**Доклади на Българската академия на науките**
**Comptes rendus de l'Académie bulgare des Sciences**
*Tome 75, No 8, 2022*

*AGRICULTURAL SCIENCES*

*Pest management*

# MOLECULAR IDENTIFICATION OF *Beauveria bassiana* AND *Metarhizium anisopliae* ISOLATES AND THEIR BIO-CONTROL POTENTIAL AGAINST *Acanthoscelides obtectus* AND *Sitophilus zeamais*

**Salih Karabörklü**

*Received on February 4, 2022*
*Presented by A. Atanassov, Member of BAS, on March 29, 2022*

**Abstract**

*Acanthoscelides obtectus* and *Sitophilus zeamais* are among the most damaging stored-product insect pests. This study was carried out to identify *Beauveria bassiana* and *Metarhizium anisopliae* and to evaluate their insecticidal potential against *A. obtectus* and *S. zeamais* adults. Fungal isolates were characterized molecularly based on ITS1, 5.8S and ITS2 regions of rDNA. *B. bassiana* YK23 and YK26, and *M. anisopliae* YK41 and YK45 were sprayed onto adults of *A. obtectus* and *S. zeamais* at $1\times10^5$, $1\times10^6$ and $1\times10^7$ conidia $mL^{-1}$ doses. At the end of the 10th day of application, the numbers of dead adults were counted. Mortality rates (%) and lethal times ($LT_{50}$ and $LT_{90}$) were calculated for each isolate. Fungal isolates displayed promising insecticidal activity against *A. obtectus* and *S. zeamais*. *B. bassiana* and *M. anisopliae* caused 100% mortality against *A. obtectus*, even at the lowest dose. However, *B. bassiana* and *M. anisopliae* caused 100% mortality on *S. zeamais* at higher doses. Depending on the increasing doses, significant decreases were observed at the lethal times. Median lethal time ($LT_{50}$) decreased up to 64.24 h (2.67 days) and 53.69 h (2.24 days) in *A. obtectus* exposed to *B. bassiana* and *M. anisopliae*, respectively. $LT_{50}$ decreased to 61.04 h (2.54 days) and 53.89 h (2.25 days) in *S. zeamais* exposed to the same fungi. *B. bassiana* and *M. anisopliae* can be taken into account in the control of those pests.

**Key words:** bean weevil, entomopathogenic fungi, lethal time, maize weevil, mortality

DOI:10.7546/CRABS.2022.08.18

**Introduction.** Stored-product insect pests such as the bean weevil, *Acanthoscelides obtectus* (Say) (Col.: Chrysomelidae) and maize weevil, *Sitophilus zeamais* (Motschulsky) (Col.: Curculionidae) are major problem in agriculture worldwide [1–4]. They reduce the quantity, nutritional quality, commercial and agronomic value of harvested and stored products. The damage caused by such insect pests may reach up to 40% depending on the condition of region, climate, harvesting and storage [2,5]. Oviposition and growth of both weevils is continuous. The adults reproduce either in the field or in the stored seeds in a continuous cycle and the larvae feed on seeds. Various synthetic insecticides are still the main control agents protecting stored products [5]. Concerns about the insecticide resistance on insect pests, environmental pollution and impact of insecticide residuals on ecosystems and human health have intensified the search for alternative eco-friendly strategies in pest management [5,6].

In this context, entomopathogenic microorganisms have come to the fore in the fight against insect pests as important eco-friendly alternative to chemical insecticides. Among these microorganisms, entomopathogenic fungi (EPFs) have great potential as promising microbial control agents against insect pests, because of their high reproductive abilities, target-specific activities, short production times and ability to produce saprobic phases that allow them to survive longer even in the absence of an available host [7]. *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikof) Sorokin are among the most important entomopathogenic fungi as about 80% of the commercial bio-insecticides produced are based on species from the genera *Beauveria* and *Metarhizium* [8,9].

The present study was carried out to molecularly identify entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* from soil, collected from Duzce province of Turkey and to evaluate their insecticidal potential against the bean weevil, *Acanthoscelides obtectus* and the maize weevil, *Sitophilus zeamais*.

**Materials and methods.** ***Test insects.*** The test insects, *A. obtectus* and *S. zeamais* cultures were maintained in the Department of Plant Protection, Faculty of Agriculture, University of Duzce. Females of both insects laid eggs on *Phaseolus vulgaris* L. and *Zea mays* L. seeds, respectively, and the larvae developed inside the seeds until adult emergence in l L plastic jars. To allow air passage, jars were covered with a sterile tulle cloth. Insect cultures were maintained at $23 \pm 2\,^{\circ}\mathrm{C}$, $60 \pm 5\%$ humidity and 16:8 h photoperiod (light/dark) in a climate room.

***Fungal isolates.*** Isolates of *B. bassiana* and *M. anisopliae* were obtained from soils of Duzce, Turkey. Geographical origin of fungal isolates is given in Table 1. Isolation and morphological characterization of fungal isolates were performed according to our previous study [10].

DNA was extracted from fungal spores (conidia) using commercially available DNA extraction kit (GeneMATRIX plant & Fungi DNA Purification Kit,

Table 1

Geographic origin of fungal isolates

| Isolate | Locality | Habitat | Altitude (m) | Coordinates |
|---|---|---|---|---|
| *B. bassiana* YK23 | Akçakoca/Duzce | Hazelnut orchard | 19 | 41°01′56.71″N 30°59′57.47″E |
| *B. bassiana* YK26 | Kaynaşlı/Duzce | Forest | 797 | 40°45′04.37″N 31°22′19.16″E |
| *M. anisopliae* YK41 | Duzce centrum | Walnut orchard | 211 | 40°54′53.05″N 31°16′12.98″E |
| *M. anisopliae* YK41 | Yığılca/Duzce | Corn fields | 286 | 40°56′43.03″N 31°21′36.05″E |

EURx, Gdańsk, Poland) following the manufacturer's instruction. The universal fungal primers ITS1 (CGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) were employed to amplify the ribosomal ITS1, 5.8S, and ITS2 regions by conventional polymerase chain reaction, PCR [11].

DNA suspension (3 µL) was added to a PCR (in Kyratec thermocycler) mixture containing PCR buffer (1X), $MgCl_2$ (1.5 mM), dNTP mixture (0.2 mM each), primers (each 0.3 mM), Taq polymerase (5 U/µL) and distilled water to a final volume of 35 µL. PCR protocol was set up as follows: 95 °C for 5 min, followed by 40 cycles of 95 °C for 45 s, 57 °C for 45 s and 72 °C for 1 min, and final extension at 72 °C for 5 min. After amplification, PCR product was loaded on a 1.5% agarose gel and electrophoresed. Electrophoresed PCR products were purified using commercially available MAGBIO "HighPrep® PCR Clean-up System" (AC-60005) purification kit.

The purified PCR products were sequenced by commercial company BMLabosis (Ankara, Turkey). Two replicates were sequenced per isolate. Sequences were aligned using NCBI's (National Centre for Biological Information) Basic Local Alignment Search Tool (BLAST) and compared with known sequences on NCBI database. Molecular Evolutionary Genetics Analysis (MEGA) software version 11 program was used to evaluate phylogenetic and molecular evolutionary analyses and phylogenetic trees were constructed using the neighbour-joining (NJ) and bootstrap tree methods (based on 1000 bootstrap replications).

***Spore suspensions.*** Fungal isolates of *B. bassiana* YK23 and YK26, and *M. anisopliae* YK41 and YK45 were used to prepare spore suspensions. All isolates were taken from stock cultures, inoculated onto Potato Dextrose Agar (PDA) medium, and kept in a climate chamber at 24 °C, 60 ± 5% relative humidity and full darkness for 10–15 days. Following the developmental period, the conidia were collected from rearing medium and transferred into distilled water including 0.03% Tween 80. Three different spore doses ($1 \times 10^5$, $1 \times 10^6$ and $1 \times 10^7$ conidia $mL^{-1}$) were prepared using a hemocytometer (0.100 mm × 0.0025 $mm^2$) under light microscope.

***Insecticidal activity assay.*** In order to test the insecticidal activity of *B. bassiana* and *M. anisopliae* on the adults (< 48 h age) of *A. obtectus* and *S. zeamais*, ten adults were put into plastic jars (1 L). Ten bean and maize seeds were added to each jar for feeding the adults of *A. obtectus* and *S. zeamais*, respectively. Spore suspensions of fungal isolates were added to 50 mL spraying bottles and sprayed onto adults. Only distilled water including 0.03% Tween 80 was sprayed onto control group. Three replicates were used per isolate and control. After the spraying process, the plastic jars were covered with thin tulle and transferred to climate room. At the end of the 10th day of application, the numbers of live and dead adults were counted. Mortality rates (%) were corrected using Abbott's formula [12] for the mortalities in the controls for each fungal isolate and test insect.

***Statistical analysis.*** Mortality data was compared with analysis of variance (one-factor ANOVA) and grouped with Tukey–Kramer HSD post-test at a 5% level of significance using SPSS (SPSS 17.0 commercial software, SPSS, Inc., Chicago, IL) program. Probit analysis was carried out to estimate the $LT_{50}$ and $LT_{90}$ values of the fungal isolates at the SPSS.

**Results.** ***Molecular identification.*** Molecular identification of fungal isolates was carried out based on the ITS1, 5.8S and ITS2 regions of rDNA and these regions were amplified via PCR method. Nucleotide fragments of 552 and 562 bp for *B. bassiana* YK23 and YK26, and 551 and 446 bp for *M. anisopliae* YK41 and YK45 were sequenced and clustered together with other entomopathogenic fungi in NCBI database. BLASTN homology search of rDNA regions of *B. bassiana* YK23 and YK26 indicated 99.82 and 100% identity with BbL_1 (MT533246) and SHU.M.161 (KU158472) isolates of *B. bassiana*, respectively (Fig. 1). *M. anisopliae* YK41 and YK45 indicated 99.55 and 99.82% identity with isolate CNYN1 (FJ545304) and strain 687 (HM055426) of *M. anisopliae*, respectively (Fig. 1). Accession numbers of *B. bassiana* YK23 (MZ781314) and YK26 (MZ781311), and *M. anisopliae* YK41 (MZ781310) and YK45 (MZ781315) were given in NCBI gene bank.

***Insecticidal activity.*** In insecticidal activity assays, entomopathogenic fungi, *B. bassiana* and *M. anisopliae* caused high mortality on two important stored-product insect pests, *A. obtectus* and *S. zeamais* ($P < 0.05$) compared to the control. Both fungal isolates were very effective against *A. obtectus* adults (Table 2) with 100% mortality even at the lowest dose ($1 \times 10^5$ conidia $mL^{-1}$).

Compared to *A. obtectus* adults, the *S. zeamais* adults were more resistant to *B. bassiana* and *M. anisopliae* (Table 2). The highest mortality ratios were obtained from *M. anisopliae* YK41 and *B. bassiana* YK23 with 93.33 and 90.00% against *S. zeamais* adults at the lowest dose. However, *B. bassiana* YK26, *M. anisopliae* YK41 and YK45 reached to 100% mortality against *S. zeamais* adults at higher doses ($1 \times 10^6$ and $1 \times 10^7$ conidia $mL^{-1}$).

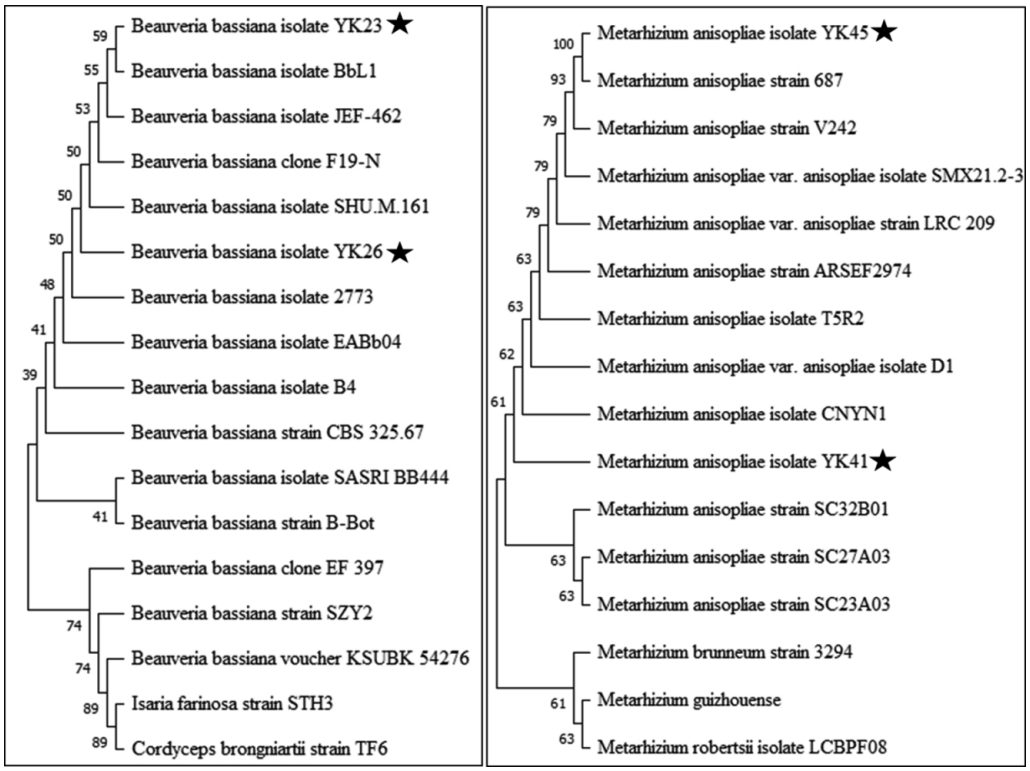


Fig. 1. Phylogenetic relationship of entomopathogenic fungi with YK23 and YK26 isolates (left), and YK41 and YK45 isolates (right). Trees were reconstructed by the neighbour-joining method

It was observed that the lethal time ($LT_{50}$ and $LT_{90}$) values – duration required to kill half and 90% of the insect pest population – decreased significantly depending on the increasing doses of *B. bassiana* and *M. anisopliae* isolates (Table 3). The lowest $LT_{50}$ value, calculated for *A. obtectus*, was 101.91 h for the lowest dose of *M. anisopliae* YK41 and this value decreased to 53.69 h for the highest dose of the same isolate. Similarly the lowest $LT_{90}$ value was 146.76 h at the lowest dose, but this time decreased to 95.44 h at the highest dose in the same isolate. In other isolates, $LT_{90}$ values fluctuated from 127.63 to 95.47 h depending on the increasing dose *A. obtectus*.

Therewithal, in *S. zeamais* adults, the lowest $LT_{50}$ was 148.19 h at the lowest dose of *M. anisopliae* YK41, but this value decreased to 53.89 h at the highest dose. Similarly the lowest $LT_{90}$ value was 242.59 h at the lowest dose decreased to 98.29 h at the highest dose. $LT_{90}$ values fluctuated from 177.73 to 109.22 h for other isolates (Table 3).

**Discussion.** The bean and maize weevils, *A. obtectus* and *S. zeamais* are two important stored-product insect pests, causing significant crop losses. Entomopathogenic fungi can be evaluated as environmentally safe alternatives to

Table 2

Insecticidal activity of *B. bassiana* and *M. anisopliae* isolates on the *A. obtectus* and *S. zeamais* adults

| Treatment | Dose | Mortality (% ± SE) | |
|---|---|---|---|
| | | *A. obtectus* | *S. zeamais* |
| Control | — | 3.33 ± 1.92b* | 0.00 ± 0.00c |
| *Beauveria bassiana* YK23 | $1 \times 10^5$ | 100.00 ± 0.00a | 76.67 ± 8.82b |
| | $1 \times 10^6$ | 100.00 ± 0.00a | 86.67 ± 3.33ab |
| | $1 \times 10^7$ | 100.00 ± 0.00a | 93.33 ± 3.33ab |
| *Beauveria bassiana* YK26 | $1 \times 10^5$ | 100.00 ± 0.00a | 90.00 ± 5.77ab |
| | $1 \times 10^6$ | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | $1 \times 10^7$ | 100.00 ± 0.00a | 100.00 ± 0.00a |
| *Metarhizium anisopliae* YK41 | $1 \times 10^5$ | 100.00 ± 0.00a | 93.33 ± 3.33ab |
| | $1 \times 10^6$ | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | $1 \times 10^7$ | 100.00 ± 0.00a | 100.00 ± 0.00a |
| *Metarhizium anisopliae* YK45 | $1 \times 10^5$ | 100.00 ± 0.00a | 86.67 ± 3.33ab |
| | $1 \times 10^6$ | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | $1 \times 10^7$ | 100.00 ± 0.00a | 100.00 ± 0.00a |

*Different letters indicate statistically significant differences between the means in the same column ($P \leq 0.05$). SE: Standard error

chemical control in the control of stored-product insect pests. In this study the entomopathogenic fungi, *B. bassiana* and *M. anisopliae* were very effective against insect pests *A. obtectus* and *S. zeamais* adults. Both caused 100% mortality against *A. obtectus* adults, even at the lowest dose ($1 \times 10^5$ conidia mL$^{-1}$). It was found that a commercial product of *B. bassiana* (Nostalgist BL®, Agrobest Group) showed good killing activity against *A. obtectus* in dipping method [13]. A significant decrease was observed in the number of the eggs produced by *A. obtectus* and the number of adults that emerged [13].

Rodríguez-González et al. [14] reported that *B. bassiana* demonstrated a good inhibitory activity (over 80.0%) on the egg hatching of *A. obtectus*. *B. bassiana* and *M. anisopliae* displayed significant insecticidal activity against *Callosobruchus maculatus* (96 and 100% mortality at $1 \times 10^7$ conidia mL$^{-1}$, respectively) [15].

Although the *S. zeamais* is relatively resistant according to *A. obtectus* adults, *B. bassiana* and *M. anisopliae* caused complete mortality at higher doses ($1 \times 10^6$ and $1 \times 10^7$ conidia mL$^{-1}$). Similarly, Dal Bello et al. [16] reported that *A. obtectus* were more sensitive to *B. bassiana* than *S. oryzae*. While dry conidia of *B. bassiana* caused lower mortality in *S. oryzae*, 75% mortality occurred in *A. obtectus* without the requirement of high humidity. The killing ability of *B. bassiana* and *M. anisopliae* on *A. obtectus* and *S. zeamais* adults increased with increasing

Table 3

$LT_{50}$ and $LT_{90}$ values of *B. bassiana* and *M. anisopliae* isolates calculated for *A. obtectus* and *S. zeamais* adults

| Treatment | Dose | $LT_{50}$ (h) | $LT_{90}$ (h) |
|---|---|---|---|
| *A. obtectus* | | | |
| *Beauveria bassiana* YK23 | $1 \times 10^5$ | 122.04 | 177.40 |
| | $1 \times 10^6$ | 98.14 | 151.69 |
| | $1 \times 10^7$ | 79.30 | 127.63 |
| *Beauveria bassiana* YK26 | $1 \times 10^5$ | 110.76 | 164.70 |
| | $1 \times 10^6$ | 89.63 | 138.68 |
| | $1 \times 10^7$ | 64.24 | 110.29 |
| *Metarhizium anisopliae* YK41 | $1 \times 10^5$ | 101.91 | 146.76 |
| | $1 \times 10^6$ | 59.41 | 103.43 |
| | $1 \times 10^7$ | 53.69 | 95.44 |
| *Metarhizium anisopliae* YK45 | $1 \times 10^5$ | 116.37 | 169.46 |
| | $1 \times 10^6$ | 62.25 | 106.31 |
| | $1 \times 10^7$ | 55.80 | 95.47 |
| *S. zeamais* | | | |
| *Beauveria bassiana* YK23 | $1 \times 10^5$ | 148.81 | 272.51 |
| | $1 \times 10^6$ | 115.81 | 265.62 |
| | $1 \times 10^7$ | 82.64 | 177.73 |
| *Beauveria bassiana* YK26 | $1 \times 10^5$ | 149.55 | 291.57 |
| | $1 \times 10^6$ | 79.55 | 141.78 |
| | $1 \times 10^7$ | 61.04 | 109.22 |
| *Metarhizium anisopliae* YK41 | $1 \times 10^5$ | 148.19 | 242.59 |
| | $1 \times 10^6$ | 92.70 | 150.68 |
| | $1 \times 10^7$ | 53.89 | 98.29 |
| *Metarhizium anisopliae* YK45 | $1 \times 10^5$ | 162.11 | 284.46 |
| | $1 \times 10^6$ | 101.94 | 164.55 |
| | $1 \times 10^7$ | 61.33 | 114.28 |

LT: Lethal Time, h: hour

doses. Similarly, Adane et al. [17] indicated that mortality was dependent on the conidial dose of *B. bassiana* in *S. zeamais* adults and mortality increased up to 100% with increasing conidial dose. Rehman et al. [18] reported that *B. bassiana* and *M. anisopliae* also displayed 100 and 96% killing activity against *S. oryzae* by immersion method (in $1 \times 10^8$ conidia $mL^{-1}$). Isolates of *B. bassiana* (H20) and *M. anisopliae* (Met-A) also complete killed *S. granarius* adults at the same dose [19]. Batta [20] reported that different formulations of *M. anisopliae* caused significant mortality in adults of *S. oryzae*.

In the current study, depending on the increasing doses, significant decreases were observed at the lethal times ($LT_{50}$ and $LT_{90}$) of isolates. Similar results were reported by Adane et al. [17] and they reported that median lethal time ($LT_{50}$)

decreased drastically based on the increasing dose of *B. bassiana* in *S. zeamais*. A similar trend was observed in different formulations of *B. bassiana* against *A. obtectus* and *S. oryzae* [16].

**Conclusions.** As a result, the local Turkey isolates of *B. bassiana* and *M. anisopliae* displayed promising insecticidal activity against stored-product pests *A. obtectus* and *S. zeamais*. *B. bassiana* and *M. anisopliae* can be evaluated in the fight against these pests. However, additional investigations should be carried out to prevent loss of effectiveness and to improve their performance in warehouse or field conditions.

**Acknowledgements.** The author is grateful to Dr. Nedim Altin and Ahmet Faruk Uygun for their support and assistance in laboratory trials.

## REFERENCES

[1] Ayvaz A., O. Sagdic, S. Karaborklu, I. Ozturk (2010) Insecticidal activity of the essential oils from different plants against three stored-product insects, J. Insect Sci., **10**, 21.

[2] Peschiutta M. L., F. Achimón, V. D. Brito, R. P. Pizzolitto, J. A. Zygadlo et al. (2022) Fumigant toxicity of essential oils against *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae): a systematic review and meta-analysis, J. Pest Sci., **95**(3), 1037–1056.

[3] Sserunjogi M., C. J. Bern, T. J. Brumm, D. E. Maier (2021) Periodic disturbance time interval for suppression of the maize weevils, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) in stored maize (*Zea mays* L.), J. Stored Prod. Res., **94**, 101875.

[4] Lazarević J., A. Radojković, I. Kostić, S. Krnjajić, J. Mitrović et al. (2018) Insecticidal impact of alumina powders against *Acanthoscelides obtectus* (Say), J. Stored Prod. Res., **77**, 45–54.

[5] Shaaya E., M. Kostjukovski, J. E., Eilberg, C. Sukprakarn (1997) Plant oils as fumigants and contact insecticides for the control of stored-product insects, J. Stored Prod. Res., **33**(1), 7–15.

[6] Wakil W., N. G. Kavallieratos, M. Usman, S. Gulzar, H. A. El-Shafie (2021) Detection of phosphine resistance in field populations of four key stored-grain insect pests in Pakistan, Insects, **12**(4), 288.

[7] Sinha K. K., A. K. Choudhary, P. Kumari (2016) Entomopathogenic Fungi. In: Ecofriendly Pest Management for Food Security (ed. Omkar), Amsterdam, Elsevier, 475–505.

[8] Karaborklu S. (2022) Biocontrol potential of *Beauveria bassiana* and *Metarhizium anisopliae* isolates from Turkey against *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae) larvae under laboratory and field conditions, Biosci. J., **38**, e38015.

[9] Meyling N. V., S. Arthur, K. E. Pedersen, S. Dhakal, N. Cedergreen et al. (2018) Implications of sequence and timing of exposure for synergy between the pyrethroid insecticide alpha-cypermethrin and the entomopathogenic fungus *Beauveria bassiana*, Pest Manag. Sci., **74**(11), 2488–2495.

[10] Karaborklu S., N. Altin, Y. Keskin (2019) Native entomopathogenic fungi isolated from Duzce, Turkey and their virulence on the mealworm beetle [*Tenebrio molitor* L. (Coleoptera: Tenebrionidae)], Philipp. Agric. Sci., **102**(1), 82–89.
[11] White T. J., T. Bruns, S. Lee, J. Taylor (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A Guide to Methods and Applications (eds M. A. Innis, D. H. Gelfand, J. J. Sninsky, T. J. White), New York, Academic Press, 315–322.
[12] Abbott W. S. (1925) A method for computing the effectiveness of an insecticide, J. Econ. Entomol., **18**, 265–267.
[13] Sönmez E. (2022) Effect of nostalgist BL® on biology of *Acanthoscelides obtectus*' (Say) (Coleoptera: Bruchidae), Pakistan J. Zool., **54**(4), 1549–1555.
[14] Rodríguez-González Á., S. Mayo, Ó. González-López, B. Reinoso, S. Gutierrez et al. (2017) Inhibitory activity of *Beauveria bassiana* and *Trichoderma* spp. on the insect pests *Xylotrechus arvicola* (Coleoptera: Cerambycidae) and *Acanthoscelides obtectus* (Coleoptera: Chrisomelidae: Bruchinae), Environ. Monit. Assess., **189**(1), 12.
[15] Ozdemir I. O., C. Tuncer, I. Erper, R. Kushiyev (2020) Efficacy of the entomopathogenic fungi; *Beauveria bassiana* and *Metarhizium anisopliae* against the cowpea weevil, *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae: Bruchinae), Egypt. J. Biol. Pest Control, **30**(1), 1–5.
[16] Dal Bello G., S. Padín, P. Juárez, N. Pedrini, M. De Giusto (2006) Biocontrol of *Acanthoscelides obtectus* and *Sitophilus oryzae* with diatomaceous earth and *Beauveria bassiana* on stored grains, Biocontrol Sci. Technol., **16**(2), 215–220.
[17] Adane K., D. Moore, S. A. Archer (1996) Preliminary studies on the use of *Beauveria bassiana* to control *Sitophilus zeamais* (Coleoptera: Curculionidae) in the laboratory, J. Stored Prod. Res., **32**(2), 105–113.
[18] Rehman S. U., N. Ahmed, A. H. Lahori, J. N. Feng, D. Wang (2019) Use of indigenous isolates of *Metarhizium*, *Isaria* and *Beauveria* as potential bio-control agents against *Sitophilus oryzae* under laboratory conditions, Pakistan J. Agricult. Sci., **56**(4), 1037–1044.
[19] Mantzoukas S., I. Lagogiannis, M. Mpekiri, I. Pettas, P. A. Eliopoulos (2019) Insecticidal action of several isolates of entomopathogenic fungi against the granary weevil *Sitophilus granarius*, Agriculture, **9**(10), 222.
[20] Batta Y. A. (2004) Control of rice weevil (*Sitophilus oryzae* L., Coleoptera: Curculionidae) with various formulations of *Metarhizium anisopliae*, Crop Prot., **23**(2), 103–108.

*Department of Plant Protection*
*Faculty of Agriculture*
*Sakarya University of Applied Sciences*
*Sakarya, Turkey*
*e-mail*: salihkaraborklu@subu.edu.tr