



## RESEARCH ARTICLE

# Fungal and Bacterial Bioagents Efficiency on the Control of Potato Pest *Phthorimaea operculella* via Ingestion or Contact

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## ABSTRACT

Potatoes are one of the most important food products in the world and considered a main human nutrition sources source. Potato tuber moth (*Phthorimaea operculella* Zeller (PTM) (Lepidoptera: Gelechiidae)) causes remarkable economic losses to important crop, both in field and under storage conditions. In this study, the insecticidal efficiency of the following bioagents: *Brevibacillus brevis* (FD-1), *Bacillus atrophaeus* (FD 17), *Bacillus sphaericus* (FD 49), *Bacillus cereus* (FD 63), *Vibrio hollisae* (FD 70), *Bacillus thuringiensis* subsp. *kenyae* (FDP 8) bacteria strains and *Beauveria bassiana* fungal isolate (ET 10), were evaluated on their efficacy to control *P. operculella*, under controlled conditions. In addition to insecticidal efficacy evaluations, analyses were also carried out to determine the differences between bioagents action mode: (1) uptake (ingestion as a gastric poison) and (2) contact. For (1), 20 larvae were fed on the tubers immersed in these suspensions to analyze efficacy by ingestion; for (2), suspensions of  $1 \times 10^8$  CFU/ml of bacteria and  $5.7 \times 10^5$  conidia/ml of fungus were prepared and sprayed to 20 larvae. FD-63 (91.67%) and FD-17 (88.33%) taken up by diet gave the most effective results against the pest.

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## 1. Introduction

Potato (*Solanum tuberosum* L.), which belongs to the Solanaceae family, is one of the most important food crops in the world (Khorrami, 2018). According to FAO (2019) data, 370.4 million tons of potatoes were produced on 17.3 million hectares of land worldwide. 28.3% of the world's potato cultivation areas are in China, 12.1% in India and, 6.9% in Russia, and these three countries account for 47.3% of the

world potato production. In 2019, 91.8 million tons of potatoes were produced in China, 50.1 million tons in India, and 22.07 million tons in Russia (FAO, 2019). According to 2019 production data, 4 million 980 thousand tons of potatoes were produced in 141 thousand hectares of land in Turkey (TUIK, 2020).

There are some important insect species and nematodes as well as fungal, bacterial and viral disease factors that cause

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economic losses in potato production both in the field and in storage conditions (GTHB, 2016). The potato tuber moth (PTM), *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae) is known to be one of the potato pests causing significant economic losses by damaging the crop in the field and the warehouse (Tsedaley, 2015; Rondon, 2010). This pest probably first appeared in the tropical highlands of South America (Graf 1917), and was later recorded as an important and widespread potato pest in 90 countries in Africa, Asia, Central and South America with tropical and subtropical climates (Sporleder et al., 2004; Jensen et al., 2005; Lacey et al., 2010). Its hosts primarily include potatoes and tomatoes (*Lycopersicon esculentum* (Miller)), other cultivated plants belonging to Solanaceous genus such as tobacco (*Nicotiana tabacum* L.), eggplant (*Solanum melogena* L.) and bell peppers (*Capsicum annuum* L.) and wild species belonging to the genera of *Solanum*, *Datura*, *Nicotiana*, *Fabina*, *Hyoscyamus*, *Physalodes*, *Lycium* and *Nicandra* (Schaub & Kroschel, 2018).

PTM females lay their eggs on leaves and exposed tubers near the eye, while their larvae dig tunnels during feeding, causing approximately 100% damage to both unharvested tubers in the field and post-harvest potato tubers held in storage (Visser, 2005; Sporleder et al., 2008). In addition, the larvae enter the leaflets, form transparent bubbles, injure the leaves, and then pass into the root tissue, causing the death of the plant (Sabbour & Raheem, 2015). However, it is stated that the damage to the green parts is not economically significant, and the main damage takes place in the warehouse as a result of the larvae's feeding on the tubers. Potatoes' edible and seed properties deteriorate in contaminated warehouses, causing loss of quality and weight (Okello et al., 2017). The extent of the damage increases rapidly when several generations develop during the storage period (Sharaby et al., 2019).

To date, various control methods have been utilized to prevent and control the damage of PTM (Clough et al., 2010). The high adaptability of PTM to daily and seasonal changes, its high reproductive potential and its survival even in extremely hot conditions pose difficulties in the control of this pest (Dođramacı & Tingey, 2008). In addition, it has developed

resistance to insecticides such as pyrethroid, carbamate and organophosphate, posing challenges to chemical pest control (Kroschel, 2006; Clough et al., 2008; Hafez, 2011; Tsedaley, 2015; Yan et al., 2019). In warehouses, timing is critical to ensure that newly hatched PTM larvae are killed before they enter the potato tubers and tunnel through (Tsedaley, 2015). The risks of chemicals for the environment, animal and human health have also driven awareness about the environment and the search for alternative control methods. Biological control, which is one of these methods, is an important control method that is environmentally friendly, has side effects on human and animal health; ensures the protection of the existing natural balance; does not cause resistance; does not cause residue problems in nutrients and does not pollute soils as well as groundwater. Studies have been conducted using entomopathogens, natural enemies, essential oils and plant extracts to reduce the damage caused by PTM.

In this study, insecticidal effects on *Phthorimaea operculella* larvae of *Brevibacillus brevis* (FD 1), *Bacillus atrophaeus* (FD 17), *B. sphaericus* (FD 49), *B. cereus* (FD 63), *Vibrio hollisae* (FD 70), *B. cereus* (FDP 8) bacterial strains and *Beauveria bassiana* (ET 10) fungal isolate (Saraç et al., 2011; Tozlu et al., 2011; Dadasoglu et al., 2013; Dadasoglu et al., 2014; Tozlu et al., 2020a,b; Tozlu et al., 2021), which were found to be effective on various pests in previous studies, were tested.

## 2. Materials and Methods

### 2.1. Insect, Bioagent Bacterial and Fungal isolates

Potato tubers contaminated with PTM larvae wintering during the larva period under climate conditions of Erzurum (Turkey), were obtained during the survey studies conducted at the time of potato harvest in September 2019, brought to Atatürk University Faculty of Agriculture, Department of Plant Protection Pest Systematics laboratory and stored in a 30×45×30 cm desiccator at 25±2 °C under 65%±5 proportional humidity and 16-h light: 8-h dark conditions by providing daily fresh food and humidity check (Figure 1).



**Figure 1.** Original field collected and laboratory stored potato tuber showing severe infestation and damaged by *Phthorimaea operculella* larvae.

The fungal and bacterial isolates considered in this study had previously demonstrated effective biocontrol in different pests as shown in Table 1. Bacterial biocontrol isolates were grown in Nutrient Broth (NB; Difco) containing 15% glycerol at -80°C in the Culture Collection in Atatürk University Faculty

of Agriculture Plant Protection Department Plant Clinical Laboratory, while fungal biocontrol isolate was stored in oblique Potato Dextrose Agar (PDA; Difco) medium at +4°C in Atatürk University Faculty of Agriculture Plant Protection Department Mycology Laboratory (Table 1).

**Table 1.** Bacterial strains and fungal isolate used in the study.

<b>Bacterial strains</b>					
Strains	Isolated from	MIS* Identification results	S**	HR***	Reference
FD 1	<i>Malacosoma neustria</i>	<i>Brevibacillus brevis</i>	0.625	-	Tozlu et al. 2011
FD 17	<i>Yponomeuta evonymella</i>	<i>Bacillus atrophaeus</i>	0.459	-	Tozlu et al. 2011
FD 49	<i>Culex</i> sp.	<i>Bacillus sphaericus</i>	0.681	-	Dadaşođlu et al. 2016
FD 63	<i>Yponomeuta evonymella</i>	<i>Bacillus cereus</i>	0.241	-	Tozlu et al. 2011
FD 70	<i>Melolontha melolontha</i>	<i>Vibrio hollisae</i>	0.476	-	Tozlu et al. 2019
FDP 8	<i>Bemisia tabaci</i>	<i>Bacillus cereus</i>	0.652	-	Tozlu et al. 2011
<b>Fungal isolate</b>					
Isolate	Isolated from	ITS Identification Result	S**	ITS 1 sequences****	Reference
ET 10	<i>Sphenoptera antiqua</i>	<i>Beauveria bassiana</i>	0.99	GB KY806126	Tozlu et al. 2017

\*MIS: Microbial Identification System, \*\*S: Similarity index, \*\*\*HR: Hypersensitivity test, -: Negative reaction, \*\*\*\*GenBank Accessed Number.

## 2.2. Preparation of the Bacterial Suspension

The tested bacterial strains were cultured in 4 phases in Nutrient Agar (NA) medium at 30 °C for 24 hrs to obtain fresh cultures. A single bacterial colony taken from these cultures in sterile loops was inoculated into Erlenmeyer flasks with 300 ml of NB medium and incubated for 24 hours at 250 rpm and 27 °C in a horizontal shaker incubator. The bacterial density of the resulting aqueous culture was adjusted to  $1 \times 10^8$  CFU/ml by BIOLOG turbidimeter and transferred to sterile spray vials.

## 2.3. Preparation of the Fungal Conidial Suspension

Conidia production was achieved by incubation of *B. bassiana* ET 10 isolate in Sabourth Dextrose Agar (SDA) medium at 25 °C, 80% humidity for 2-3 weeks. Then, a stock suspension was prepared by washing the surface of the culture into bottles containing sterile water with 0.2 ml/l Tween-80 solution (Quesada-Moraga et al., 2006). Using a hemocytometer, the concentration of conidia suspension to be applied was found to be  $5.7 \times 10^5$  conidia/ml.

## 2.4. Insecticidal Effect Test in Controlled Conditions

Within the scope of the study, blotting paper was placed on the bottom of each petri dish (12 cm). In order to determine the stomach poison effect of entomopathogens, potato tuber pieces cut in a rectangular shape (1.5×7×5 cm) were placed in petri dishes after dipping them into bioagent bacteria and fungus suspensions for 5 minutes (Figure 2). 20 PTM larvae were placed in each petri dish under a binocular microscope. To determine their efficacy by contact, potato tuber pieces cut in the same sizes were placed in petri dishes without any application, 20 PTM larvae were placed under a binocular microscope, and bioagent suspensions were sprayed on the larvae. Petri dishes were then kept under controlled conditions

at  $25 \pm 2$  °C, 65-70% RH and a 16:8 (light:dark) photoperiod. The number of dead larvae was recorded regularly every 24 hours and the death rates were determined by the formula below.

$$\text{Mortality rate (\%)} = \frac{100 \times \text{the number of dead adults in treatment}}{\text{Total adult in treatment}} \quad (1)$$

In the study, a sterile NB medium was used as negative control and commercial chemical Red Sunny WP (25% Diflubenzuron) was used a positive control. The trial was performed in triplicate for each combination on the same day.

## 2.5. Data Analysis

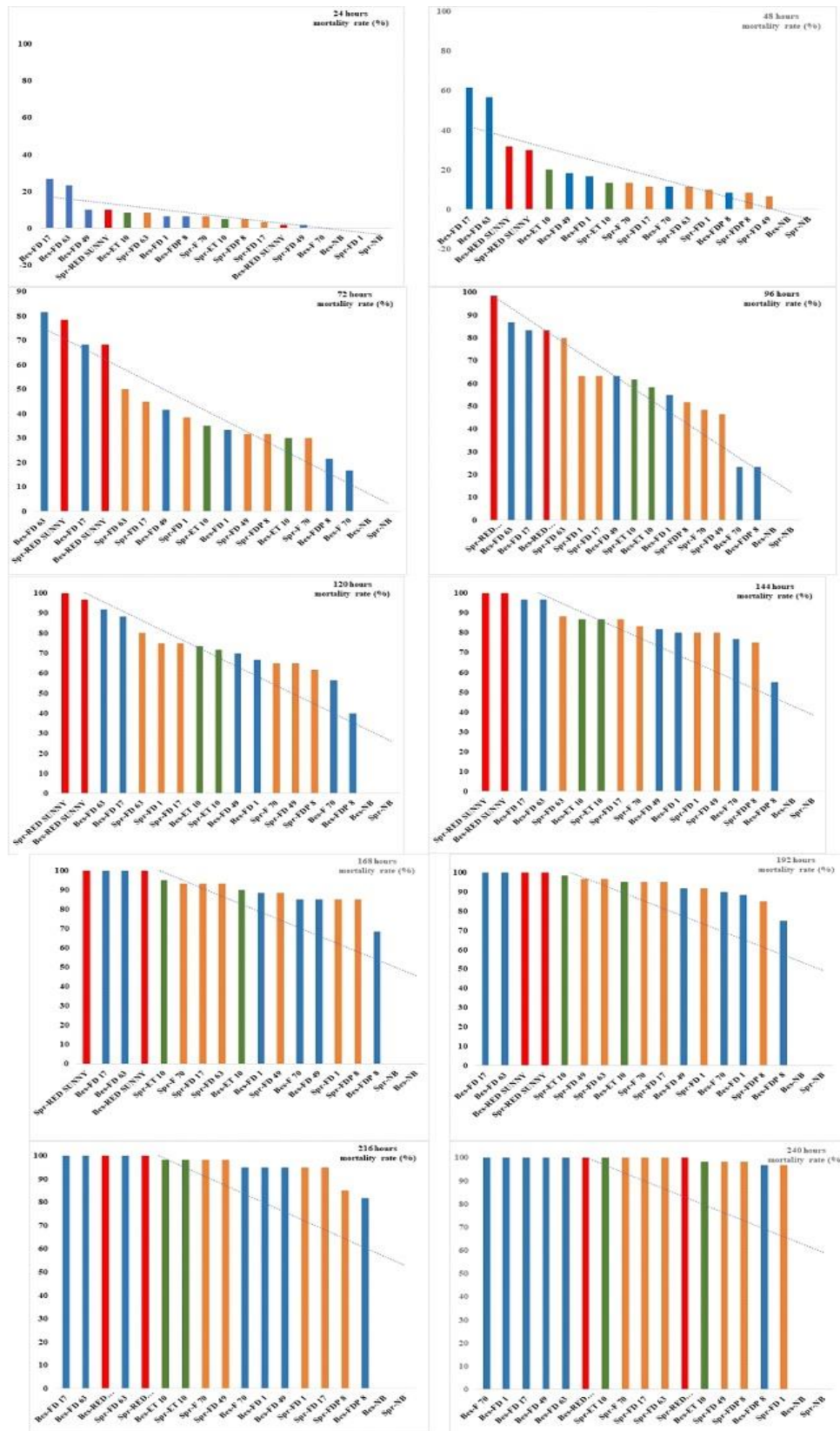
Analysis of variance was applied to the values related to the results obtained, and the differences between the means were compared to the LSMeans Student test at a significance level of  $P < 0.01$ . Data analyses were performed using the statistical software package JMP IN (SAS Institute, Cary, NC, .0% PC version).

## 3. Results

According to the results obtained, it was determined that all bacterial and fungal isolates had a pesticidal effect at varying rates. While there was no death in the negative controls, the death rate in the positive control was 100%. In the first 24 hours of the study, over 20% of death was detected in FD-17 and FD-63 isolates, and 10% of death were in FD-49 isolates, which were all applied to potato pieces. Treatments with FD-17 and FD-49 isolates, which had the highest death rate in the 24-hour evaluation, in the 48 hours the results were 61.67% and 56.67%, respectively. These two isolates gave the best results in all periodic counts. In the counts, death rates for FD-17 and FD-49 isolates were respectively as follows: 68.33% and 81.67% at 72 hours; 83.33% and 86.67% at 96 hours; 88.33% and 91.67% at

120 hours; 96.67% for both at 144 hours and 100% for both at 168 hours (Figure 2). In the study, the standard deviation values in the application to potato pieces varied 0.00 (FD-1, FD-17, Red Sunny WP (25% Diflubenzuron), FD-63, FD-49, F 70),

2.89 (FDP-8, ET 10), 25.17 (NB) and in the sprayed 0.00 (ET 10, F-70, FD-63, FD-17, Red Sunny WP (25% Diflubenzuron)), 2.89 (FDP-8, FD-49, NB), 5,77 (FD-1) according to the 240 hours (Figure 2).



**Figure 2.** Percentages of mortality of *Phthorimaea operculella* larvae in response to some entomopathogenic bacterial strains and fungal isolate by hours (Bes= Ingestion; Spr= Contact).



#### 4. Discussion

Entomopathogens are among the most influential factors regulating pest populations. Many biopesticides are commonly used worldwide in the biological control of pests in greenhouse products, ornamental plants, stored products, forest products and horticultural products (vegetables and fruit) (Lacey et al., 2001). There are many studies on the use of entomopathogenic bacteria and fungi in biological pest control (Inglis et al., 2001; Wraight et al., 2001; Lacey et al., 2001; Vestergaard et al., 2003; Copping, 2004; Aslantas et al., 2008; Khachatourians & Sohail, 2008; Lacey et al., 2010).

Among bacteria, species belonging to the genus *Bacillus* are among the most important species in biological control. Among these species, especially *Bacillus thuringiensis*, *B. brevis*, *B. cereus*, *B. circulans*, *Bacillus megaterium* and *B. subtilis* are known to be used for biotechnological and industrial applications (Xu & Côté, 2003; Rooney et al., 2009). Furthermore, *B. thuringiensis* has also been reported to be used against *P. operculella* in various parts of the world to reduce pesticide use and create a rich natural enemy complex in the fields (Alvarez et al., 2005; Chandel et al., 2020).

In a study investigating the means to biologically control the larvae of PTM in the tubers, 960 g of sand was mixed with 40 g of *B. thuringiensis* subsp. *kurstakii* and 96% effective control were achieved (Kroschel & Koch, 1996). Imam and Ghiet (2019) evaluated insecticide properties against PTM larvae of *B. thuringiensis* isolates and *Artemisia judaica* extract and reported that the death rates of PTM larvae caused by 1.25 and 10 CFU/ml of *B. thuringiensis* were in the range of 14-58%, respectively and those caused by 125 and 1000 ppm of *Artemisia judaica* were in the range of 34-76%, respectively. Gökürk et al. (2018)'s study, on the other hand, investigated the insecticidal effect against nymphs and adults of *Ricania simulans* of 10 bacteria and 1 fungus isolates, including *B. brevis* CP 1, FD 1, *B. thuringiensis* FDP 1, *Bacillus thuringiensis* subsp. *kenyae* FDP 8, FDP 42, *Bacillus thuringiensis* subsp. *kurstakii* FDP 41, BAB 410, *Bacillus subtilis* EK 7, *Pseudomonas chlororaphis* NEM 28 and *B. sphaericus* FD 49 and *B. bassiana* ET 10, reported that *B. thuringiensis* subsp. *kenyae*, *B. brevis*, and *B. sphaericus* were effective against nymphs, while *B. thuringiensis* subsp. *kurstakii*, *P. chlororaphi*, and *B. brevis* were effective against adults. In addition, they noted that the mortality rate varied in the range of 19.58 to 42.08% in nymph applications and 6-18% in adult applications. Tozlu et al. (2019) investigated the insecticidal effect against nymphs of *Halyomorpha halys* of *Brevibacillus*, *Bacillus*, *Pantoea*, *Vibrio*, *Pseudomonas*, and *Beauveria* under controlled conditions and determined that bacterial isolates were 75-100% effective and *B. bassiana* fungus isolate was 76.19% effective. They also reported that bacterial isolates of *B. cereus* and *Pantoea agglomerans* had a success potential of 100%. Tozlu et al. (2020a) determined that

bacterial isolates of *Bacillus pumilus* TV 67C, *B. brevis* CP 1, and *B. megaterium* TV 91C against *Pseudaulacaspis pentagona* caused death in the range of 41.68 to 89.04%. Similarly, in the current study, the isolates of *B. brevis* FD 1 (100%-96.67%), *B. atrophaeus* FD 17 (100%-100%), *B. sphaericus* FD 49 (100%-98.33%), *B. cereus* FD 63 (100%-100%) - FDP 8 (96.67%-98.33%) and *V. hollisae* FD 70 (100%-100%) species were effective against PTM at varying rates.

Fungi as well as bacteria are used in biological control against pests (Lacey & Neven, 2006). Entomopathogenic fungi that colonize plants as endophytes have lethal and non-lethal pathological effects on insect pests (Mutune et al., 2016; Zhang et al., 2021). *B. bassiana*, which is one of the most widely and extensively studied entomopathogenic fungi species, is the active ingredient of many products currently in use and under development worldwide (Tangtrakulwanich et al., 2014). The use of *B. bassiana* in Integrated Pest Management (IPM) programs is of great importance due to its environmentally friendly nature, bio-persistence, and ability to kill pests at various developmental stages of their life cycle (Kumar & Sultana, 2015). The spores of *B. bassiana* adhere to the cuticle of the insect, germinate, and the formed hyphae multiply by penetrating the body of the insect. Attacked insects die after about 3-5 days, and infected cadavers serve as a spore source for the secondary spread of the fungus. Previous studies reported various death rates in a variety of pest groups to which *B. bassiana* was applied (Sahab & Sabbour, 2011; Abdel-Raheem et al., 2015).

Sabbour and Raheem (2015) tried to determine the efficacy of *Beauveria brongniartii* and *Nomuraea riley* against PTM and reported that the number of eggs per female PTM decreased significantly compared to the control group under laboratory conditions, while the number of adults decreased in their applications in field plots, thus the yield of potatoes increased. Similarly, Zhang et al. (2021) determined that the survival of the larvae of *P. operculella* fed on potato plants inoculated with *B. bassiana* and the number of eggs laid by the females were quite low, and they noted that *B. bassiana* could be a potential biological control agent in the control of this species.

Zelege et al. (2015) showed that *B. bassiana* significantly reduced PTM larvae infestation of both leaves and tubers, compared to *M. anisopliae* and *V. lecanii* and concluded that the number of larvae and damage to leaves significantly decreased with increasing fungus concentration levels. In addition, many researchers demonstrated that *B. bassiana* and *M. anisopliae* isolated from *P. operculella* larvae caused death of larvae (Yuan et al., 2017; Yuan et al., 2018; Gao, 2018). Hui-guo et al. (2018) reported a death rate of  $90.3\% \pm 2.1$  for PTM larvae treated with *B. bassiana* ( $1 \times 10^7$  conidia mL<sup>-1</sup>) and further argued that *B. bassiana* not only has a high pathogenicity against PTM larvae but also causes non-lethal effects such as shortening the development of a generation,

reducing the fertility of the female offsprings and affecting the population parameters. Khorrani et al. (2018) tested the efficacy of *M. anisopliae* IRAN 2252, *Nomura earileyi* IRAN 1020C and *Paecilomyces tenuipes* IRAN 1026C isolates against PTM larvae and eggs, and suggested that  $1 \times 10^3$  conidia/ml of *N. rileyi* gave the most effective results and that individual fungus applications reduced adult emergence and first generation progeny of PTM. Based on their data, they stated that these entomopathogenic fungi have lethal effects on eggs and larvae of *P. operculella* and recommended using these fungi in integrated pest management (IPM) programs.

Tozlu et al. (2017) isolated *B. bassiana* ET 10 isolate, the effect against PTM of which was investigated in the current study, from *Sphenoptera antiqua* larvae in a previous study and tried to determine its effect on many pests. In one of these studies, they applied  $10^6$ ,  $10^7$ , and  $10^8$  spore suspensions of ET 10 against *S. parreyssii* and reported death rates as 82.72%, 83.95%, and 90.12%, respectively (Tozlu et al., 2017). Tozlu et al. (2019) reported a death rate of 76.19% for *Halyomorpha halys* nymphs treated with *B. bassiana* at 264 hours. In another study, Tozlu et al. (2020b) noted a death rate of % 100 for *Icerya purchasi* nymphs treated with *B. bassiana* and 80% for the adults. Similarly, at the end of the current study, it was established that *B. bassiana* ET 10 fungal isolates showed various levels of efficacy (98.33%-100%) against PTM.

## 5. Conclusion

In the study, it was determined that environmentally friendly control agent entomopathogens can be used successfully as an alternative to chemicals in the control of *P. operculella*. Considering that the efficacy of the microorganisms used in these controls may change under field conditions, the efficacy of entomopathogens should also be tested under field conditions.

## Conflict of Interest

The authors declare that they have no conflict of interest.

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