



Potential Use of *Beauveria bassiana* ET 10 in Biological Control of *Sitophilus zeamais* (Mots.) (Coleoptera: Curculionidae)

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Abstract: The use of entomopathogenic fungi as biological control agents against storage pests has become an effective method to reduce postharvest losses. This study aimed to determine the insecticidal effect of *Beauveria bassiana*, an important entomopathogenic fungus, on *Sitophilus zeamais* adults, known as the most important storage pest in maize. *B. bassiana* ET 10 isolate prepared in three different spore suspensions (10^6 , 10^7 and 10^8 conidia/ml + Tween 80 (0.02%)) was applied to *S. zeamais* adults by the spraying method. The experiment was set up in three replicates according to the randomized plot design. For each suspension, one experimental group was set with corn grains and 10 adult *S. zeamais* adults, the number of dead insects was recorded every 24 hours and the data was recorded until the conclusion 264 hours. As a result of the application, mortality were observed in all suspensions from the first 24 hours, reaching 100% in the 10^8 conidia/ml suspension at the 144th hour. At the end of 264 hours, the mortality rates were recorded as 26.7% for 10^6 conidia/ml, 36.7% for 10^7 conidia/ml, 100% for 10^8 conidia/ml. The most effective dose among the application suspensions was determined to be 10^8 conidia/ml. It has been proven that *B. bassiana* ET 10 isolate can be used as a potential bioagent as an alternative in the biological control of *S. zeamais* and that biological control with entomopathogenic fungi can be a correct and positive strategy.

Keywords: *Beauveria bassiana*, biological control, maize weevil, mortality rate, *Zea mays* L.

Sitophilus zeamais (Mots.) (Coleoptera: Curculionidae)'in Biyolojik Mücadelesinde *Beauveria bassiana* ET 10'nun Kullanılma Potansiyeli

Öz: Entomopatojen fungusların depo zararlılarına karşı biyolojik mücadele ajanı olarak kullanımı, hasat sonrası kayıpları azaltmak için etkili bir yöntem haline gelmiştir. Bu çalışmada mısırdan önemli depo zararlısı olarak bilinen *Sitophilus zeamais* (Mots.) (Coleoptera: Curculionidae)'in erginleri üzerinde önemli bir entomopatojen fungus olan *Beauveria bassiana*'nın insektisidal etkisinin belirlenmesi amaçlanmıştır. Üç farklı spor süspansiyonunda (10^6 , 10^7 ve 10^8 konidi/ml+ Tween 80 (%0.02)) hazırlanan *B. bassiana* ET 10 izolatu spreyleme yöntemi ile *S. zeamais* erginlerine uygulanmıştır. Deneme tesadüf parselleri desenine göre üç tekerrürlü olarak yürütülmüştür. Her süspansiyon için mısır taneleri ile 10 adet ergin *S. zeamais* bireyi içeren deney grubu hazırlanmış, her 24 saatte bir ölü böcek sayıları kaydedilmiş ve 264 saatin sonuna kadar veriler kaydedilmiştir. Uygulama sonucu ilk 24 saatten itibaren tüm süspansiyonlarda ölümler gözlenmiş, 144. saatte 10^8 konidi/ml süspansiyonda %100'e ulaşmıştır. 264 saat sonunda ölüm oranları 10^6 konidi/ml için %26.7, 10^7 konidi/ml için %36.7, 10^8 konidi/ml için %100 olarak kaydedilmiştir. Uygulama süspansiyonları arasında en etkili dozun 10^8 konidi/ml olduğu belirlenmiştir. *B. bassiana* ET 10 izolatinın *S. zeamais*'in biyolojik mücadelesinde alternatif olarak potansiyel bir biooajan olarak kullanılabileceği ve entomopatojen funguslarla biyolojik mücadelenin doğru ve olumlu bir strateji olabileceği kanıtlanmıştır.

Anahtar kelimeler: *Beauveria bassiana*, biyolojik mücadele, mısır biti, ölüm oranı, *Zea mays* L.

INTRODUCTION

Corn (*Zea mays* L.), is one of the important agricultural products in the family Gramineae (Poaceae) that can be cultivated almost everywhere in the world except Antarctica (Babaoğlu, 2019). The production of corn, which is the most cultivated product after wheat and rice, has been

~1.2 billion tons (annual grain) globally and ~8.5 million tons domestically (FAO, 2024).

Corn, which has a very important place in human nutrition, is used as a raw material in many different sectors other than agriculture (Şahin, 2001). The nutritional content of corn is 72% starch, 10% protein and 4% fat, providing 365 kcal of energy per 100 grams. Corn is used as the main

component of various products such as flour, sweeteners, oil, beverages, adhesives, industrial alcohol and fuel ethanol (Orhun, 2013; Ranum et al., 2014).

From the production of the corn plant to the public offering process, significant economic losses occur due to many diseases and pests. Approximately 15% of annual post-harvest yield losses are losses that occur during the storage process (Gwinner et al., 1996; Scheepens et al., 2011; Chen et al., 2018). Losses that occur during the storage process are caused by microorganisms, insects, mites and rodents (Casas, 1987). Insects are the most important of these pests (Ertugay & Certel 1991; Rajendran, 2002; Keskin & Özkaya, 2013; Chen et al., 2018). Species belonging to the orders Coleoptera, Lepidoptera and Psocoptera cause significant damage to stored products (Rees, 2004). Approximately 130 species of insects that cause postharvest economic losses have been recorded to the present day, and the majority of these are species belonging to the Coleoptera and Lepidoptera orders to the present day (Khare, 1994; Hagstrum & Subramanyam, 2009; Morales-Quiros et al., 2019). Among these species, the maize weevil *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) stands out as the species that causes the greatest damage to corn grains during storage in the world (Danho et al., 2002; Costa et al., 2006; Carneiro, 2019; Sebayang et al., 2023). In addition to corn, this pest also attacks many other stored agricultural products such as sorghum, rice, wheat, pasta and processed food products such as biscuits (Sebayang et al., 2023). The maize weevil, *S. zeamais*, is so harmful that it is reported to cause damage ranging from 20-90% in corn if precautions are not taken (Denning et al., 2009; Issa et al., 2011; Noosidum & Sangprajan, 2014). *S. zeamais* consumes the endosperm and/or germ of the corn grain during the larval stage and eats the grain from the outside during the adult stage, causing weight loss, decreased in nutritional value, and deterioration of quality (Keskin & Özkaya, 2013). Some studies report that this pest can cause 18-20% loss in corn, and in high water content conditions, the damage rate can reach up to 30-40%. This pest attack reduces product and nutritional quality, seed weight and germination percentage, which reduces the market value of the product (Sebayang et al., 2023).

Proper storage conditions and use of chemical pesticides have been shown to be control methods that can reduce postharvest insect reproduction in stored corn (Arthur & Subramanyam, 2012; Karim et al., 2017; Sikirou et al., 2018). Due to residues from chemical pesticide applications, the development of resistance of pests to pesticides, and harmful effects on non-target organisms, human health and the environment, many researchers have intensified the search for alternative environmentally friendly strategies (Udo, 2005; Salem et al., 2007; Mahdi & Rahman, 2008; Phillips & Throne, 2010; Wakil et al., 2021). Among

alternative control methods, the use of entomopathogenic fungi in the control of stored product pests is a promising strategy that has the potential to eliminate the negative effects of pesticides, provides long-term protection, has a high ability to adapt to different environmental conditions, is reliable for human and environmental health, has a high reproductive capacity, and has a low risk of developing resistance (Nboyine et al., 2015; Sinha et al., 2016; Abd-Elgawad, 2019; Baker et al., 2020; Azhar et al., 2023; Khan & Khan, 2023). Entomopathogenic fungi penetrate the cuticle layer of the pest insect with their mycelium through both physical and enzymatic mechanisms, reach the internal organs and kill the pest (Altinok et al., 2019). The potential of entomopathogenic fungi to be used in different environments and against different pests has been demonstrated by many studies (Athanassiou et al., 2008; Kavallieratos et al., 2014; Abdel-Raheem et al., 2015; Mbata et al., 2018; Ak, 2019; Tozlu et al., 2019; Uçar et al., 2020; Wakil et al., 2021; Khan & Khan, 2023; Tekiner et al., 2023; Yaman & Güvendik, 2024). *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae) is one of the most widely used and commercially produced entomopathogenic fungi with proven activity against a wide range of pests (Lord, 2001; Meyling et al., 2018; Karabörklü, 2022a; 2022b).

The aim of this study to determine the insecticidal potential of different spore concentrations of *B. bassiana* ET 10 against adult *S. zeamais* in vitro conditions.

MATERIAL AND METHOD

Maize weevil rearing: Insects obtained from the stock culture of *S. zeamais* (Atatürk University Faculty of Agriculture, Department of Plant Protection, Erzurum, Türkiye) were reared on cracked corn in the laboratory at 27±2°C and 65-75% humidity under 16:8 hours (h) light/dark photoperiod.

Production of conidia of *Beauveria bassiana* ET 10 isolate: The entomopathogenic fungus *B. bassiana* ET 10 isolate used in this study was isolated from *Sphenoptera antiqua* (Coleoptera, Buprestidae) adults that damage *Onobrychis sativa* L. (Fabacea), plant, was used as an entomopathogenic fungus (Tozlu et al., 2017). *B. bassiana* ET 10 isolate was obtained from the fungus culture collection of the Mycology Laboratory in the Plant Protection Department of Atatürk University Faculty of Agriculture. *B. bassiana* ET 10 isolate, which was preserved in Sabouraud Dextrose Agar (SDA, BD Difco), was transferred to petri (90 mm) dish containing Potato Dextrose Agar (PDA, BD Difco) and grown in an incubator for 2 weeks at 25±1°C for conidia production. Fungus spores were then collected in 10 ml of sterile water containing Tween 80 (0.02%). Three different spore doses (10⁶, 10⁷ and 10⁸

conidia/ml) were prepared using a hemocytometer (0.100 mm×0.0025 mm²) under light microscope (Quesada-Moraga & Alain, 2004).

Insecticidal activity assay: For each application, 10 adult maize weevil *S. zeamais* were placed on corn seeds in a petri dish (90 mm) containing sterile filter paper (Whatman No. 1). Then, 10⁶, 10⁷ and 10⁸ conidia/ml suspensions of the *B. bassiana* ET 10 isolate were sprayed onto 10 *S. zeamais* adults. As a control, sterile water containing 0.2 ml/l Tween-80 was applied. The treated petri dishes were covered with parafilm and incubated at 27±2°C with 65-75% humidity and 16:8 hour light/dark photoperiod. The laboratory experiment was designed in a randomized design with three replications. The counts were continued for 264 h at 24 h intervals from the application and during which the number of dead adult insects was recorded.

Percentage mortality rate (%) = 100 X Number of dead adults in the application / Total number of adults, in the

application, calculated according to the formula (Abbott, 1925). According to Koch's Postulates, re-isolation was performed on infected insect adults with spores of the entomopathogenic fungus and the results were recorded.

Statistical analysis: The data obtained from the experiment conducted under controlled conditions in the laboratory were statistically analyzed using the JMP 5.0.1 program and the means were separated with LS Means Student's tests (P≤0.01). Differences between the applications were recorded after the analysis.

RESULTS AND DISCUSSION

In the study conducted to determine the insecticidal effect of *B. bassiana* on *S. zeamais* adults, data were recorded by counting dead and live insects between 24 and 264 hours (Table 1).

Table 1. Insecticidal effects of three different spore doses of *Beauveria bassiana* ET10 against *Sitophilus zeamais* adults under laboratory conditions.

Percentage Mortality Rate (%)												
Spore doses/hour (h)	24	48	72	96	120	144	168	192	216	240	264	Average
10 ⁶ Conidia/ml	6.7	6.7	10	13.3	13.3	13.3	13.3	13.3	23.3	26.7	26.7	15.15±6.87 B
10 ⁷ Conidia/ml	6.7	6.7	6.7	10	10	13.3	13.3	13.3	26.7	36.7	36.7	16.37±10.9 B
10 ⁸ Conidia/ml	13.3	60	80	83.3	83.3	100	100	100	100	100	100	82.12±25.4 A
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.3	3.3	3.3	0.9±1.47 C
CV: 0.49												
LSD: 11.37												

According to the obtained data, the mortality rate of the treated insects was found to be significantly higher than that of the control group. It was determined that all different spore suspensions of *B. bassiana* ET 10 isolate had an insecticidal effect on *S. zeamais*. The insecticidal activities of the three different conidia suspensions tested were determined to be statistically different from the control at 11 different times and according to the results of the statistical variance analysis. The difference between the treatments was found to be statistically significant (P≤0.01). The differences between the groups were also determined using the LSMeans Differences Student's test (Percent mortality rate of *S. zeamais* F treatment: 48.8913; CV: 0.49; LSD treatment: 11.37; P≤0.01).

When the insecticidal effect of *B. bassiana* ET 10 isolate on *S. zeamais* is examined in terms of mortality rate, it was recorded that the effect started within the first 24 hours. At 144 h (6th day) *B. bassiana* ET 10 isolate's mortality rate in 10⁸ conidia/ml suspension was 100%. The lowest mortality rate was observed in the control group.

It was observed that the mortality rate of *S. zeamais* treated with *B. bassiana* ET 10 isolate increased with increasing concentrations. When three different spore suspensions were evaluated, it was determined that the most effective dose was 10⁸ conidia/ml (82.12%) and the least effective dose was 10⁶ conidia/ml (15.15%). It was also revealed that the insecticidal effects of different spore

doses of the *B. bassiana* ET 10 isolate varied compared to the control (Table 1).

Entomopathogenic fungi are reliable alternative control agents that do not threaten the ecosystem, environment and human health in the control of stored product pests (Aminae et al., 2010; Tozlu et al., 2017; Karabörklü, 2022b). *B. bassiana* is one of the most widespread and extensively studied species among entomopathogenic fungi and is the active agent of biopesticides that are in use and under development worldwide (Zimmermann, 2007; Mascarín & Jaronski, 2016; Javed et al., 2019; Tozlu et al., 2019; Tekiner et al., 2023; Erol & Erdoğan 2024).

Many researchers have applied *B. bassiana* against various storage pests and observed different mortality rates (Meikle et al., 2001; Kassa et al., 2002; da Paz Junior et al., 2012; Athanassiou et al., 2017; Batta, 2018; Uçar et al., 2020; Acheampong et al., 2023). Different researchers have reported that many factors (host specificity, physiological condition of the host, entomopathogenic fungus species, virulence of the entomopathogenic fungus species, application method and environmental factors) affect different levels of effectiveness (Wakefield, 2006; Padmini & Padmaja, 2010; Abdel-Raheem et al., 2015). Kaoud (2010) reported that after the death of insects treated with entomopathogenic fungi, the fungus can grow on the insect

and produce more spores, which can increase the mortality rate of other mobile insects in the same environment.

Adane et al. (1996) recorded the insecticidal effect of *B. bassiana* against *S. zeamais* with a mortality rate of approximately 88% on the eighth day. In this study, a mortality rate of 100% was recorded by the sixth day at a concentration of 10^8 conidia per ml of *B. bassiana* ET 10 isolate. Similar to our results, other researchers also confirmed that *B. bassiana* was effective against *S. zeamais* and reported that periodic counts were made for 24 h or longer (Meikle et al., 2001; Barra et al., 2013; Mbata et al., 2018). In Teshome and Tefera (2009), a single dose (10^8 conidia/ml) was used under laboratory conditions to determine the effectiveness of 11 local *M. anisopliae* and 6 *B. bassiana* isolates against *S. zeamais* and the most virulent isolates. They noted that the most virulent isolates (3 *M. anisopliae*, 2 *B. bassiana*) achieved a mortality rate between 84.4% to 98.3%.

Karabörklü (2022b) applied 10^6 , 10^7 , 10^8 conidia/ml doses to adults of *B. bassiana* YK23 and YK26 and *M. anisopliae* YK41 and YK45, *S. zeamais* and *Acanthoscelides obtectus* by spraying and counting the dead insects for 10 days. *B. bassiana* and *M. anisopliae* caused 100% mortality against *A. obtectus* at the lowest dose (10^5 conidia/ml), while it was found that it caused 100% mortality in *S. zeamais* at higher doses (10^6 , 10^7 conidia/ml). The effectiveness of *B. bassiana* has also been proven useful in the biological control of *S. oryzae*, a rice storage pest belonging to the same genus as the corn pest (Kavallieratos et al., 2014; Er et al., 2018; Atmaca et al., 2022). Rehman et al., (2019) reported that the application of 10^8 conidia/ml of *B. bassiana* and *M. anisopliae* by the immersion method showed 100% and 96% mortality rate against *S. oryzae* under controlled conditions. In our study, it was determined that the most effective dose against *S. zeamais* was 10^8 conidia/ml and, this finding was similar to other studies in the literature.

In addition to being effective against storage pests of different *Sitophilus* genera, *B. bassiana* has been reported to effectively control important maize storage pests of different genera (*Prostephanus truncatus* and *Tribolium* spp.) with high mortality rates by different researchers (Meikle et al., 2001, 2002; Kassa et al., 2002; Acheampong et al., 2016; Akmal et al., 2020; Tekiner et al., 2023).

Isolates of the species *B. bassiana* are also used in the control of insects that damage different plants in addition to storage pests. Tozlu et al., (2020) tested fungal and bacterial biological control agents under controlled conditions on nymphs and adults of the pouched cochineal (*Icerya purchasi*), which is harmful to mimosa plants (*Acacia dealbata*). The fungal bioagent *B. bassiana* ET 10 was shown to cause mortality of up to 100% in nymphs and

80% in adults. Similarly, in the biological control of the boxwood moth, which damages boxwood trees, Tozlu et al., (2022) used bacterial (*Bacillus cereus* FD-63, *B. brevis* FD-1 and *Vibrio hollisae* FD-70) and fungal (*B. bassiana* ET 10) biological control agents, recording 100% mortality after 8 and 9 days in 10^8 conidia/ml suspension under laboratory conditions.

As a result, it has been demonstrated that entomopathogenic fungi have high potential if they are offered for commercial use in environmentally friendly pest management. In this way, dependency on pesticides can be reduced. In recent years, there has been a need to identify novel entomopathogenic fungi or to investigate more effective isolates in the biological control of stored product pests. According to the data obtained in this study, testing the storage applications of ET 10 isolate against *S. zeamais* in future studies by considering different temperature and humidity conditions in future studies (the effectiveness of entomopathogenic fungi may vary depending on the conditions) may be an important alternative method to control this pest. In addition, it has been concluded that ET 10 isolate is effective against many different pests and has a promising potential in this respect. It can be used as an alternative mycoinsecticide to chemical insecticides.

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