# Virulence of Entomopathogenic Fungi and Bacteria Against Stored Product Pests

Ali Sevim[1]*, Elif Sevim[2], Meryem Demirci[3]

[1]Ahi Evran University, Faculty of Engineering and Architecture, Genetic and Bioengineering, Kırşehir-40100, Turkey, Tel: +90 386 2803809, ali.sevim@ahievran.edu.tr
[2]Ahi Evran University, Faculty of Engineering and Architecture, Genetic and Bioengineering, Kırşehir-40100, Turkey, Tel: +90 386 2803809, esevim@ahievran.edu.tr
[3]Recep Tayyip Erdoğan University, Faculty of Arts and Sciences, Department of Biology, 53100-Rize, Turkey, meryemdmrc88@hotmail.com
*Corresponding author / İletişimden sorumlu yazar

Recieved: 29th July, 2015
Accepted: 11th December 2015
DOI: http://dx.doi.org/10.18466/cbujos.71031

**Abstract**

Entomopathogenic microorganisms such as bacteria, fungi, viruses, nematodes and protozoa play an important role for regulation of insect pest populations and, this leads to use these microorganisms as biological control agents against pest species as an alternative to chemicals insecticides. In this study, we tested different bacteria originated from stored product pests and fungi isolated from different sources against two important stored product pests, *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae) and *Callosobruchus maculatus* (F) (Coleoptera: Bruchidae), under laboratory conditions. Based on the laboratory screening, the highest mortality against *A. obtectus* within bacteria and fungi was obtained from *Staphylococcus kloosii* Fbe-10 with 73% and *Lecanicillium muscarium* ARSEF3600, *Beauveria pseudobassiana* ARSEF8664 and, *Beauveria bassiana* ARSEF8668 with 100%, respectively. Also, the highest mycosis within fungi was obtained from *Lecanicillium muscarium* ARSEF3600 and *Beauveria bassiana* ARSEF8668 with 100%. The highest mortality against *C. maculatus* within bacteria and fungi was obtained from *Bacillus pumilus* Be-2with 57% and *Lecanicillium muscarium* ARSEF3600, *Beauveria pseudobassiana* ARSEF8664 and, *Beauveria bassiana* ARSEF8668 with 100%, respectively. Also, the highest mycosis within fungi was obtained from *Beauveria bassiana* ARSEF8668 with 90%. These results showed that the fungal isolates used in this study seem to be more effective than bacteria and, should be further investigated in terms of developing microbial control agent against stored product pests.

**Keywords**— Bacteria, fungi, microbial control, stored product pests

# Depolanmış Ürün Zararlılarına Karşı Entomopatojenik Fungus ve Bakterilerin Virulansları

**Özet**

Bakteri, fungus, virus, nematod ve protozoa gibi entomopatojenik mikroorganizmalar zararlı böcek popülasyonlarının düzenlenmesinde önemli rol oynamaktadırlar ve bu entomopatojen mikroorganizmaların kimyasal insektisidlere alternative olarak zararlı böceklere karşı biyolojik mücadele etmeni olarak kullanımına yol açmaktadır. Bu çalışmada, depolanmış ürün zararlılarından izole edilen farklı bakteriler ve farklı kaynaklardan izole edilen funguslar iki önemli depolanmış ürün zararlısı olan *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae) ve*Callosobruchus maculatus* (F) (Coleoptera: Bruchidae)'a karşı laboratuar koşulları altında test edilmiştir. Laboratuar tarama testlerine göre, *A.*

*obtectus*'a karşı en yüksek ölüm %73 ile bakteriler arasından *Staphylococcus kloosii* Fbe-10'dan ve %100 ile funguslar arasından*Lecanicillium muscarium* ARSEF3600, *Beauveria pseudobassiana* ARSEF8664 ve*Beauveria bassiana* ARSEF8668'den elde edilmiştir. Funguslar arasından en yüksek mikozlanma oranı ise %100 ile *Lecanicillium muscarium* ARSEF3600 ve *Beauveria bassiana* ARSEF8668'den elde edilmiştir. *C. maculatus*'a karşı ise en yüksek ölüm %57 ile bakteriler arasından *Bacillus pumilus* Be-2'den ve %100 ile funguslar arasından *Lecanicillium muscarium* ARSEF3600, *Beauveria pseudobassiana* ARSEF8664 ve*Beauveria bassiana* ARSEF8668'den elde edilmiştir. Funguslar arasında en yüksek mikozlanma oranı ise %90 ile *Beauveria bassiana* ARSEF8668'den elde edilmiştir. Bu sonuçlar bu çalışmada kullanılan fungal izolatların bakterilerden daha etkili olduğunu ve bu bakterilerin depolanmış ürün zararlılarına karşı mikrobiyal mücadele etmeni olarak geliştirilmesi amacıyla daha fazla araştırılması gerektiğini göstermektedir.

**Anahtar kelimler**— Bakteri, fungus, mikrobiyal mücadele, depolanmış ürün zararlıları

## 1 Introduction

Infestation of stored products by insects results in various damages and economic losses in agriculture. These insect pests can cause damage by physical loss of commodity, spoilage and loss of quality, encouragement of mould growth, contamination of commodities with insect bodies and, safety and environmental concerns throughout the world [1]. These insects can be hidden in inaccessible places and, survive on small amount of food particles. Subsequently, they might move from these places into bulk-stored products [2]. The order of Coleoptera comprises approximately 250.000 insect species and, the members of 40 families within this order have been recorded in stores worldwide. Many species known as stored product pests is located in the families of Bostrichidae, Bruchidae, Cucujidae, Curculionidae, Dermestidae, Silvanidae and, Tenebrionidae [3]. Within the family of Bruchidae, two species known as *Acanthoscelides obtectus* and *Callosobruchus maculatus* (F) are major concerns for bean and cowpea, respectively [4-5].

The control of stored product pests is conducted with using many strategies such as physical control, inert dust, ionizing irradiation, light and sound, thermal control, ozonation, fumigation, semiochemicals and some kind of repellents [6]. Also, chemical pesticides such as the group of organophosphorus pesticides have been used against storage pests to protect bulk-stored products [7]. However, these chemicals have undesirable effects to human health and environment. Therefore, biological control of these insect pests is considered an interesting alternative to traditional and chemical control methods.

Entomopathogenic microorganism such as bacteria, fungi, nematodes, viruses and protozoa have been used as microbial control agents against various pests species in both agriculture and forestry [8-9]. The use of these microorganisms in the control of insect pests is favorable because they kill undesirable agricultural and forest pests without harming the environment and humans [8]. The use of microbial insecticides is growing at a rapid rate of 10-25 per cent per year. Among entomopathogens, *Bacillus thuringiensis* (*Bt*), the entomopathogenic spore forming bacterium, is the most widely used microbial pest control agents and, has been the principle target of product development and accounts for most sales in US $75 million global market for biological control products [10-12]. Also, the entomopathogenic fungi include approximately 750 fungal species belonging to 56 genera attack terrestrial and aquatic arthropods. In terms of microbial control, these fungi as a biological control agent against pest species have not made a good impact so far, compared to entomopathogenic bacteria (especially *Bt*) [11]. However, fungal entomopathogens are unique pathogens because they are able to infect their host via the external cuticle. Therefore, there is no need to be ingested to initiate infection with few exceptions such as *Ascosphaera*. This makes them primer candidates for use against plants sucking insects [13-15]. Moreover, there are many fungal species that are in commercial or experimental production stages in USA, Brazil, UK, India and some other countries. The most common species used are *Beauveria, bassiana and Metarhizium anisopliae* [11].

In this study, we aimed to test different bacteria originally isolated from stored product pests (*A. obtectus* and *C. maculatus*) and entomopathogenic fungi from

different sources against stored product pests under laboratory conditions to find possible biological control agent against these pests.

## 2 Material and Methods

### 2.1 Bacteria and Fungi

The bacterial isolates used in this study were previously isolated from *Acanthoscelides obtectus* and *Callosobruchus maculatus* and identified based on traditional and molecular techniques (unpublished data). The fungal isolates were provided from Dr. Richard Humber (The USDA-ARS Collection of Entomopathogenic Fungal Cultures (ARSEF), Ithaca, New York). All bacterial and fungal isolates and their sources are given in Table 1.

**Table 1.** Bacterial and fungal isolates used in this study and their origin.

| | Fungal isolates | Isolate no | Origin |
|---|---|---|---|
| 1 | *Metarhizium anisopliae* | ARSEF8341 | Soil |
| 2 | *Metarhizium anisopliae* | ARSEF8432 | Soil |
| 3 | *Metarhizium anisopliae* | ARSEF8433 | Soil |
| 4 | *Lecanicillium muscarium* | ARSEF3600 | *Lymantria dispar* (Lepidoptera: Lymantriidae) |
| 5 | *Beauveria pseudobassiana* | ARSEF8664 | Soil |
| 6 | *Beauveria pseudobassiana* | ARSEF8666 | Soil |
| 7 | *Beauveria bassiana* | ARSEF8668 | *Rhynchites baccus* (Coleoptera: Curculionidae) |
| | Bacterial isolates | Isolate no | Origin |
| 1 | *Staphylococcus kloosii* | Fbe-1 | *Acanthoscelides obtectus* |
| 2 | *Staphylococcus* sp. | Fbe-2 | *A. obtectus* |
| 3 | *Enterococcus faecalis* | Fbe-3 | *A. obtectus* |
| 4 | *S. kloosii* | Fbe-4 | *A. obtectus* |
| 5 | *S. saprophyticus* | Fbe-5 | *A. obtectus* |
| 6 | *Staphylococcus* sp. | Fbe-6 | *A. obtectus* |
| 7 | *S. kloosii* | Fbe-7 | *A. obtectus* |
| 8 | *E. faecalis* | Fbe-8 | *A. obtectus* |
| 9 | *Staphylococcus* sp. | Fbe-9 | *A. obtectus* |
| 10 | *S. kloosii* | Fbe-10 | *A. obtectus* |
| 11 | *Staphylococcus* sp. | Fbe-11 | *A. obtectus* |
| 12 | *Bacillus pumilus* | Be-1 | *Callosobruchus maculatus* |
| 13 | *B. pumilus* | Be-2 | *C. maculatus* |
| 14 | *B. pumilus* | Be-3 | *C. maculatus* |
| 15 | *Staphylococcus* sp. | Be-4 | *C. maculatus* |
| 16 | *Pantoea* sp. | Be-5 | *C. maculatus* |
| 17 | *Staphylococcus* sp. | Be-6 | *C. maculatus* |
| 18 | *B. pumilus* | Be-7 | *C. maculatus* |
| 19 | *Staphylococcus* sp. | Be-8 | *C. maculatus* |
| 20 | *Pantoea* sp. | Be-9 | *C. maculatus* |
| 21 | *B. pumilus* | Be-10 | *C. maculatus* |
| 22 | *B. pumilus* | Be-11 | *C. maculatus* |

### 2.2 Preparation of bacterial and fungal suspensions for bioassays

For the bacterial isolates, each isolate was initiallystreaked on nutrient agar plates to obtain single colony. After that, each isolate coming from single colony was inoculated into 4 ml of Laura Bertani broth (LB broth) and, incubated at 30°C over night. After incubation, the bacterial cells were centrifuged at 5.000 rpm for 20 min and, the pellets were dissolved in 5 ml of sterile phosphate buffer solution (PBS). Finally, the cell density was measured at OD (optical density)$_{600\ nm}$ absorbance and adjusted to 1.89 ($1.8 \times 10^9$ cfu $ml^{-1}$) [16-18]. Subsequently, these samples were used for the bacterial bioassay.

For fungal isolates, each isolate was propagated from single colony using fungal stock solutions ($1 \times 10^6$ $ml^{-1}$ spor/ml) at -20°C. To do this, each fungal stock solution was platedon PDAY (potato dextrose agar + 1% yeastextract; Merck, Darmstadt, Germany) and incubated at 25 °C for 4 to 5 days under a12-h L / 12-h D photoperiod. At the end of the incubation period, single colony for each isolate was taken and, transfrred to another fresh PDAY plate and incubated at 25 °C for 4 to 5 weeks until plates were fully overgrown and sporulated. After sporulation, the conidial suspensions of the fungal isolates were prepared by scraping conidia from petri-dishes into distiled water with 0.01% Tween-80 (Applichem, Darmstadt, Germany). The conidial suspensions were filtered through two layers of the sterile cheesecloth to remove mycelial and agar particles. The concentration of conidia in the final suspensions was determined using a Neubauer Haemocytometer and, was adjusted to $1 \times 10^7$ $ml^{-1}$conidia/ml using sterile 0.01% Tween-80. The viability of conidia was determined by enumerating the percentage of the germinated conidia 24 h after spreading 100 µL of conidial suspensions ($1 \times 10^6$ $mL^{-1}$) on PDAY medium. Conidia were considered to have germinated if the germ tube was longer than the diameter. Isolates with higher germination rates of 95% were used for bioassay experiments [19].

### 2.3 Experimental infection

For the bioassay experiments, insect samples were grown on bean and pea in glass jars (1.000 ml volume) in the laboratory at 28°C under dark. Among growing

insects, randomly selected adults were used in bioassays. For the bacterial bioassay, the bacterial isolates from *Acanthoscelides obtectus* were tested on it and, the isolates from *Callosobruchus maculatus* were tested on it. Firstly, bean particles for *A. obtectus* and pea particles for *C. maculatus* were contaminated with the bacterial solutions prepared as described above for each isolate. The control groups were treated with sterile PBS. Following this, the contaminated seeds were put into a plastic box (20 mm) with ventilated lids to permit airflow. Finally, randomly selected adults of *A. obtectus* and *C. maculatus* were put into each box of bean and pea, respectively. The bacterial bioassays were conducted during ten days and dead insects were checked at $10^{th}$ day.

For the fungal bioassay, adult individuals of *A. obtectus* and *C. maculatus* were dipped into the 10 ml of spore suspensions ($1 \times 10^7$ $mL^{-1}$) as described previously to contaminate insects with spores [20]. The control group was treated with sterile 0.01% tween 80. After that, they were put on filter paper to remove excessive spore suspension for 2-3 seconds. Finally, they were put into plastic boxes (200 mm) with bean seeds (for *A. obtectus*) and pea (for *C. maculatus*). The fungal bioassays were conducted during two weeks and dead insects were checked at $14^{th}$ day.

Both bacterial and fungal bioassay experiments were performed with 10 adults per replicate and each isolate, and all experiments were repeated 3 times on different occasions. All treated and untreated adults were kept in rearing boxes at 25 °C for 10 days (for bacterial isolates) and for two weeks (for fungal isolates) under dark. At the end of the fungal bioassay, dead insects were counted and cadavers were immediately surface sterilized with 1% sodium hypochlorite for 30 s, followed by 3 rinses with sterile distilled water. They were placed on wet filter paper in sterile plastic petri dishes (15 mm), sealed with Parafilm and incubated at 25 °C to induce sporulation on the cadavers. Finally, virulence results fromthree replicate assays (30 adults / treatment) werecombined and mortality data were corrected using Abbott's formula (Abbott 1925) and the percentage of mycosed cadavers was calculated for the fungal bioassays.

### 2.4 Statistical analysis

Mortality data were corrected according to Abbott's formula [21] and percent mycosis values for the fungal bioassays were calculated based on the mycelia growth outside cadaver. The data were subjected to analysis of variance (ANOVA)followed by LSD multiple comparison tests to compare test isolates with each other and the control group with respect to mortality and mycosis (for the fungal bioassays) ($P < 0.05$). Before performing the ANOVA, all data set were tested for homogeneity of variance using Levene's statistic. Computations for all experiments were performed using SPSS 16.0.

## 3 Results

For the bacterial bioassay, there was a significant difference among the bacterial isolates from *A. obtectus* with respect to mortality against on it ($F$= 3.8, $df$= 11, $p<0.05$). Among the *A. obtectus* isolates, the highest mortality was obtained from *S. kloosii* Fbe-10 with 73% mortality ($F$= 3.8, $df$= 11, $p<0.05$). Other isolates caused mortality values raging from 50 to 20%. The isolates of *Staphylococcus* sp. Fbe-6, *E. faecalis* Fbe-8 and, *Staphylococcus* sp. Fbe-11 caused the same mortality with control ($p>0.05$) (Figure 1). There was also a significant difference among *C. maculatus* isolates with respect to mortality against on it ($F$= 3.557, $df$= 11, $p<0.05$). Among *C. maculatus* isolates, the highest mortality was obtained from *B. pumilus* Be-2 with 57% ($F$= 3.557, $df$= 11, $p<0.05$). The other isolates caused the same mortality with the control ($p<0.05$) (Figure 1)

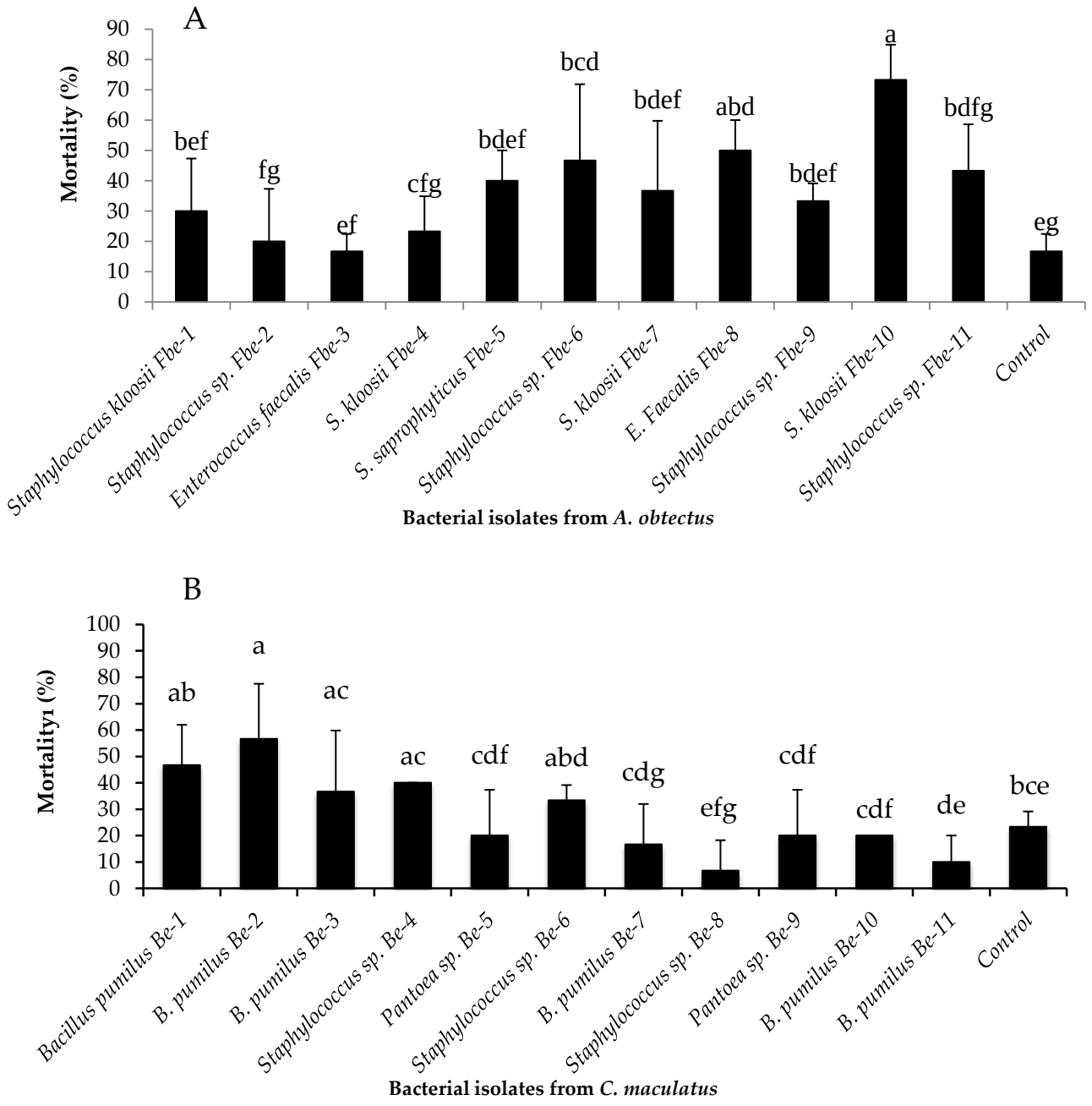


**Figure 1.** Mortality values of the bacterial isolates within ten days after inoculation under laboratory conditions. Mortality data were calculated based on the Abbott's formula [21]. Bars indicate standard deviations. Different letters indicates statistically differences among the isolates. Control: PBS. A; the mortality values of the bacterial isolates from *A. obtectus* against on it. B; the mortality values of the bacterial isolates from *C. maculatus* against on it.

For the fungal bioassay, there was a significant difference among treatments with respect mortality against *A. obtectus* ($F$= 58.56, $df$= 7, $p<0.05$). The isolates of *L. muscarium* ARSEF3600, *B. pseudobassiana* ARSEF8664 and, *B. bassiana* ARSEF8668 caused the highest mortality against *A. obtectus* with 100% ($F$= 58.56, $df$= 7, $p<0.05$). Other fungal isolates caused produced mortality ranging from 93 to 70%. There was also a signifi-

cant difference among treatments with respect mycosis on the outside of *A. obtectus* cadavers ($F$= 36.753, $df$= 7, p<0.05). The highest mycosis value was obtained from *Lecanicillium muscarium* ARSEF3600 and *Beauveria bassiana* ARSEF8668 with 100% ($F$= 36.753, $df$= 7, p<0.05) (Figure 2).

The fungal isolates produced different mortalities on *C. maculatus* ($F$= 13.835, $df$= 7, p<0.05). The highest mortality was obtained from *L. muscarium* ARSEF3600, *B. pseudobassiana* ARSEF8664 and, *B. bassiana* ARSEF8668 with 100% ($F$= 13.835, $df$= 7, p<0.05). Other isolates caused mortalities, ranging from 93 to 83%. Also, the fungal isolates produced different mycosis values on the outside of *C. camulatus* cadavers ($F$= 23.065, $df$= 7, p<0.05). The highest mycosis value was obtained from *B. bassiana* ARSEF8668 with 90% ($F$= 23.065, $df$= 7, p<0.05) (Figure 2).

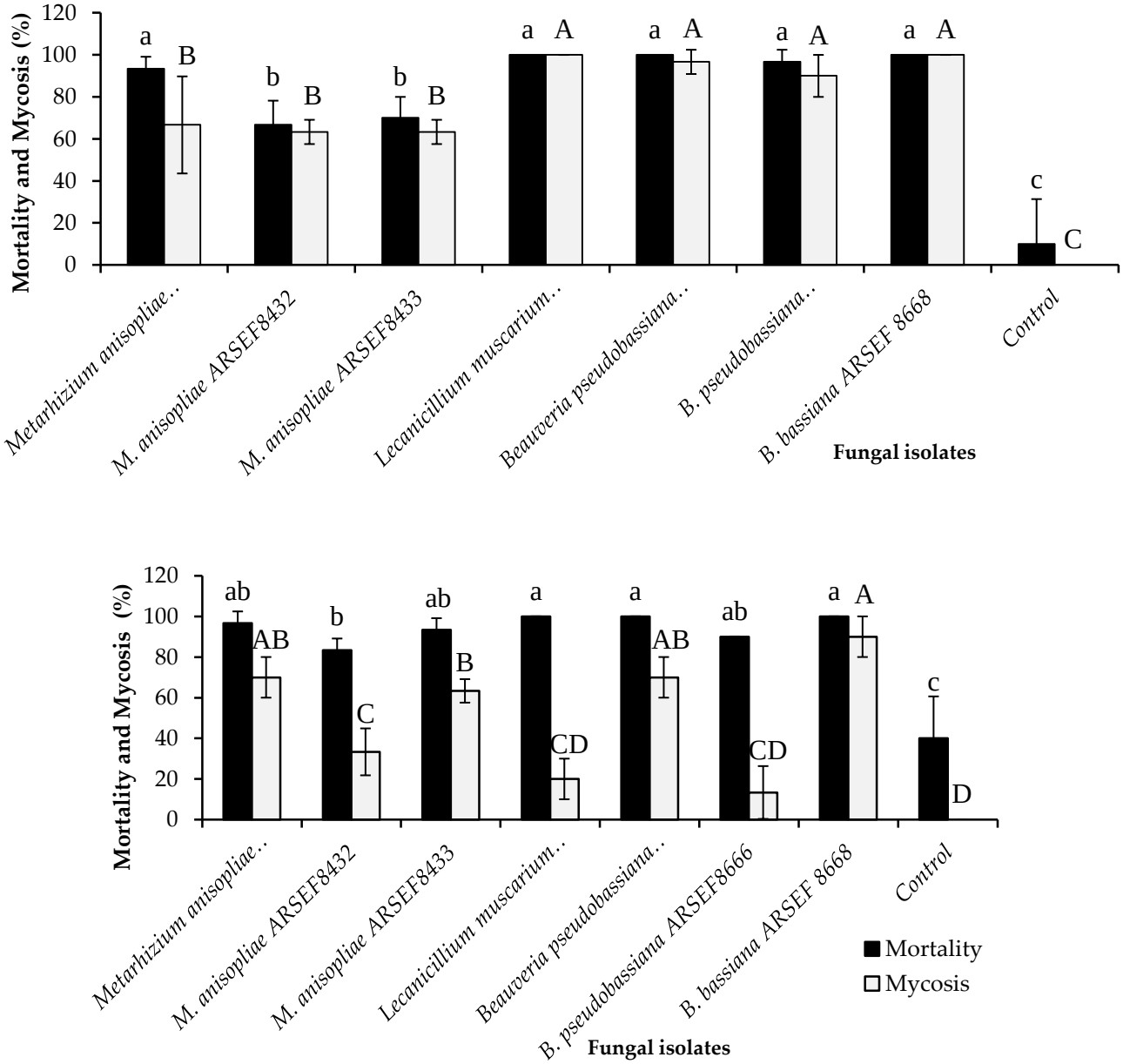


**Figure 2.** Mortality values of the fungal isolates within 15 days after application of 1 × $10^7$ conidia $mL^{-1}$. Mortality data were calculated based on Abbott's formula [21]. Different uppercase and lowercase letters represent statistically significant differences among mortality and mycosis, respectively, between treatments according to LSD multiple comparision test (P < 0.05). Bars show standard deviation. Control, 0.01% tween 80. A; mortalities of *A. obtectus*. B; mortalities of *C. maculatus*.

## 4 Discussion

There is a global interest to find and develop microbial control agents against insect pests in both agriculture and forestry because the main interest in microbial insecticides as opposed to chemical insecticides is their high specificity and thus less damage to non-target organisms. The control of stored product pests in turkey mainly relies on fumigation which is a method of pest control using almost gaseous chemical pesticides. In Turkey, approximately 297 tons of chemical pesticides has been used for controlling of stored product pests based on nonofficial data in 1998 [22]. So, there is a need to find safer and more effective control method in combating with stored product pests. For this reason, we tested different possible entomopathogenic bacteria which were originally isolated from *A. obtectus* and *C. maculatus* and fungi from different sources against the aforementioned stored product pests.

Until now, different species of the genus of *Staphylococcus* have been isolated from different insects belonging to different orders such as Coleoptera, Lepitoptera, Diptera and, Homoptera [23-27]. However, there is no any study that *Staphylococcus* species are insect pathogen. In this study, we found that two *Staphylococcus* species (*Staphylococcus* sp. Fbe-6 and *Staphylococcuskloosii* Fbe-10) showed significant mortality against *A. obtectus* under laboratory conditions. This suggest that some of *Staphylococcus* species might be entomopathogen but further studies such more detailed bioassays are certainly needed to prove this.

*E. faecalis* is a commensal bacterium inhabiting the gastrointestinal tracts of humans and other mammals [28]. There are also some studies that species of the genus of Enterococci can be found in insect digestive tracks [16,29]. Channaiah et al. (2010)isolated 154 enterococcal strains from stored product insects and, they found that *E. faecalis* comprises 7% of all isolates [29]. They also suggested that stored product insects can serve as potential vectors in disseminating antibiotic-resistant and potentially virulent enterococci. Although many Enterococci species are known as common symbionts in the gastrointestinal tracts of domestic animals, with this study, we showed for the first time that *E. faecalis* Fbe-8 has an important mor

tality value (50%) against *A. obtectus*.

The entomopathogenic bacteria belonging to the genus of *Bacillus* are natural agents which are used for biological control of many insect pests worldwide [30]. Within this genus, *B. pumilus* a gram positive, aerobic, spore forming bacillus that can be commonly found in soil [31]. This bacterium has been also isolated from many insect species and, it has been showed that it had pathogenic effects against insects [32-33]. Molina et al. (2010) found that some strains of *B. pumilus* showed important mortality against *Ceratitis capitata* (Wiedemann (Diptera: Tephritidae)). In this study, we also showed that *B. pumilus* Be-2 caused 57% mortality against *C. maculatus* [34].

Entomopathogenic fungi are considered environmentally safe and natural mortality agents of many insect pests such as *Corythucha ciliata* (Say) (Hemiptera: Tingidae) [19]. There is worldwide interest in the use of these fungi for the biological control of insect pests and other arthropod species [35]. There are many commercially available bioinsecticides based on entomopathogenic fungi to control insect pests such as the banana weevils (*Cosmopolites sordidus* (Germar (Coleoptera: Curculionidae)) and the pine caterpillars (*Dendrolimus*spp.) [13]. In this study, we showed that entomopathogenic fungi containing different species caused important mortality values against both A. *obtectus* and *C. maculatus*. Also, we considered and observed important mycosis values from these fungi since sporulation is an important factor for the dissemination of fungi in the field [13, 36].

In conclusion, we tested different bacteria (originally isolated from test insects) and entomopathogenic fungi against two important stored product pests under laboratory conditions. In the event, we observed promising results from both some bacterial species (especially *Staphylococcus* sp. Fbe-6, *Staphylococcuskloosii* and, *B. pumilus*) and entomopathogenic fungi (for all species). These microorganisms should be further investigated for the future biocontrol of *A. obtectus* and *C. maculatus*.

## References

[1] Rees, D. Insects of Stored Products; Collingwood VIC, Csiro Publishing: Australia, 2004; 128 p.
[2] Campbell, J.F.; Arthur, F.H.; Mullen, M.A. Insect Management in Food Processing Facilities. Adv. Food.

Nutr. Res. 2004; 48, 240-295.
[3] Rees, D. Coleoptera. In Integrated Management of Insects in Stored Products; Subramanyam, B., Hagstrum, D.W., Eds., Marcel Dekker: New York, 1996; 1-40.
[4] Özdem, A. Researches on the Biology of the Bean Weevil [Acanthoscelides obtectus (Say) (Col.: Bruchidae)] in Eskişehir. Bull. Crop. Proc.1997; 37(3-4), 111-118.
[5] Kang, J.K., Pittendrigh, B.R., Onstad, D.W. Insect Resistance Management for Stored Product Pests: A Case Study of Cowpea Weevil (Coleoptera: Bruchidae). J. Econ. Entomol. 2013; 106(6), 2473-2490.
[6] El-Aziz, S.E.A. Control Strategies of Stored Product Pests. J. Entomol. 2011; 8(2), 101-122.
[7] Wakefield, M.E. In Factors affecting storage insect susceptibility to the entomopathogenic fungus Beauveria bassiana,Proceedings of the 9th International Working Conference on Stored Product Protection, Campinas, São Paulo, Brazil, Oct 15-18, 2006; Lorini, I. et al., Eds.; Brazilian Post-harvest Association - ABRAPOS, 2006.
[8] Khetan, S.K. Microbial Pest Control. Marcel Dekker:NY, USA, 2001, 320 p.
[9] Lacey, L.A.; Frutos, R.; Kaya, H.K.; Vail, P. Insect Pathogens as Biological Control Agents: Do They Have a Future? Biol. Cont. 2001; 21, 230-248.
[10] Starnes, R.L.; Liu, C.L.; Marrone, P.G. History, Use and Future of MicrobialInsecticides. Am. Entomol. 1993; 39, 83-91.
[11] Koul, O.; Dhaliwal, G.S. 2002. Microbial pesticides: An introduction. In Microbial Pesticides; Koul, O. et al., Eds., Taylor and Francisz: New York, 2002; 1-11.
[12] Lacey, L.A.; Siegel, J.P. Safety and Ecotoxicology of Entomopathogenic Bacteria. In Entomopathogenic Bacteria: from Laboratory to Field Application; Charles, J.F. et al., Eds., Kluwer Academic Publisher: Dordrecht, 2000; 253-273.
[13] Goettel, M.S.; Eilenberg, J.; Glare, T. Entomopathogenic fungi and their role in regulation of insect populations. InComprehensive Molecular Insect Science; Gilbert, L.I. et al., Eds., Elseiver: Amsterdam, 2005; 361-405.
[14] St Leger, R.; Roberts, D.W. Engineering Improved Mycoinsecticides. Trend Biotechnol. 1997; 15, 83-86.
[15] Sevim, A.; Donzelli, B.G.G.; Wu, D.; Demirbag, Z.; Gibson, D.M.; Turgeon, B.G. Hydrophobin Genes of the Entomopathogenic Fungus, Metarhizium brunneum, are Differentially Expressed and Corresponding Mutants are Decreased in Virulence. Curr. Genet. 2012; 58, 79-92.
[16] Sevim, A.; Demirbag, Z.; Demir, I. A New Study on the Bacteria of Agrotis segetum Schiff. (Lepidoptera: Noctuidae) and Their Insecticidal Activities. Turk. J. Agric. For. 2010; 34, 333–342.
[17] Ben-Dov, E.; Boussiba, S.; Zaritsky, A. Mosquito Larvicidal Activity of Escherichia coli with Combinations of Genes from Bacillus thuringiensis subsp. israelensis. J. Bacteriol. 1995; 10, 2581-2587.
[18] Moar, W.J.; Pusztzai-Carey, M.; Mack, T.P. Toxicity of Purified Proteins and the HD-1 Strain from Bacillus thuringiensis Against Lesser Cornstalk Borer (Lepidoptera: Pyralidae). J. Econ. Entomol. 1995; 88 (3), 606-609.
[19] Sevim, A.; Demir, I.; Sönmez, E.; Kocaçevik, S.; Demirbağ, Z. Evaluation of Entomopathogenic Fungi Against the SycamoreLace Bug, Corythucha ciliata (Say) (Hemiptera: Tingidae).Turk. J. Agric. For. 2013; 37 (5), 595-603.
[20] Sevim, A.; Demir, I.; Höfte, M.; Humber, R.A.; Demirbag, Z. Isolation and Characterization of Entomopathogenic Fungi from Hazelnut-Growing Region of Turkey. BioControl. 2010; 55, 279-297.
[21] Abbott, W.S. A Method of Computing the Effectiveness of an Insecticide. J. Econ. Entomol. 1925; 18, 265-267.
[22] Emekci, M.; Ferizli, A.G. Current Status of Stored Product Protection in Turkey. IOBC WPRS Bull. 2000; 23 (10), 39-45.
[23] Danışmazoğlu, M.; Demir, İ.; Sevim, A.; Demirbağ, Z.; Nalçacıoğlu, R. An Investigation on the Bacterial Flora of Agriotes lineatus (Coleoptera: Elateridae) and Pathogenicity of the Flora Members. Crop Prot. 2012; 40, 1-7.
[24] Demirci, M.; Sevim, E.; Demir, İ.; Sevim, A. Culturable Bacterial Microbiota of Plagiodera versicolora (L.)(Coleoptera: Chrysomelidae) and Virulence of the Isolated Strains. Folia Microbiol. 2013; 58, 201-210.
[25]Sevim, E.; Çelebi, Ö.; Sevim, A. Determination of the Bacterial Flora as a Microbial Control Agentof Toxoptera aurantii (Homoptera: Aphididae).Biologia 2012; 67(2), 397-404.
[26] Özkan Çakıcı, F.; Sevim, A.; Demirbağ, Z.; Demir, İ. Investigating Internal Bacteria of Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae) Larvae and Some Bacillus Strains as Biocontrol Agents.Turk. J. Agric. For. 2014; 38, 99-110.

[27] Kuzina, L.V.; Peloquin, J.J.; Vacek, D.C.; Miller, T.A. Isolation andIdentification of Bacteria Associated with Adult Laboratory MexicanFruit Flies, Anastrepha ludens (Diptera: Tephritidae). Curr. Microbiol. 2001; 42, 290-294.
[28] Gilmore, M. The Enterococci: Pathogenesis, Molecular Biology, and Antibiotic Resistance. American Society for Microbiology Press: Washington DC, USA, 2002; 439 p.
[29] Channaiah, L.H.; Subramanyam, B.; McKinney, L.J.; Zurek, L. Stored-Product Insects Carry Antibiotic-Resistant and Potentially Virulent Enterococci. FEMS Microbiol. Ecol. 2010; 74, 464-471.
[30] Hajek, A. Natural enemies: An introduction to biological control. Cambridge University Press: Cambridge, United Kingdom, 2004; 396 p.
[31] Priest, F.G.Systematics and Ecology of Bacillus. In Bacillus subtilis and Other Gram-Positive Bacteria: Biochemistry, Physiology, and Molecular Genetics; Sonenshein, A.L. et al., Eds., ASM Press: Washington DC, 1993; 3–16.
[32] Molina, C.A.; Cana-Roca, J.F.; Osuna, A.; Vilchez, S. Selection of a Bacillus pumilus Strain Highly Active against Ceratitis capitata (Wiedemann) Larvae. Appl. Environ. Microbiol. 2010; 76(5), 1320-1327.
[33] Sreerag, R.S.; Jayaprakas, C.A.; Ragesh, L.; Kumar, S.N. Endosymbiotic Bacteria Associated with the Mealy Bug, Rhizoecus amorphophalli (Hemiptera: Pseudococcidae). Int. Sch. Res. 2014, DOI: 10.1155/2014/268491.
[34] Molina, C.A.; Vilchez, S. Cooperation and Bacterial Pathogenicity: An Approach to Social Evolution. Rev. Chil. Hist. Nat. 2014; 87, 14.
[35] Gul, H.T.; Saeed, S.; Khan, F.Z.A. Entomopathogenic Fungi as Effective Insect Pest Management Tactic: A Review. Appl. Sci. Bus. Econ. 2014; 1(1), 10-18.
[36] Sevim, A.; Demir, İ.; Demirbağ, Z. Molecular Characterization and Virulence of Beauveria spp. from the Pine Processionary Moth, Thaumetopoea pityocampa (Lepidoptera: Thaumetopoeidae). Mycopathologia 2010; 170, 269-277.