Fruit Fly Mortality*

In the experiments, blotting paper was placed on the inner surface of the petri dishes. Five last instar larvae were left in each petri dish, and *B. bassiana* isolates Bb-1, Bb-18, ET-101 and ET-10 were sprayed onto these larvae with a hand sprayer from a distance of 20 cm at a spore concentration of 10^8 conidia mL^{-1}. Sterile pure water containing only 0.05% Tween 80 was applied to control petri dishes. Jujube fruit pieces were added to petri dishes so that the larvae could be fed during the experiments. Petri dishes were placed in climatic chambers (25±1°C temperature, 60±5% relative humidity and a photoperiod of 16:8 h L: D) conditions. The number of live individuals and mortality rates were recorded in the groups treated with entomopathogenic fungi and the control group on the 1st, 3rd, 5th and 7th days after the application. Experiments were conducted on a total of 25 larvae in a randomized plot design with 5 replicates for each isolate.

D. *Statistical analysis*

One-way analysis of variance (One-Way ANOVA) was applied to the data obtained after angle transformation. Differences between means were determined by Tukey's multiple comparison test at the $P\leq0.05$ significance level (Tukey, 1949). Statistical analyses were performed using the SPSS® Statistics (Version 20.0, August 2011, SPSS Inc., Chicago, Illinois, USA) package program. In addition, the estimated time (LT_{50}) to kill 50% of the insects was determined by the Probit analysis program (Throne et al., 1995).

III. Results And Discussion

The percentage mortality values of the last stage larvae of the Medfly of *B. bassiana* Bb-1, Bb-18, ET-101 and ET-10 isolates are given in Figure 1. The mortality rate in Medfly larvae increased with increasing time in daily counts in all applied isolates. In the first day counts, the highest mortality rate was recorded in the ET-10 isolate of *B. bassiana* with 40% and was statistically in the different group with Bb-1, Bb-18 and ET-101 isolates. In the third day counts, the highest mortality rate was recorded in the ET-10 isolate of *B. bassiana* with 84% and was statistically in the same group with Bb-18 and ET-101 isolates. In the fifth day counts, the highest mortality rate was detected in the ET-10 isolate (88%) of *B. bassiana* and all isolates were statistically in the same group. In the seventh day counts, 100% mortality rate was detected in all applied *B. bassiana* isolates and all isolates were statistically in the same group (Table 1).

The results were consistent with the results of other research that found that the larval stage of *C. capitata* was the most susceptible to EPF. Dimbi et al., (2003) reported mortality rates ranging from 7-100% as a result of application of local *B. bassiana* and *M. anisopliae* isolates to *C. capitata* adults. In another study, the effect of *B. bassiana* and *M. anisopliae* isolates on Medfly pupae and adults was recorded as 30-100% (Quesada-Moraga et al., 2006). Ekesi et al. (2010) determined *M. anisopliae* and *B. bassiana* isolates also induced large deferred mortality in emerging adults following treatment as late third instar larvae of the Medfly. In another study, *M. anisopliae* was tested at four doses, against fourth larval stage and adults (male & female) of *C. capitata* under laboratory conditions. The mortality rate of the fourth larvae on the seventh day following inoculation was 89. 05% for the maximum dose indicated as 52 x 10^5 spore mL^{-1} (Boudjelida and Soltani, 2011).

Beris et al. (2013) native isolates of *B. bassiana*, *Isaria fumosorosea* and *M. anisopliae* caused 18.7-23.9% mortality in pupae and 41.9-88.0% mortality in *C. capitata* adults was detected. Qazzaz et al. (2015) under laboratory conditions, when native *B. bassiana* isolates (P. Bv32, P. Bv39, P. Bv41, P. Bv51, and P. Bv52) were applied to adults, mortality rates of 77%, 65%, 65%, 65%, and 58% were recorded, respectively. Chergui et al. (2020) obtained the pathogenicity of the Turkish isolate *B. bassiana* BMAUM M6-4 was evaluated against immature stages and adult of *C. capitata* under laboratory conditions. It achieved an infection rate of 33.33 and 43.5% of the 3rd instar larvae treated on filter paper and in the soil, respectively. Sevinç and Karaca (2024) the

effects of native *B. bassiana* and *Cordyceps fumosorosea* isolates on the last instar larvae of the *C. capitata* were investigated, and the mortality rates in Petri dishes were recorded as 30-60% for LD.2016 (*B. bassiana*), 30-46% for M6-4 (*B. bassiana*), and 65-100% for IFR (*C. fumosorosea*), depending on the doses. Chergui et al. (2020) obtained the pathogenicity of the Turkish isolate *B. bassiana* BMAUM M6-4 was evaluated against immature stages and adult of *C. capitata* under laboratory conditions. It reached an infection rate of 33. 33 and 43. 5% for the 3rd instar larvae exposed on filter paper and in the soil, respectively.

LT_{50} values of Bb-1, Bb-18, ET-101 and ET-10 isolates of *B. bassiana* applied to *C. capitata* larvae exposed to 10^8 conidia mL^{-1} concentration are given in Figure 1. LT_{50} values for *B. bassiana* isolates Bb-1, Bb-18, ET-101 and ET-10 were recorded as 3.28, 3.17, 2.52, and 2.26 days, respectively. Among the applied *B. bassiana* isolates, the most effective isolate on *C. capitata* was determined ET-10 and LT_{50} value was recorded as 2.26 days (Figure 1). The pathogenicity of the Turkish isolate *B. bassiana* BMAUM M6-4 was evaluated against adult of *C. capitata* under laboratory conditions. LT_{50} was shorter in adult males (6.54 and 10.54 days) than in adult females (10.87 and 14.68 days) in oral and contact bioassay (Chergui et al., 2020). *C. capitata*, *C. cosyra* and *C. fasciventris*, the lethal time to 50% mortality (LT_{50}) values ranged between 2.6 to 2.9 days in male flies after applications *Metarhizium anisopliae* (Dimbi et al., 2003). Konstantopoulou and Mazomenos (2005) authors obtained LT_{50} values of 13.4 days for adults *C. capitata* inoculated as oral bioassay with *B. bassiana*.

IV. Conclusion

As a result of the study, all native isolates (Bb-1, Bb-18, ET-101, and ET-10) of *B. bassiana* showed an effect (100%) against *C. capitata* larvae on the 7th day. Based on the findings from the study, *B. bassiana* isolates appeared to be effective in managing *C. capitata*. However, the interactions of this isolate with environmental factors and pesticides used in agriculture need to be investigated and expanded to include studies on direct soil application of EPF. In addition, developing the use of *B. bassiana* isolates in combating the Medfly will be beneficial in terms of organic agriculture, good agricultural practices and integrated management.

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TABLE I. Mortality rates (%) of *Beauveria bassiana* isolates on last instar larvae of *Ceratitis capitata.*

***Beauveria bassiana* isolates**	**Mortality (%) ± SE**																			
	Days after treatment																			
	1st				**3rd**				**5th**				**7th**							
Bb-1	20.00	±	12.64	ab*	48.00	±	20.59	ab	76.00	±	16.00	a	100.00	±	0.00	a				
Bb-18	12.00	±	4.89	ab	68.00	±	17.43	a	72.00	±	13.56	a	100.00	±	0.00	a				
ET-101	32.00	±	10.19	ab	80.00	±	15.49	a	84.00	±	11.66	a	100.00	±	0.00	a				
ET-10	40.00	±	10.95	a	84.00	±	7.48	a	88.00	±	8.00	a	100.00	±	0.00	a				
Control	0.00	±	0.00	b	0.00	±	0.00	b	0.00	±	0.00	b	0.00	±	0.00	b				

*The difference between the means represented by columns with the same letter is statistically insignificant (Tukey's HSD test, P<0.05), SE:± standard error)

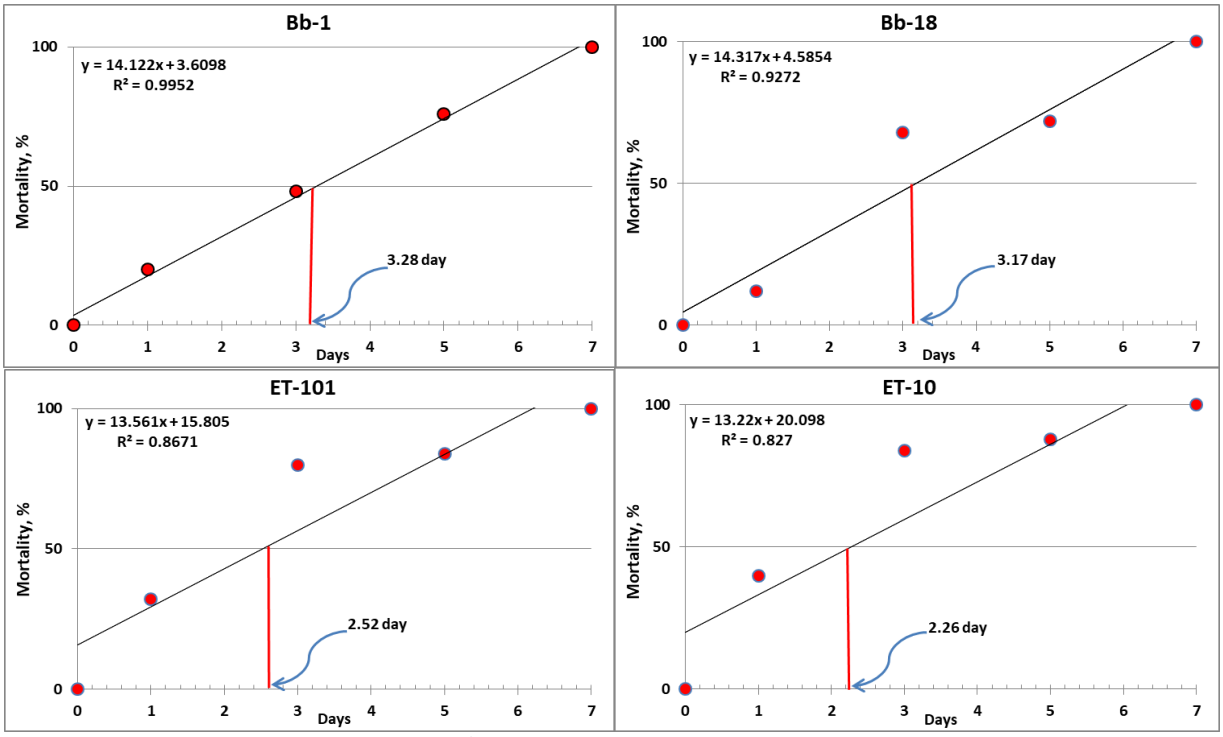

Fig. 1. The mean LT_{50} values of *Beauveria bassiana* isolates applied to *Ceratitis capitata* larvae.