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Effect of entomopathogenic *Beauveria bassiana* (Balls.) Vuill. isolates on *Myzus persicae* (Sulzer) (Hemiptera: Aphididae)

Alime Bayındır Erol[1] **Oktay Erdoğan**[2]

[1]Pamukkale University, Faculty of Applied Sciences, Department of Organic Farming Business Management Çivril-Denizli, Turkey

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Corresponding Author
Alime Bayındır Erol
✉ abayindir@pau.edu.tr

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Abstract

The aim of the study was to determine the lethal effect of 1 x 10^8 conidia mL^{-1} concentration of local *Beauveria bassiana* isolates (ET 10, BMAUM-M6-4, Bb 1) against *M. persicae* under laboratory conditions. For the spraying method, *B. bassiana* isolates was applied to *M. persicae* nymphs with a hand spray. In this context, the experiments were carried out in a randomized plots experimental design with ten replicates with ten nymphs in each Petri plate. After the applications, the number of live individuals was recorded by counting the 1st, 3rd, 5th and 7th days and the percentage mortality rate was calculated. On the third and fifth days of the experiment, the highest mortality rates of 64 and 95% were recorded for the Bb 1 isolate of *B. bassiana*, respectively. In the seventh day counts, 100% mortality rates were determined for Bb 1 and ET 10 isolates and 99% for the BMAUM-M6-4 isolate. In addition, the mortality date (LT_{50}) values were calculated as 3.62 days for ET 10 isolate, 3.60 days for BMAUM M6-4 isolate, and 2.93 days for Bb 1 isolates, respectively. As a result, it is thought that *B. bassiana* isolates can be used in biological control practices within the scope of integrated pest management program against *M. persicae*.

Keywords: Entomopathogenic fungi, *Beauveria bassiana*, *Myzus persicae*, Mortality, Biological control

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INTRODUCTION

Aphids (Hemiptera: Aphididae) are important pests of many vegetables and fruits (Tang et al., 2017). More than 5000 aphid species have been identified worldwide (Satrio Arinanto et al., 2024). The most damaging and widespread species of aphids include *Myzus persicae* (Sulzer, 1776); *Brevicoryne brassicae* L.; *Macrosiphum euphorbiae* Thomas; and *Aphis gossypii* Glover (Hemiptera: Aphididae) (Javed et al., 2019). Among these species, *M. persicae* is one of the most important aphid species that is harmful to agricultural areas and greenhouses. Green peach aphid *M. persicae* (Hemiptera: Aphididae) is a polyphagous insect pest, cause damage more than 400 plant species in more than 50 families and economic losses in many horticulture crops. Due to its rapid reproductive capacity, *M. persicae* prevents the growth rate and development of the plant from decreasing (Blackman and Eastop, 2000; Tang et al., 2017; Kumar and Paul, 2017). *M. persicae* individuals are harmful by sucking the sap of plants and release toxic substances into the plant during sucking. It also causes fumagine by releasing a honey-like substance. *M. persicae* is the vector of more than 100 plant viruses in approximately 30 different families (Diaz et al., 2009; Torres-Quintero et al., 2013). It is stated that the pest is resistant to many chemicals used in chemical control, including organophosphates, carbamates and pyrethroids (Devonshire et al., 1998). Chemical pesticides cause undesirable side effects on products, beneficial organisms, humans and the environment. Due to these negative effects, the need for sustainable alternative control methods is increasing (Kingsley Nwosu and John, 2022; Nicolopoulou et al., 2016). One of these changes is the use of entomopathogenic fungi (EPF). Entomopathogenic fungal pesticides are important in Integrated Pest Management Programs (IPM) and cause diseases in insects, suppressing their growth and reproduction rates (Thomas and Read, 2007). Considering the studies carried out; various EPFs such as *Lecanicillium* sp. (Jung et

al., 2006, Steenberg and Humber, 1999), *Beauveria bassiana* (Quesada-Moraga et al., 2006), *Metarhizium anisopliae* (Shia and Feng, 2004) and *Isaria farinosa* (Shia and Feng, 2004). They are reported to be effective biocontrol agents used in the control against aphids and many other pests. Although *B. bassiana* (Bals.) Vuill. one of these EPFs, is generally isolated from infected insects (Vega et al., 2008, Allegrucci et al., 2020), it is also obtained from various soil types such as peat bogs, mountain soil and desert soil (Zimmermann, 2007). It is reported that *B. bassiana* has 707 different hosts, including 521 genera, 149 families and 15 orders. *B. bassiana* causes disease in the orders Lepidoptera, Coleoptera, Hymenoptera, Diptera, Hemiptera, Orthoptera, Siphonaptera, Isoptera, Thysanoptera, Mantodea, Neuroptera, Dermaptera, Blattariae and Embioptera (Zimmermann, 2007). Entomopathogenic fungi have many benefits, such as being effective in all development stages of the insect, not creating resistance in pests, not being poisonous to mammals, controling in nature for a long time, being applied together with insecticides, and being cheap and easy to apply (Sevim et al., 2015). For these reasons, entomopathogenic fungi have a significant advantage in the control against harmful insects.

The objective of the present study was to evaluate the lethal effect of 1 x 10^8 conidia mL^{-1} concentration of ET 10, BMAUM-M6-4, Bb 1 isolates of the entomopathogenic fungus *B. bassiana* against *M. persicae* under laboratory conditions. Thus, it was discussed whether it is possible to use these EPF isolates within the scope of integrated pest management.

MATERIALS AND METHODS

Production of pepper plants and *Myzus persicae*

Mostar green pepper cv (*Capsicum annuum* L.) from the Solanaceae family was used for host plant production. Pepper seedlings obtained from a private company were transplanted into plastic pots (20 cm diameter) containing a mixture of soil, perlite and peat (1:1:1) and transplanted. Then, these pots which the pepper seedlings were planted in were kept in the growth chamber. No external fertilizer or pesticide application was made during the growing of the seedlings. However, diseased and harmful seedlings were removed from the plant growth chamber.

Aphids used in the experiment were grown on pepper plants in the plant growth chamber. Pepper leaves containing *M. persicae* were propagated by transferring them onto pepper seedlings that had reached a certain height and number of leaves. Fresh pepper seedlings were transferred periodically to ensure aphid stock culture. The production of *M. persicae* individuals and pepper plants was carried out in the plant growth chamber [25±1°C, 60±5% RH and photoperiod 16:8 h (L:D)] in the Entomology laboratory within the Department of Organic Farming Business Management, Faculty of Applied Sciences, Pamukkale University.

Preparation of EPF isolates and spore suspensions

The EPF isolates of *B. bassiana* used in this study are given in Table 1. Three local isolates of *B. bassiana* were obtained from different hosts and locations in Türkiye. All *B. bassiana* isolates were developed within the dark at 25±1°C for 7–15 days and after that subcultured on potato dextrose agar (PDA-Difco). Conidia were harvested from 7-15 days old entomopathogenic *B. bassiana* cultures by adding 10 mL of 0.01% Tween 20 to cultures on agar plates (100 mm) and gently scraping the surface of the cultures with a sterile inoculating loop to dislodge the conidia from the surface of the agar plates. Spor suspension was stirred by a magnetic shaker for 10 min. In order to calculate the spore density from the prepared suspension, a 10^{-2} dilution was made and counted with a Neubauer hemocytometer under light microscope, and spore suspensions with a density of 1 x 10^8 conidia mL^{-1} were prepared for each *B. bassiana* isolate (Fancelli et al., 2013).

Table 1. Fungal isolates, sources and locality of the EPF isolates used for bioassay study

Isolates	Source	Locality	References
Beauveria bassiana/ ET 10	*Sphenoptera antiqua*	Erzurum, Türkiye	Tozlu et al. (2017)
Beauveria bassiana/ BMAUM-M6-4	Field soil	Isparta, Türkiye	Baydar et al. (2016)
Beauveria bassiana/ Bb 1	Forest soil	Düzce, Türkiye	Erdoğan and Sağlan (2023)

Application of *B. bassiana* isolates to *M. persicae* nymph

In the experiments, thinly cut sponge and sterile blotting paper were placed on the bottom of plastic Petri plates (100 mm diameter). A pepper leaf was placed on the blotting paper and a ring (2 cm diameter) was placed in the middle of this leaf, to which the aphids could be transferred. There was a ring in each Petri plate, and the second stage nymphs of *M. persicae* were transferred into these rings under a binocular stereo microscope with the help of a fine-tipped brush. By spraying method, spore suspensions of *B. bassiana* isolates (ET 10, BMAUM-M6-4 and Bb 1) containing 1 x 10^8 conidia mL^{-1} were applied to the transferred aphid nymphs with a hand sprayer, 2 mL per petri plate. As a control, sterile distilled water containing 0.01% Tween 20 was sprayed onto the second stage nymphs. Experiments were carried out with 10 replicates and 10 nymphs in each

replication. Numbers of live nymph were recorded at 1st, 3rd, 5th, and 7th after spraying. The experiment was carried out in a plant growth chamber with 25±1°C temperature, 60±5% RH, photoperiod 16:8 (L: D) conditions.

Statistical analysis

One-way analysis of variance (One-Way ANOVA) was applied to the data obtained after angle transformation. The differences between the means were determined by the Tukey's multiple comparison test at the P≤0.05 significance level (Tukey, 1949). Statistical analyzes were carried out with the SPSS® 20.0 package program. ın addition, LT_{50} values were determined by the Probit analysis program (Throne et al., 1995).

RESULTS AND DISCUSSION

The mortality rates of ET 10, BMAUM-M6-4 and Bb 1 isolates of *B. bassiana* to second instar nymph of *M. persicae* by spraying method are given in Figure 1. After applying ET 10, BMAUM-M6-4 and Bb 1 isolates of *B. bassiana*, the differences between the mortality rates in *M. persicae* nymphs and the mortality rates in nymphs in the control group on the 1st, 3rd, 5th and 7th days were found to be statistically significant. On the first day after application, the highest mortality rate recorded in the BMAUM-M6-4 isolate with 16%, followed by ET 10 and Bb 1 isolates with a 12% mortality rate, and EPF isolates were statistically in the same group. The highest mortality rates were detected in the Bb1 isolate on the third and fifth days of application, with 64 and 95%, respectively. BMAUM-M6-4 (50-67%) and ET 10 (41-59%) isolates followed the Bb 1 isolate, respectively. EPF isolates were found to be statistically different in the 5th day counts. In the seventh day counts, a 100% mortality rate was recorded for Bb 1 and ET 10 isolates, and a 99% mortality rate for BMAUM-M6-4 isolate, and all EPF isolates were statistically in the same group (Figure 1). LT_{50} values of ET 10, BMAUM-M6-4 and Bb 1 isolates of *B. bassiana* applied to *M. persicae* nymphs exposed to 10^8 conidia mL^{-1} concentration are given in Figure 2. Percentage mortality rates increased with time after the application. LT_{50} values for ET 10, BMAUM-M6-4 and Bb 1 isolates were calculated as 3.62, 3.60 and 2.93 days, respectively.

The fungi causing pathogenecity in insects have been observed to cause mortality in insect pest populations and therefore studied for their use as biological control agents (Hesketh et al., 2008; Freed et al., 2012). Studies conducted on *Beauveria*, *Metarhizium* and *Isaria* spp. and some *Lecanicillium* spp. isolates were highly effective on aphids (Hayden et al., 1992; Vu et al., 2007; Kim and Kim, 2008). The entomopathogenic fungi potential against target aphid populations is different for different isolates and also varies from strain to strain. The study showed that *B. bassiana* is effective for the control of aphids on different crops at different concentrations. As a result of application of *B. bassiana* isolates 5493, JW-1 and GHA to the first instar nymphs of *M. persicae*, mortality rates of 91%, 100% and 56% were recorded (Jandricic et al., 2014). In another study, 100% mortality rate was recorded as a result of the application of *B. bassiana* isolate at a concentration of 1.0 x 10^8 conidia mL^{-1} to the third stage nymphs of *M. persicae* (de Loureiro and Moino, 2006). Berber and Birgücü (2020) recorded 98 and 92% mortality rates, respectively, nine days after treatment of second and third instar nymphs of *M. persicae* with LD.2016 and M6-4 isolates of *B. bassiana* at a concentration of 1.0 x 10^8 conidia mL^{-1}. It is reported that *B. bassiana* isolate applied at different concentrations throughout the study increased the percentage mortality value depending on the dose increase. Seven days after treatment of *M. persicae* individuals with *B. bassiana* ART41 and ART2580 isolates, mortality rates of 92 and 98% were recorded, respectively (Lefort et al., 2014).A mortality rate of over 80% occurred on the tenth day after the application of a dose of 10^7 conidia mL^{-1} of the *B. bassiana* isolate to *M. persicae*. In other studies, mortality rates of over 75% were recorded in adult *M. persicae* adults exposed to *Beauveria* isolates (Hesketh et al., 2008; Shan and Feng, 2010; Tesfaye and Seyoum, 2010). In another study, treatment of *M. persicae* individuals with Bb-72 and Bb-252 isolates of *B. bassiana* resulted in 91 and 95% mortality (Nazir et al., 2019). As a result of application of *B. bassiana* strains (Bb-202) (1.0×10^2 to 6.75×10^5 conidia mm^2) to *M. persicae* individuals, 100% mortality was detected (Bugti et al., 2018). As a result of another study conducted with the *B. bassiana* LPSC 1067 isolate, an increase in the mortality rate and a decrease in the formation of new nymphs were recorded in *M. persicae* adult individuals (Allegrucci, et al., 2020). LT_{50} values of LD.2016 and M6-4 isolates of *B. bassiana* were 6.19 and 5.5 days, respectively at 10^8 conidi mL^{-1} dose (Berber and Birgücü, 2020). Accordingly, the LT_{50} values of 1×10^5 , 1×10^6 , 1×10^7 , and 1×10^8 conidia/mL^{-1} concentrations of *Beauveria bassiana* strain 202 were calculated for *M. persicae* that were determined as 5.2~8.24, in days (Bugti et al., 2018). Our results are parallel to the results obtained in previous studies.

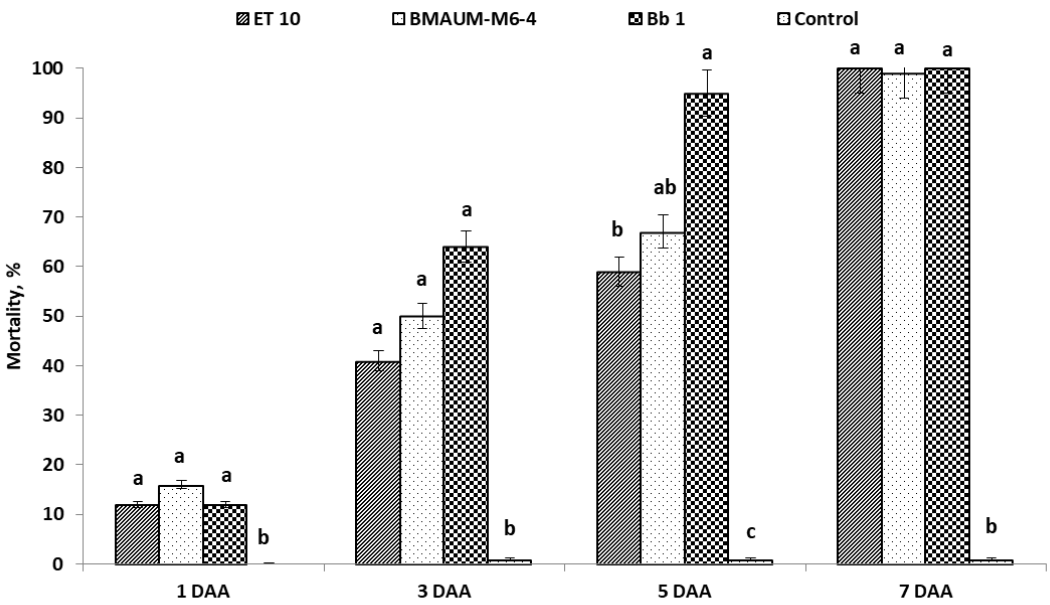

Figure 1. Mortality (%) of *Myzus persicae* nymphs inoculated with *Beauveria bassiana* isolates (1×10^8 conidia mL^{-1}. Different lowercase letters represent statistically significant differences among mortality, between treatments according to Tukey's HSD test ($P \leq 0.05$). DAA= days after application

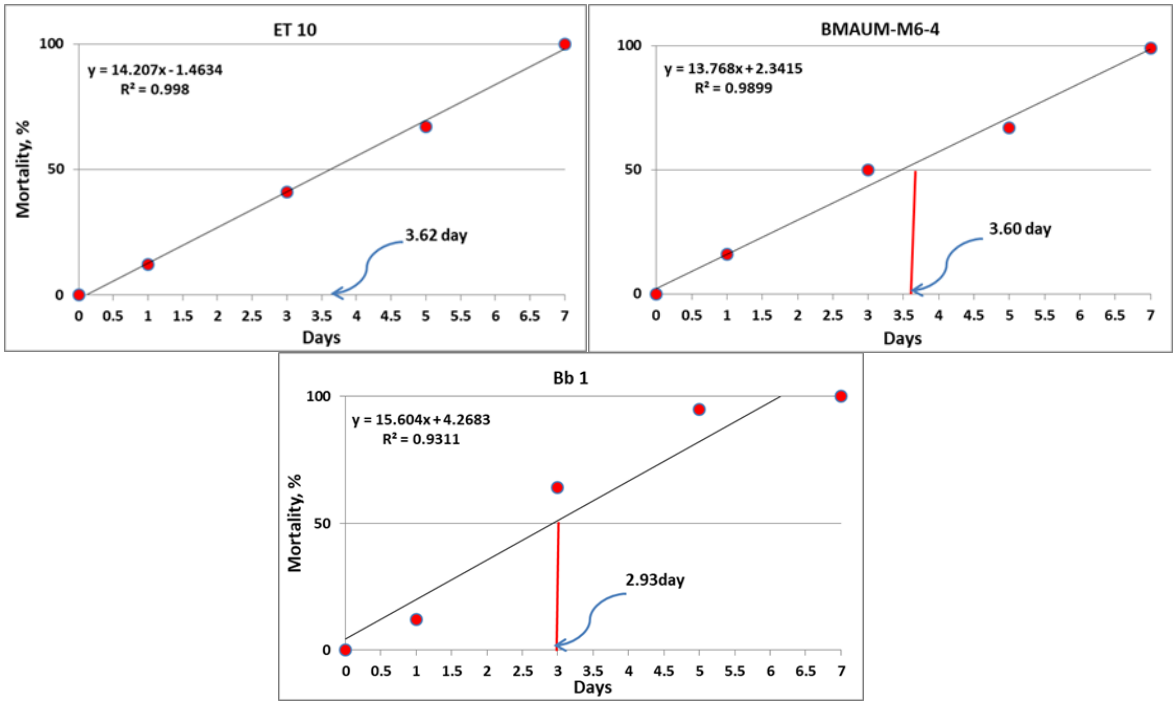

Figure 2. The mean LT_{50} values of ET 10, BMAUM-M6-4 and Bb 1 isolates of *Beauveria bassiana* applied to *Myzus persicae* nymphs

CONCLUSION

As a result of the study, ET 10, BMAUM-M6-4 and Bb 1 isolates of *B. bassiana* showed an effect against *M. persicae* from the 1st day of application, and this effect reached 99-100% on the 7th day. According to the results obtained from the study, the use of *B. bassiana* isolates in the control against *M. persicae* was found to be promising. Entomopathogenic fungi are a suitable alternative to chemicals since they do not have any toxic effects on mammals. However, efficacy trials need to be conducted with *B. bassiana* isolates under field and greenhouse conditions. Under field conditions, 60-80% reduction in *M. persicae* population was recorded after

the application of *B. bassiana* (CG-864 and PL-63) isolates (Filho et al., 2011). It is reported that relative humidity of 90% and above in greenhouse conditions increases the activity of *B. bassina* (Shipp et al., 2003). Additionally, temperature and other factors were found to be effective in the growth and speed of fungi (Orozco-Avitia et al., 2013). If these isolates are found successful in the studies, these isolates can be used in biological control applications within the scope of an integrated pest management program against *M. persicae*.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

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