RESEARCH ARTICLE

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The effect of two isolates of *Beauveria bassiana* (Bals.) Vull. on the larvae of confused flour beetle [*Tribolium confusum* du Val., 1863 (Coleoptera: Tenebrionidae)]

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Abstract

The confused flour beetle cause economic losses in stored products, especially in products obtained from wheat. Although using insecticides against storage pests is an effective method, their frequent and continuous use can lead to resistance and toxicity issues in non-target organisms. In this study, the effect of local *Beauveria bassiana* isolates (BMAUM LD.2016 and BMAUM M6-4) on the 3rd instar larvae of confused flour beetle (*Tribolium confusum*) was determined under laboratory conditions. As a result of the experiment, both isolates of *B. bassiana* were found to be more effective on the larvae in the spraying method compared to the dipping method. In the spraying method, mortality rates on the 9th day were 72% in BMAUM LD.2016 isolate, 34% in the dipping method, respectively. Mortality rates of BMAUM M6-4 isolate were recorded as 96% in the spraying method and 8% in the dipping method. In the spraying method, the mortality date (LT_{50}) was determined as 6.09 days for BMAUM LD.2016 isolate and 3.90 days for BMAUM M6-4 isolate. The LT_{50} value could not be calculated in the dipping method, since the mortality rates were below 50% for both isolates. BMAUM M6-4 isolate caused higher mortality in larvae than BMAUM LD.2016 isolate. As a result, *B. bassiana* isolates have shown a high level of effectiveness against *T. confusum*, demonstrating that they can be used as isolates for insect control.

Keywords: *Beauveria bassiana,* Biological control, Entomopathogenic fungi, Mortality rate, *Tribolium confusum*

INTRODUCTION

Grain production ranks first among cultivated crops in Türkiye and in the world. Wheat and barley are the leading grains produced in Türkiye The annual production of wheat, which is cultivated on an area of approximately 6 million 800 thousand hectares, is 18 million tons while that of barley, which is cultivated on an area of approximately 3 million 200 thousand hectares, is 6 million tons annually (TSI, 2021). After harvest, grains need to be stored and protected with minimal losses for a long time until consumption. For this, it is important to identify the organisms that adversely affect the quality and quantity of the products (Bağcı et al., 2014). Stored-grain insects cause damage to by feeding on grain prodcuts directly and indirectly, resulting in a decrease in the seed quality weight. nutritional values, and consequently commercial value (Boxall, 2001). Every year, approximately 10% to 40% of stored grains around the world are damaged both qualitatively and quantitatively by insects, especially in tropical and subtropical regions of developing or underdeveloped countries (Tripathi et al., 2009).

Stored foods are destroyed by mites and various harmful beetles and moths insects

(Rajendran and Sriranjini, 2008). The beetles (Coleoptera) include many harmful species such as *Sitophilus granarius* (L.) (Curculionidae), *Tribolium castaneum* (Herbst) (Tenebrionidae), *Oryzaephilus surinamensis* (L.) (Silvanidae), and *Rhyzopertha dominica* (Bostrichidae), *Tribolium confusum* du Val. (Tenebrionidae) (Hill, 1990; Hodges et al., 1996; Lord, 2007). Confused flour beetles, particularly in products derived from wheat (such as flour, etc.), pasta, dried fruits, biscuits, and nuts, causes damage and leads to losses (Karunakaran et al., 2004).

Farmers use different insecticides to control stored-product insects, such as malathion (Arthur and Zettler 1991, 1992), deltamethrin (Arthur, 1997), cyfluthrin (Arthur, 1994; 1999), bioresmethrin (Ardley, 1976) and chlorpyrifos-methyl (LaHue, 1997)]. Although these chemicals are effective their frequent and continuous use can lead to serious problems such as resistance and toxicity on non-target organisms (Isman, 2006; Daglish, 2008; Watts and Williamson, 2015). Hence, there has been a trend towards using safer alternative control methods (Upadhyay and Ahmad, 2011). In this context, pathogens have been used as biological control agents to control stored product insects. Such pathogens include entomopathogenic fungi, protozoa, viruses, and nematodes (Moore et al., 2000). Field and laboratory studies have shown that entomopathogenic fungi (EPF) are quite successful in controlling many pests of stored grains (Batta, 2004; Vassilakos et al., 2006; Sabbour et al., 2012).

Currently, approximately 700 species of entomopathogenic fungi belonging to 90 genera have been identified (Roberts and Humber, 1981). Among these, EPFs such as *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metschn.) Sorokin 1883, and *Isaria fumosorosea* Wize (=*Paecilomyces fumosoroseus* (Wize) A.H.S. Br. & G. Sm.), have been used in various studies for the control of stored-product insects (Bello et al., 2001; Padin et al., 2002; Khashaveh et al., 2011; Shafighi et al., 2014; Kubilay Er et al., 2016). *B. bassiana* and *M. anisopliae* are reported as the most extensively studied fungi species in the control of stored-product insects (Rumbos and Athanassiou, 2017). Particularly, it has been determined that *B. bassiana* isolates are effective against stored grain insects, including *Sitophilus oryzae*, *Rhyzopertha dominica*, and *Tribolium castaneum* (Padin et al., 1996; Bello et al., 2001).

In this study, the effects of *B. bassiana* BMAUM LD.2016 and BMAUM M6-4 isolates obtained from Isparta province (Western Turkey) on the 3rd instar larvae of of the confused flour beetle, *T. confusum*, were investigated under laboratory conditions.

MATERIALS AND METHODS

Rearing of *Tribolium confusum*

The adult individuals of *T. confusum* were obtained from infested seeds of wheat varieties in Pamukkale University, Faculty of Applied Sciences, Department of Organic Agricultural Management, Genetic Stock Unit. The larvae were cultured in a laboratory conditions in plastic pots (20 x 20 cm in size) containing a mixture of bran and flour. These containers were kept in plant growth chambers with a temperature of 25°C, relative humidity of 60±5%, photoperiod 16:8 (light:dark) lighting conditions.

Preparation of *Beauveria bassiana* isolates and spore suspensions

The study utilized the *B. bassiana* BMAUM M6-4 isolate, which was isolated using the Galleria trap method from soil samples collected from agricultural fields in the city center of Isparta (Zimmermann, 1986), and the *B. bassiana* BMAUM LD.2016 isolate, which was isolated from adult *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) collected on the campus of Süleyman Demirel University and found to be highly pathogenic (Baydar et al., 2016).

B. bassiana isolates used in the experiment were cultured in potato dextrose agar (PDA-39 g/l, Difco) medium. For this purpose, PDA was prepared using distilled water (dH_2O) and poured into glass Erlenmeyer flasks (500 ml) and sterilized in an autoclave at 121°C for 15 minutes. PDA medium cooled at room temperature was poured into plastic Petri dishes (90 mm) at a volume of 20 ml. Spore discs (0.5 cm) of BMAUM M6-4 and BMAUM LD.2016 isolates were transferred to the center of Petri dishes containing PDA medium in a sterile cabinet. The Petri dishes, sealed with Parafilm, were incubated for 14 days in a dark, cooled incubator at a temperature of 25°C and a relative humidity of 75%. After 14 days of incubation, the spores developed on PDA plates of BMAUM M6-4 and BMAUM LD.2016 cultures were gently scraped and collected. These spores were then added to 50 ml of sterile distilled water containing 0.05% Tween 80 to prepare spore suspensions. In order to calculate the spore density from the prepared suspension, a 10^{-2} dilution was made and counted with the help of Thoma Slide under the light microscope, and spore suspensions were prepared at a density of 1 x 10^8 conidia/ml for each *B. bassiana* isolate (Fancelli et al., 2013).

Application of *B. bassiana* isolates to *T. confusum* larvae

Each *B. bassiana* isolate was applied to five 3rd instar larvae of *T. confusum* using spraying and dipping methods. Each experiment was carried out with 10 replications according to the randomized plots design.

Spraying method

In the spraying method, spore suspensions of *B. bassiana* isolates were sprayed on the 3rd instar larvae in a Petri dish (90 mm) from a distance of 20 cm with a hand sprayer. As a control, sterile distilled water containing 0.05% Tween 80 was sprayed onto the 3rd instar larvae. In order to provide high humidity, the bottoms of Petri

dishes were covered with blotting paper, and 1 ml of sterile distilled water was impregnated on these papers. Experiments were carried out with 10 replicates and 5 individuals in each replication. Numbers of live larvae were recorded at 1, 3, 5, 7 and 9 days after spraying. The spraying experiment was carried out in a plant growth chambers with 25±1°C temperature, 60±5% humidity, photoperiod 16:8 (light:dark) lighting conditions.

Dipping method

In the dipping method, the larvae of the 3rd instar larvae were placed in a cheesecloth and kept in spore suspensions of *B. bassiana* isolates for 5 seconds, and then transferred to Petri dishes containing moistened blotting paper. In control applications, third instar larvae were dipped in sterile distilled water containing 0.05% Tween 80. Experiments were carried out with 10 replicates and 5 individuals in each replication. Numbers of live larvae were recorded at 1, 3, 5, 7 and 9 days after dipping. The dipping experiment was carried out in a plant growth cabinet with 25±1°C temperature, 60±5% humidity, photoperiod 16:8 (light: dark) lighting conditions.

Data analysis

Data for this study was subjected to analysis of variance (ANOVA), and the differences between means were compared using the Tukey's multiple comparison test at a significance level of $P \leq 0.05$ (Tukey, 1949). The data analysis was performed using the IBM® SPSS® Statistics software (Version 20.0, August 2011, SPSS Inc., Chicago, IL, USA) statistical package program. Abbott formula is used to determine the percentage of mortality rates (Abbott, 1925). In addition, the estimated time (LT_{50}) to kill 50% of the insects was determined by the Probit analysis program (Throne et al., 1995).

RESULTS AND DISCUSSION

Spraying and dipping methods of *B. bassiana* BMAUM LD.2016 showed that this isolate was effective on the 3rd instar larvae of *T. confusum* and percentage mortality rates in the larvae (Fig. 1). Mortality rates increased depending on the application days in both spraying and dipping methods. The highest mortality rates in 3rd instar larvae were recorded as 72% for the spraying method and 34% for the dipping method (Fig. 1).

Percentage of mortality rates resulting from the application of the second *B. bassiana* isolate, BMAUM M6-4, on 3rd instar larvae of *T. confusum* using the spraying and dipping methods are given in Figure 2. Percentage mortality rates increased with time after the treatment. In contrast, in the dipping method, the mortality rate was recorded as 6% on the third day and 8% on the ninth day of counting. In the spraying method, the highest mortality rate of 96% was observed in the 3rd stage larvae, while in the dipping method, the highest mortality rate was recorded as 8% (Fig. 2).

The results obtained in this study are in agreement with results of previous studies. The mortality rate was determined as 40% on the 7th day after the application of *M. anisopliae* isolate at $8x10^{10}$ spore/ml concentration to *T. confusum* larvae (Michalaki et al., 2006). When applied to adults of *S. oryzae* and *T. castaneum*, *B. bassiana* isolate resulted in mortality rates of 63.3% and 26.7% respectively, while *M. anisopliae* isolate resulted in mortality rates of 50.0% and 20.2% respectively (Batta, 2008). In another study, *B. bassiana* (BbWeevil™) was applied at a concentration of 1,000 mg/kg to adults of *S. granarius*, *Oryzaephilus surinamensis*, and *T. castaneum*, resulting in mortality rates of 88.33%, 78.31%, and 64.99% respectively (Khashaveh et al., 2011). The highest mortality rate was recorded as 57.35% 21 days after the application of *B. bassiana* (RacerTM) to the 3rd instar larvae of *T. confusum* at 0.9×10^{8} conidia/kg concentrations (Rehman et al., 2018). Çetinpolat et al. (2019) reported that the application of *B. bassiana* isolate to *T. confusum* larvae resulted in 97.4% mortality at a dose of 500 ppm and 100% mortality at a dose of 1000 ppm. In a study conducted with four isolates of *B. bassiana* (GN22-1, HP15, HP5-2, HP3-1) on *T. castaneum* adults, it was reported that after 13 days of application at a concentration of 1×10^{8} conidia/ml, the mortality rates were recorded as follows: GN22-1 - 72.85%, HP3-1 - 48.88%, HP15 - 47.37%, and HP5-2 - 30.43% (Uçar et al., 2020). *B. bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae) WG-50 and WG-51 isolates, when applied at a dose of 1×10^{8} conidia/kg to *T. castaneum* adults, recorded mortality rates of 88.1% and 83.4% respectively after 21 days (Wakil et al., 2021). *B. bassiana*, when applied at a dose of 1×10^{7} conidia/ml to *T. confusum*, showed effects of 87.5%, 97.5%, and 100% at 24, 48, and 72 hours respectively (Youssra Sekrane et al., 2022).

When the mortality rates of the 3rd instar larvae of *T. confusum* were examined depending on time, the LT_{50} value, which indicates the time required for half of the *T. confusum* larvae to die in the spraying method, was calculated as 6.09 and 3.90 days in BMAUM LD.2016 and BMAUM M6-4 isolates, respectively (Fig. 3 and Fig. 4). In the dipping method, the mortality rate for both *B. bassiana* isolates remained below 50%, so the LT_{50} value could not be calculated.

Unlike the LT_{50} values we obtained in our study, Uçar et al. (2020) reported that LT_{50} values as 28.813 days for HP5-2, 17.186 days for HP3-1, 10.327 days for GN22-1, and 18.615 days for HP15 when different isolates of *B. bassiana* were applied to *T. castaneum* adults.

CONCLUSION

Results show that BMAUM M6-4 isolate of *B. bassiana*, isolated from agricultural fields, was more effective than BMAUM LD.2016 isolate on *T. confusum* larvae. The percentage of mortality rates obtained with the spraying method used in the experiment were found to be higher than the dipping method. In future studies, the interaction of these isolates with pesticides used against stored-

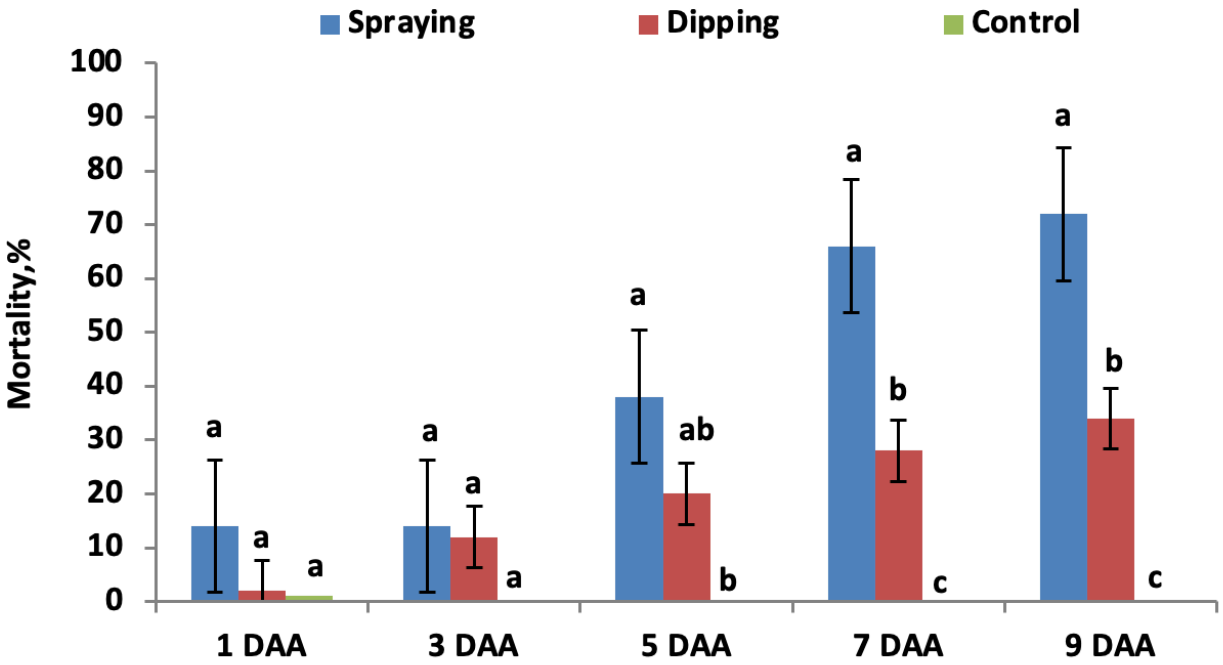

Figure 1. Percentage mortality rates of *B. bassiana* BMAUM LD.2016 isolate applied on *T. confusum* larvae with spraying and dipping methods. (The differences between the means (±standard error) of the columns indicated with different letters for each day are statistically significant (Tukey's HSD test P<0.05)). DAA: Days after application

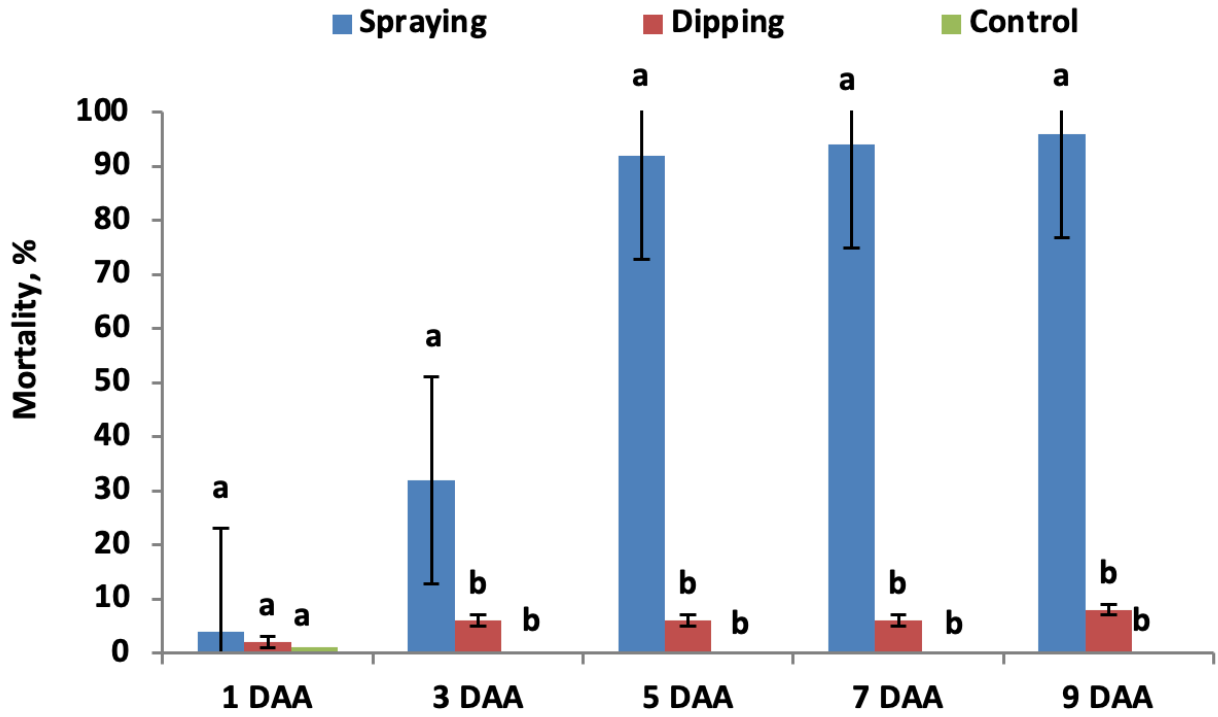

Figure 2. Percentage mortality rates of *B. bassiana* BMAUM M6-4 isolate applied on *T. confusum* larvae with spraying and dipping methods. (The differences between the means (±standard error) of the columns indicated with different letters for each day are statistically significant (Tukey's HSD test P<0.05)). DAA: Days after application

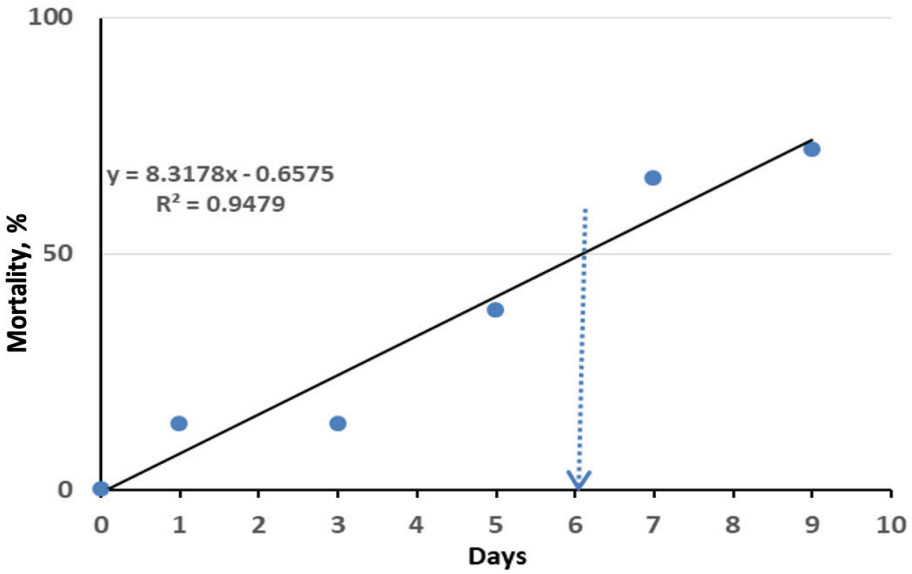

Figure 3. Mean LT_{50} values of *B. bassiana* BMAUM LD.2016 isolate in spraying method

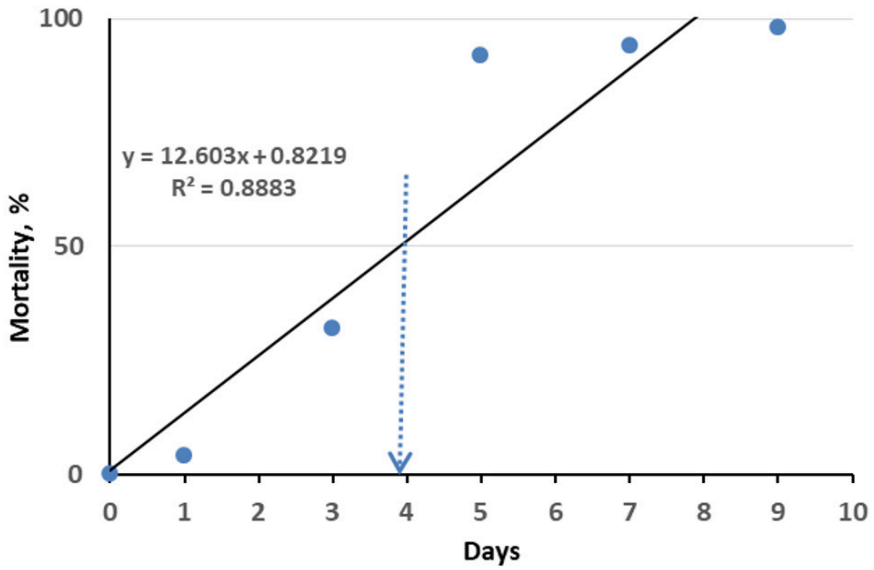

Figure 4. The mean LT_{50} values of *B. bassiana* BMAUM M6-4 isolate in spraying method

product insects should be investigated. Furthermore, the development of the use of entomopathogenic fungal isolates, including for other stored-product insects, would be beneficial for integrated pest management.

Abbreviations

ANOVA: Analysis of variance, *B. bassiana*: *Beauveria bassiana*, DAA: Days after application, EPF: Entomopathogenic fungi, PDA: Potato dextrose agar.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Declaration of interests

The authors have no conflict of interest to declare.

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.

Ethics Committee Approval

Ethics committee approval is not required. This article does not contain any studies with human participants or animals performed by any of the authors.

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Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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