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EFFICACY OF *BEAUVERIA BASSIANA* (BALS.) VUILL. ISOLATES ON DRIED FRUIT MOTH (*PLODIA INTERPUNCTELLA* [LEPIDOPTERA: PYRALIDAE])

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Abstract: The dried fruit moth, *Plodia interpunctella* (Hübner, 1813) (Lepidoptera: Pyralidae) is one of the most important pests of both dried fruits and stored grains and products. One of the alternative control methods to chemicals in the control against this pest is the use of biological control methods. Entomopathogenic fungi (EPF) stand out because they do not have any negative effects on the environment, living organism and human health, other than the target pests. In this study, ET 10 and Bb 18 isolates of *Beauveria bassiana* (Bals.) Vuill. were applied to the 4th instar larvae of *P. interpunctella* under laboratory conditions and their effectiveness was determined. EPF isolates were sprayed to the larvae in plastic petri dishes at a concentration of $1x10^8$ conidia/ml. The experiments were carried out in a randomized plots experimental design with five replicates, with five 4th instar larvae in each petri dish. After the applications, the number of live larvae was recorded by counting the 1st, 3rd, 5th and 7th days and the % mortality rate was calculated. On the fifth day of the experiment, mortality rates of 92% were recorded for the ET 10 isolate of *B. bassiana* and 84% for the Bb 18 isolate. In the seventh day counts, 100% mortality rates were determined for both isolates of *B. bassiana*. As a result, it is concluded that *B. bassiana* may have a potential effect in the biological control of stored product pests.

Keywords: *Beauveria bassiana*, *Plodia interpunctella*, Mortality rate, Biological control, Stored product

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1. Introduction

Türkiye has an important place in the world production and export of cereals, pulses, oilseeds, hazelnuts, dried fruits and their products. Among these, walnut (*Juglans regia* L.) is a hard-shelled fruit species with high commercial value that is grown in temperate regions around the world for timber and nuts (Ajazi et al., 2014; Pollegioni et al., 2015). Walnut is one of the most important sources of energy with the protein, fats and minerals (Mir and Kottaiveeran, 2018).

The production of walnut, one of the high-yielding crops in horticultural, has become quite widespread in the world with increasing demand. While 3.3 million tons of shelled walnuts are produced in an area of 13 million decares in the world, Türkiye ranks 4th with approximately 287.000 tons of walnut production. While China (2.8 million da) and the USA (1.5 million da) rank first in the world walnut production area, Türkiye ranks 3rd with a production area of 1.4 million da (FAO, 2022).

Agricultural crops must be protected and stored with minimal losses in both the domestic and foreign markets, from the harvesting process to the consumption process. Biotic and/or abiotic stress factors affect the storage process of these crops. Biotic factors that cause product loss in stored crops include microorganisms, rodents, mites and insects. These pests cause direct and indirect damage by infecting stored crops (Kahraman, 2009). *Plodia interpunctella* (Hübner, 1813), the dried fruit moth, is a pyralid moth belonging to the order Lepidoptera, family Pyralidae. *P. interpunctella* is an economically important pest of stored crops worldwide (Sedlacek et al., 1996). *P. interpunctella* larvae feed and web intensively in the environments in which they feed, causing losses in crop quality due to the wastes and residues they produce (Yıldırım et al., 1997; Boxall, 2001). The most harmful crops include dried apricots, figs, raisins, hazelnuts, chestnuts, walnuts, pistachios, peanuts, almonds and carob, etc. As can be seen, *P. interpunctella* does not exhibit food selectivity. This makes dried fruit moth infestations inevitable in many food products. The aim of protecting stored crops against *P. interpunctella* is the integrated application of cultural, mechanical, chemical, physical, biotechnical, biological and quarantine measures. However, "fumigation", a chemical control method, is widely used against stored crop pests in the world and in our country (Fields and White, 2002; Işıkber et al., 2015). Methyl bromide (MeBr) and phosphine are primarily used in fumigation

applications. MeBr, which is widely used in the control against stored crop pests, is banned all over the world due to its ozone depleting effect. Due to the ban on the use of MeBr and its negative effect on the environment, the necessity of researching alternative methods to replace MeBr has become increasingly important. In this context, biological control stands out as the most basic method in terms of human-environmental health, agricultural and environmental sustainability, and ecological balance. The use of microbial insecticides with specific effects has an important place among alternative and safe methods in biological control (Pszczola, 1997; Demirezen, 2010).

Entomopathogenic fungi (EPF) are among the most common disease-causing pathogens in agricultural and forest pests (Mueller and Schmit, 2007). Unlike other pathogens, EPFs are used as important control agents in the stages when the insect is not feeding (last larva and pupa), since they infect the pest through the integument. Since insects that die from entomopathogenic fungal infection mostly fall into the soil from the plant they are on the soil environment creates an important fungal reserve and protects fungal spores from abiotic and biotic factors, allowing them to maintain their viability for a long time (Gök et al., 2018). *Beauveria* spp. are entomopathogenic fungi (EPF) that can be easily isolated and produced from almost all ecosystems (Rehner et al., 2011). Entomopathogenic fungi (EPF) have been used as microbial control agents for more than 100 years (Roberts, 1989). Nowadays, *Beauveria bassiana* (EPF) (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae) is reported to have more than 700 hosts (Wraight et al., 2000). These hosts are contained in 521 genera, 149 families and 15 orders (Zimmermann, 2007). *B. bassiana* is an entomopathogenic fungus used in biological control against both harmful insects and plant pathogens and causes White Muscardine disease (Feng et al., 2004). *B. bassiana* produces the toxins Beauvericin and Bassianin, Bassianolide, Beauverolides, and Tennellin. These toxins kill the host by dissolving the tissues, degradation the cells, and re-germinating exiting the host body, forming the conidia and restoring the life cycle of the fungus (Sabbour, 2002). *B. bassiana* has been tested with success against various stored-product insect species in both laboratory and field trials (Lord, 2001; Padin et al., 2002; Akbar et al., 2004; Jyothi et al., 2014). Among the identified entomopathogenic fungi, *Beauveria bassiana*, *Lecanicillium* (*Verticillium*) *lecanii*, *Metarhizium anisopliae* var. *anisopliae* and *Paecilomyces farinosus* are used against dry fruit moth (Büda and Peciulyte, 2008). Faria and Wraight (2007) determined approximately 40% of the total mycoinsecticides were based on *Beauveria* spp., although many products are no longer available in the biopesticide market. Entomopathogenic fungi have important advantages such as being non-toxic to human and environmental health, having a wide host range, not developing resistance in hosts, being applied together with pesticides, being cheap, and easy to apply (Sinha et al., 2016).

The aim of this study is to determine the efficacy of ET 10 and Bb 18 isolates of *Beauveria bassiana* by applying ET 10 and Bb 18 isolates against the fourth instar larvae of *P. interpunctella* under laboratory conditions.

2. Materials and Methods

2.1. Fungi Cultures and Preparation of Spore Suspensions

Beauveria bassiana ET 10 isolate was isolated from *Sphenoptera antiqua* (Illiger, 1803) (Coleoptera: Buprestidae) in Erzurum province, Türkiye (Tozlu et al., 2017), and Bb 18 isolate was isolated from field soil in Düzce province, Türkiye (Erdoğan and Sağlan, 2023). *B. bassiana* isolates were grown on potato dextrose agar medium (PDA-39 g/l, Difco) in the dark at 25±1°C for 14 days and stored at +4°C in the refrigerator.

PDA medium was prepared and sterilized in autoclave at 121°C for 15 minutes. Approximately 25 ml of the PDA was poured into 90 mm petri dishes and allowed to cool and solidify. Mycelium plug (5 mm in diameter), taken from the leading growth edge of a 14-day-old culture of *B. bassiana* ET 10 and Bb 18 isolates grown on PDA, was placed in the center of a petri dish in a laminar flow cabinet. Parafilm-sealed petri dishes were incubated in the dark at 25±1°C for 14 days. The conidia were harvested by scraping the surface of 14 days old culture gently with inoculation needle. The conidia were suspended in distilled water containing 0.1% Tween80. The mixture 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 the help of Thoma slide under light microscope, and spore suspensions with a density of $1x10^8$ conidia/ml were prepared for each *B. bassiana* isolate (Fancelli et al., 2013).

2.2. Rearing of Tested Insect

Individuals of *P. interpunctella* were cultured in the climate cabinet (25±1°C temperature, 60±5% RH and dark conditions) in the Entomology laboratory within the Department of Organic Farming Business Management, Faculty of Applied Sciences, Pamukkale University. The nutrient medium consisted of a mixture of bran, corn flour, dry yeast, honey, milk powder and glycerin in a ratio of 2:1:0.25:0.25:0.50:0.25:0.25 respectively (Ozkan, 2006). The prepared food was taken into glass jars (1 liter) and the larvae of the pest were transferred onto the food. The mouth of the jars was covered with tulle to ensure air entry and prevent other pests from entering. In this way, the development of the pest in the nutrient medium was ensured and fourth instar larvae to be used in the experiment were obtained.

2.3. Bioassay Test

Fourth instar larvae of *P. interpunctella* were reared on their artificial diet at 25±1°C with a photoperiod of 14:10 (L:D) h, 60±5% RH in a climate cabinet. In the experiment, ET 10 and Bb 18 isolates of *B. bassiana* were applied to *P. interpunctella* larvae in plastic petri dishes

(90 mm) at a concentration of $1x10^8$ conidia/ml by spraying method. As a control, only pure water was sprayed on the pest. The experiments were carried out in a randomized plot design with five replications, with five fourth instar larvae in each petri dish. The number of live larvae was recorded by counting the 1st, 3rd, 5th and 7th days after the treatments and the % mortality rates were calculated.

2.4. Statistical Analysis

The data obtained in this study were subjected to analysis of variance (ANOVA) and the differences between the means were compared using Tukey's multiple comparison test at the P≤0.05 significance level (Tukey, 1949). Data analysis was performed using IBM® SPSS® Statistics software (Version 20.0, August 2011, SPSS Inc., Chicago, IL, USA) statistical package program. Additionally, LT_{50} values were determined by the Probit analysis program (Throne et al., 1995).

3. Results and Discussion

The mortality rates of *B.bassiana* ET 10 and Bb18 isolates to the fourth instar larvae of *P. interpunctella* by spraying method are given in Figure 1. In the experiment, except for the first counting day, % mortality rates increased depending on the treatment days. In the first day counts, mortality rate was not recorded. In the third day counts, a 40% mortality rate was recorded for the *B. bassiana* ET 10 and Bb 18 isolates. In the fifth day counts, a 92% mortality rate was recorded for the *B. bassiana* ET 10 isolate and an 84% mortality rate for the Bb 18 isolate. On the seventh day counts, a 100% mortality rate was recorded for both isolates applied. Considering the time-dependent mortality rates of *P. interpunctella* fourth instar larvae, the LT_{50} value, which indicates the time required for half of the *P. interpunctella* larvae to die, was calculated as 3.27 and 3.37 days for ET 10 and Bb 18 isolates, respectively (Figure 2 and 3).

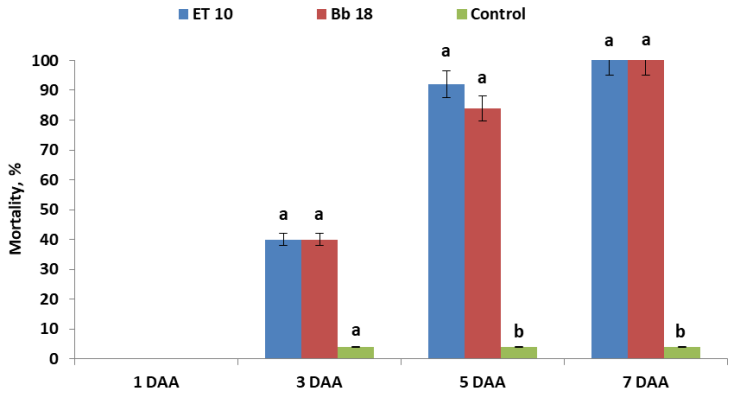

Figure 1. Percent mortality rates resulting from the application of *B. bassiana* ET 10 and Bb 18 isolates to *P. interpunctella* larvae. (The differences between the means (±standard error) of the columns indicated with different letters for each day are statistically significant (Tukey's HSD test P (Tukey's HSD test P<0.05)). DAA= days after application ($F3^{th}$=3.115, $df3^{th}$=2, 12, $P3^{th}$=0.081; $F5^{th}$=52.235, $df5^{th}$=2, 12, $P5^{th}$=0.000; $F7^{th}$=576.000, $df7^{th}$=2, 12, $P7^{th}$=0.000).

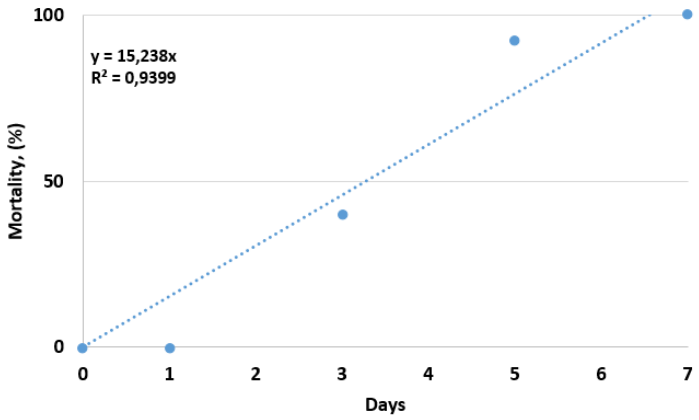

Figure 2. LT_{50} value obtained as a result of application of *B. bassiana* ET 10 isolate.

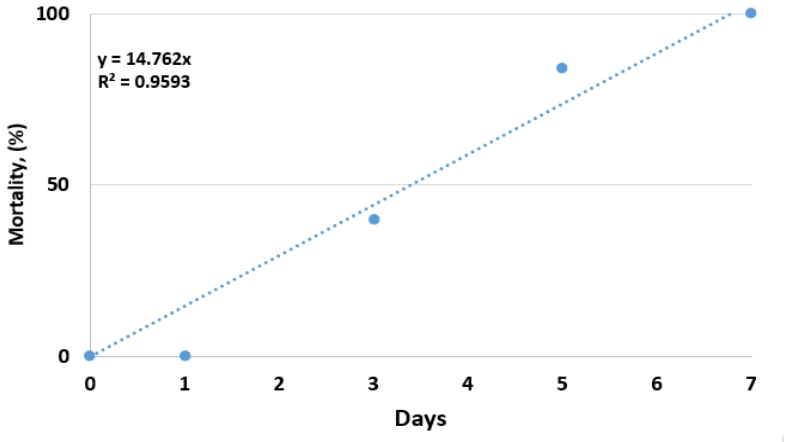

Figure 3. LT_{50} value obtained as a result of application of *B. bassiana* Bb 18 isolate.

According to literature searches, while there are studies on the effect of *B. bassiana* against storage pests in Türkiye no studies on the effect of *B. bassiana* against *P. interpunctella* have been found. There are few studies on this subject in the world. In a study by Büda and Peciulyte (2008) *B. bassiana*, *L. lecanii*, *M. anisopliae* var. *anisopliae* and *I. farinosa* entomopathogenic fungi (2.6×10^6 conidia/ml) were tested against late-stage larvae of the dried fruit moth, and the highest mortality rates for *I. farinosa* and *M. anisopliae* were recorded in the range of 35-40% at the end of the third day of the experiment. *B. bassiana* and *L. lecanii* were in the same group as the control. In another study, LC_{50} values were recorded as 138, 144 and 198, respectively, as a result of the application of *B. bassiana*, *M. anisopliae* and *I. fumosorose* isolates at 10^7 conidia/ml concentrations to the third instar larvae of *P. interpunctella* (Sabbour et al., 2012). Sedehi et al. (2014) found that there was a significant difference between the concentrations of *B. bassiana* spores on the eggs and larvae of *P. interpunctella* and determined the LC_{50} value as 7.4×10^7 conidia/ml for eggs and 6.6×10^6 conidia/ml for larvae. In a study similar to our results from the experiment, mortality rates were recorded as a result of applying *B. bassiana* (isolates TV and OZ1) and *M. anisopliae* (isolate CS1) isolates to the third instar larvae of *P. interpunctella* at a concentration of 1×10^8 conidia/ml. It has been reported that especially in these entomopathogenic fungal isolates, protease and lipase have a significant effect on the larvae (Golzan et al., 2023).

5. Conclusion

As a result of the study, ET 10 and Bb 18 isolates of *B. bassiana* isolated from different hosts showed that they could be effective in the control against storage pests, especially *P. interpunctella.* However, the control of storage pests depends not only on the characteristics of *B. bassiana* isolates, but also on biotic and abiotic factors. In addition, more detailed studies should be carried out under storage conditions in terms of the efficacy of promising isolates and pest management.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	A.B.E.	O.E.	M.S.S.
C	50	50	
D	50	50	
S	50	50	
DCP	50		50
DAI	100		
L	50		50
W	50	50	
CR	50	50	
SR	50	50	

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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