Original scientific paper
DOI: /10.5513/JCEA01/23.3.3556
Journal of Central European Agriculture, 2022, 23(3), p.526-532

Characterization of *Lecanicillium psalliotae* and *Akanthomyces muscarium* from Sunn pests (*Eurygaster* spp.)

Esra GÜL[1] (✉), Numan Ertuğrul BABAROĞLU[2], Fikret DEMİRCİ[1]

[1] Department of Plant Protection, Faculty of Agriculture, Ankara University, Ankara, Turkey

[2] Central Research Institute, Ankara, Turkey

✉ Corresponding author: esragul@ankara.edu.tr

Received: March 11, 2022; accepted: July 29, 2022

ABSTRACT

Sunn pests, which can cause significant yield losses on wheat and barley, are one of the pests whose population levels should be followed. Overwintering areas, which have an important place in the biology of the insect, provide an advantage in the development of biological or integrated control with entomopathogenic fungi. Entomopathogenic fungi are one of the most important control agents in reducing the population in overwintering areas. In this study, one *Lecanicillium psalliotae* (KK8) isolate and one *Akanthomyces muscarium* (DIKA11/1) isolate were identified according to morphologic and molecular data. Pathogenicity test was carried out with DIKA11/1 *A. muscarium* isolate. Adult insects were inoculated with a 1×10^7 conidia/ml suspension. With this current study, *A. muscarium* was reported for the first time on sunn pests with 87.7% mortality rate. The *L. psalliotae* species, which is a pathogen on other insects and sunn pests, was isolated from the sunn pests and identified from Turkey. It is thought that DIKA11/1 *A. muscarium* isolate can be used in the biological control of this insect and commercial formulation development studies.

Keywords: Entomopathogenic fungi, *Akanthomyces*, *Lecanicillium*, overwintering, graminae

INTRODUCTION

One of the most economically important pests of wheat and barley is the sunn pests (*Eurygaster* spp.), which is a member of the Pentatomidae family (Davari and Parker, 2018). Nymphs and adults of sunn pests (*Eurygaster* spp.) that can mainly feed on wheat, barley, and wild graminae can cause economic losses up to 100% and reduce the bread quality of the flour (Lodos and Önder, 1983; Hariri et al., 2000; Davari and Parker, 2018). After spending the winter season in the overwintering areas, they migrate to the grain fields and begin to feed.

The new generation development in grain areas has the potential to make epidemics if the natural enemy population of the sunn pests in the region is low and other control measures are not implemented. During this period, especially egg parasitoids are of great importance in reducing the population of the insect under suitable environmental conditions (Iranipour et al., 2010). Another factor that determines the potential of the pest to make epidemic is the density of insects migrating from overwintering areas. The higher the population of insects descending from the overwintering areas, the higher will be in the newly formed population depending on the number of ovulation. For this reason, adult counts carried out in overwintering areas are important to determine the potential of the pest to make epidemics and to plan the control studies.

The use of chemical pesticides in the control of the sunn pests can have a negative effect on the environment and also on the natural enemies of the insect. The most important natural enemies in overwintering areas are entomopathogenic fungi. The biology of the sunn pests increases the potential of using entomopathogenic fungi in their control. The pest can effectively be controlled

by using entomopathogenic fungi or their commercial formulations in the overwintering areas (Parker et al., 2003). In this way, reducing the use of chemical insecticides will eliminate the negative effects of the chemicals on the environment, human health, and natural enemies, and ensure the preservation of natural balance.

Entomopathogenic fungi can provide practical control against this insect that spends the winter collectively under plant remains. Isolates with high virulence can directly be used in these areas, and integrated control studies can be carried out by developing formulations containing a mixture of a fungicide and an entomopathogenic fungus. Thus, the insect can be effectively controlled before migrating from the overwintering areas to the grain areas.

Commercial formulations of *Akanthomyces* and *Lecanicillium* species that can be used in the biological control of different insects have been produced. There are no detailed studies on the identification and pathogenicity of *Akanthomyces* and *Lecanicillium* species on sunn pests. This study was carried out to identify *Akanthomyces* and *Lecanicillium* species isolated from the sunn pests.

MATERIALS AND METHODS

Obtaining of Isolates

Sunn pest (*Eurygaster* spp.) cadavers were obtained from the overwintering areas in the Karaman and Diyarbakır provinces of Turkey. Fungal tissue taken from cadavers was transferred to Potato Dextrose Agar (PDA and incubated for 1 day. Hyphae, taken under a binocular microscope (Leica M165C) from germinated spores, were transferred to PDA medium. Pure isolates were stored at +4 °C in agar slants and cryotubes at -80 °C in 10% glycerol.

Morphological Characterization

25 conidia and phialides from 10-day old fungal cultures were measured under 100X magnification by using with a light microscope (Leica DM1000). Morphological identifications were carried out according to Zare and Gams (2001).

Molecular Characterization

DNA extraction was performed using the DNeasy® plant mini kit (Qiagen Company, Valencia, CA, USA) from 10 day-cultures and then the DNA concentration was measured with NanoDrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). In the molecular characterization of the isolates, ITS4-ITS5 (White et al., 1990) and Bt2a-Bt2b (Glass and Donaldson, 1995) primers were used.

The Polymerase Chain Reaction (PCR) products were run on a 1.2% agarose gel in 1×TAE buffer for 50 min at 100 V and visualized under UV light. The PCR products were purified and bidirectionally sequenced by ATLAS Biotechnologies. Sequences were subjected to BLAST analyses in the NCBI gene bank. Sequences of ITS and Beta tubulin gene regions were submitted to National Center for Biotechnology Information (NCBI) and the accession numbers were taken (Table 1).

Phylogenetic Analysis

In the phylogenetic tree of the *Akanthomyces* and *Lecanicillium* species, only the sequences of the ITS gene regions were used. Sequence data were aligned with ClustalW together with other sequences obtained from the NCBI gene bank. The phylogenetic tree was inferred by using the Neighbor-Joining method based on the Kimura 2-parameter model (K2+G) (Figure 2). *Fulvia fulva* was selected as outgroup.

Pathogenicity Test

In the pathogenicity test, DIKA11/1 *A. muscarium* isolate was used. The adult insects were collected immediately after the wheat harvest in July 2015 in Ankara. Spore suspension containing 0.02% Tween 20 at a density of 1×10^7 conidia/ml was prepared using a hemocytometer from cultures grown in PDA medium for 10-14 days (Parker et al., 2003). The pathogenicity test was carried out as three replications, using 10 insects in each replication. One ml of spore suspension was applied with a hand sprayer on the insects in each plastic box of 9-13 cm dimensions. The water containing 0.02% Tween

20 was sprayed to the control boxes. On incubation days 0, 6, 9, 12, fresh wheat leaves were placed into the boxes to feed the sunn pests. The all boxes were incubated at 25 °C in a climate chamber with 80% ± 5% R.H under 8L: 16D photoperiod conditions. Dead insects in each box were counted on the 6th, 9th, 12th, and 15th days. To observe mycosis on insects, dead insects were kept in 1% NaOCl solution for 2 min, rinsed with sterile distilled water, dried between sterile filter papers, and placed in moist cells (Sevim et al., 2010).

In the evaluation of the results of pathogenicity test, the mortality percentage of the isolate was calculated. The Abbott formula (Abbott, 1925) was used to calculate the efficiency (%) of the isolate. The data were analyzed by one-way analysis of variance and Fisher test ($P<0.01$). Statistical analyses were carried out using Minitab ®17 (Minitab Inc., State College, PA, U.S.A.).

RESULTS

In this study, *Akanthomyces* and *Lecanicillium* species obtained from sunn pests samples in overwintering areas were characterized by morphological and molecular techniques. They are morphologically characterized by their verticillate branching of conidiophores. The falcate macroconidia and smaller ovoid microconidia of *L. psalliotae* were observed. *L. psalliotae* isolate (KK8) produced relatively denser red pigmentation in PDA than Malt extract agar (MEA; Merck, Germany) (Figure 1).

It was determined that the red pigmentation was the result of oosporein production by this fungus (Wainwright and Betts, 1986). *L. psalliotae* is morphologically close to *L. dimorphum*, *L. aphanocladii*, and *L. saksenae* (Zare and Gams, 2001). These species can be separated from *L. psalliotae* with the phylogeny of the ITS region. In this study, *L. psalliotae* (KK8) isolate was identified by phylogenetic tree based on the ITS region (Figure 2).

Lecanicillium species were revised by Zare and Gams (2001) based on morphological and molecular data. *L. pissodis* was identified by Kope and Leal (2005) based on this research. This species, which is very close to *A. muscarium* and *A. attenuatus*, could not be distinguished from other species in the phylogenetic analysis performed according to the ITS gene region (Figure 2).

DIKA11/1, *Akanthomyces* isolate could not be separated from *A. attenuatus* and *A. muscarium* species according to sequences of the ITS region (Figure 2). Therefore, DIKA11/1 isolate was identified based on its morphological characters. The isolate has white colonies and did not produce pigment on the PDA and MEA media. The conidial chains were not observed. Ellipsoidal and cylindrical conidia were formed in more than five fascicles at the tips of the phialides. Phialides were measured as 10-30×1.25-2 µm. This isolate was identified as *A. muscarium* according to Zare and Gams (2001).

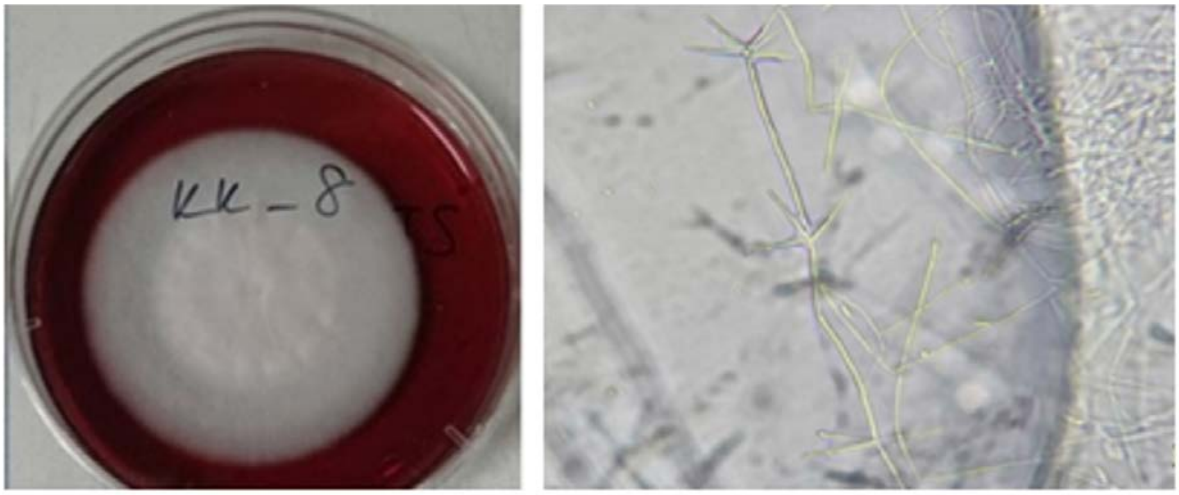

Figure 1. Culture of *Lecanicillium psalliotae* in PDA. Verticillate branching and conidia of the *Lecanicillium psalliotae* isolate

As a result, one *L. psalliotae* (KK8) isolate and one *A. muscarium* (DIKA11/1) isolate were identified according to morphologic and molecular data. Accession numbers in the NCBI gene bank and the size of the conidia of isolates was given in Table 1. In BLAST analysis, it was found that DIKA11/1 isolate was 99-100% similar with *A. muscarium* isolates, and KK8 isolate was 100-96% similar with *L. psalliotae* isolates in the NCBI gene bank.

In the pathogenicity test performed with DIKA11/1 isolate, it was determined that the isolate caused 87.7% mortality rate on the 15th day. Mycosis development was observed in all insect cadavers taken into moist cells (Figure 3).

The statistically differences among the mortality rates at 6th (24.7), 9th (52), and 15th (67) days were given in Figure 4. It was determined that the differences among the 9th, 12th, and 15th-days mortality rates were not statistically significant.

Table 1. Accession numbers in the NCBI gene bank and the size of the conidia of isolates

No	Isolate no	Provinces where isolates were obtained	Fungi species	Sizes of conidia (length-width, min-max) (µm)	Accession Numbers (ITS)	Accession Numbers (Beta tubulin)
1	DIKA11/1	Diyarbakır Karacadağ Tırbelek	*Akanthomyces muscarium*	1.5-5.25×1.25-2.5	MH193363	H287135
2	KK8	Karaman Karadağ	*Lecanicillium psalliotae*	2-7×1-2	MH192987	MH287134

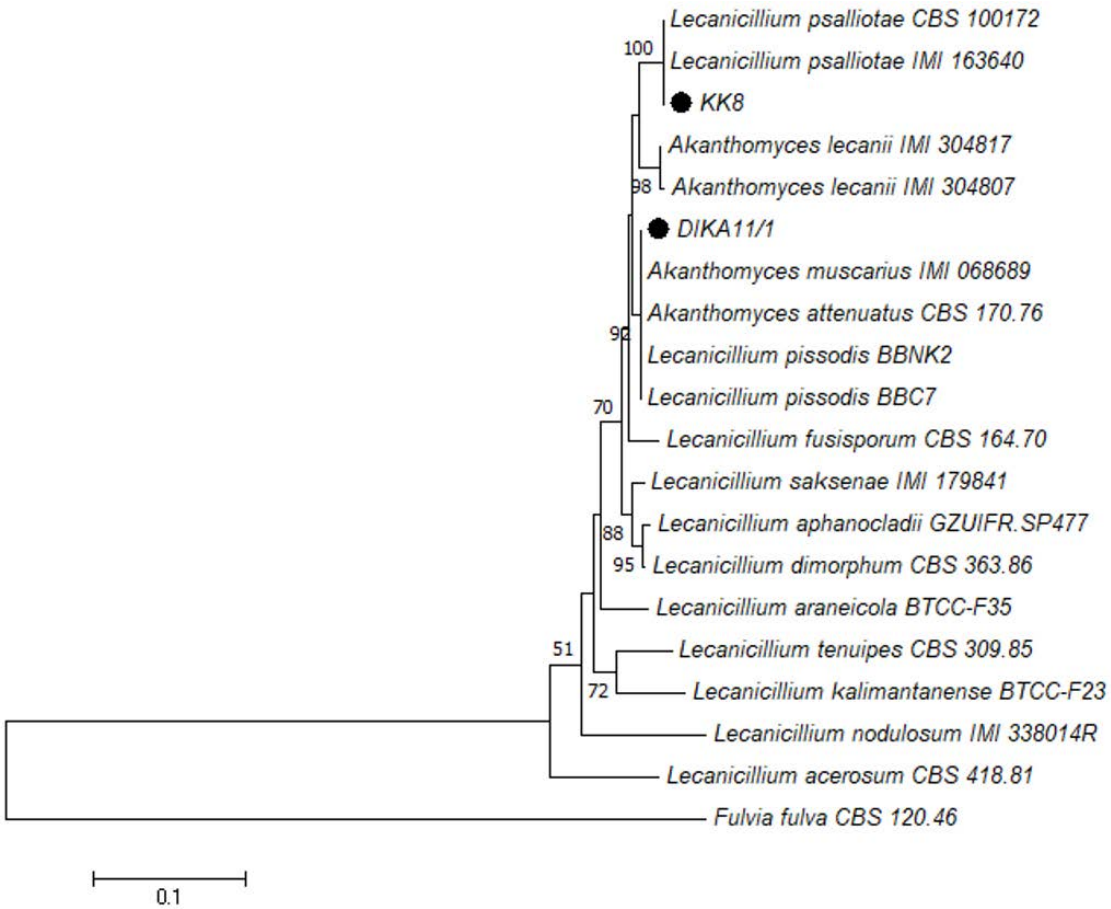

Figure 2. The phylogenetic tree was inferred using the Neighbor-Joining method with bootstrap test of 1500 replicates. The evolutionary distances were computed using the Kimura 2-parameter model (K2+G). Numbers at the nodes show bootstrap values higher than 50%. *Fulvia fulva* was selected as outgroup. Evolutionary analyses were conducted in MEGA7.

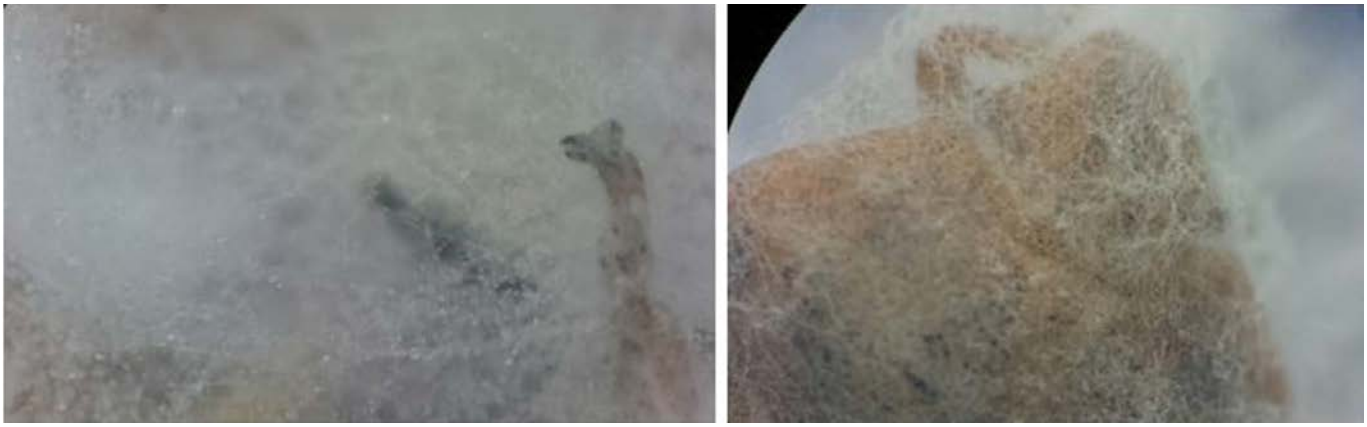

Figure 3. Growth of DIKA11/1 *Akanthomyces muscarium* isolate on sunn pest

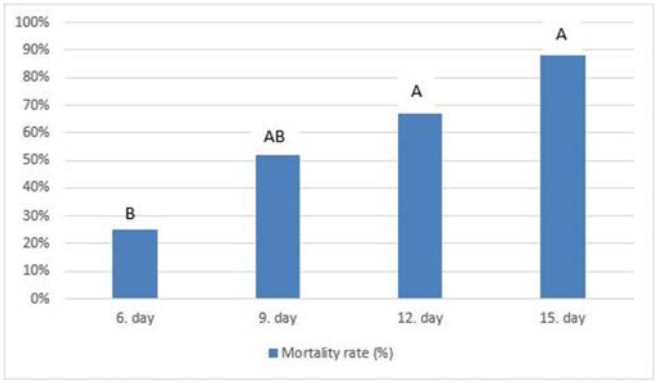

Figure 4. Mortality rates of DIKA11/1 isolate

DISCUSSION

According to recent studies, some of the *Lecanicillium* species were placed in the genus *Akanthomyces* (Kepler et al., 2017). Currently, *Lecanicillium lecani*, *L. muscarium*, and *L. attenuatum* species have been included in the Cordycipitaceae family and Acanthomyces genus (Kepler et al., 2017).

It has been reported that *A. muscarium* species, which are determined to be effective especially on aphids and whiteflies and whose commercial preparations such as Mycotol and BioCatch have been developed, was also effective on *Meloidogyne incognita* and *Heterodera schachtii* nematodes, *Scolypopa australis* (*Hemiptera: Ricaniidae*) and *Anastrepha fraterculus* (*Diptera, Tephritidae*) (Marshall et al., 2003; Hussain et al., 2017; 2018; Danilovich et al., 2020). It has also been reported that the fungus has positive effects on the shoot and root development in plants (Hussain et al., 2017). *L. psalliotae* has also been reported as pathogenic on other pests (Pirali-Kheirabadi et al., 2007; Senthil Kumar et al., 2015; Lu et al., 2016) and sunn pest (Asadolahpour et al., 2008).

It has been reported that fenitrothione, an insecticide widely used to control the sunn pests, is not compatible with the entomopathogenic fungi (Zibaee et al., 2009) while it is reported that another entomopathogenic fungus, *A. muscarium*, which was also determined to be effective on the sunn pests in our study, is compatible with insecticides (Amjad et al., 2012; Ali et al., 2017). Moreover, it has been reported that the combination of *Carum carvi* and *C. copticum* essential oils and *A. muscarium* increased the mortality rate of *Aphis gossypii* Glover (Razmjou et al., 2016). With all these aspects of this isolate, it has high potential in biological control and integrated pest management.

Fenitrothion and deltamethrin, which are widely used insecticides in the control of sunn pests, have been found to have a negative effect on the egg parasitoid *Trissolcus grandis* Thompson (Saber et al., 2005). In Turkey, mass production and release studies of egg parasitoids are successfully carried out for the control of sunn pests. For this reason, natural enemies should be taken into account in the control with insecticides.

For improvement of the integrated pest management against the sunn pests, entomopathogenic fungi formulations compatible with insecticides should be developed. Besides, more research is needed on the effects

of the combined use of different entomopathogenic fungi. Commercial preparations containing essential oils and entomopathogenic fungi, which can be used especially in organic agriculture, could be developed.

It is thought that the use of effective entomopathogenic fungi, entomopathogenic fungi combinations, fungicide-entomopathogenic fungus formulations, or essential oil-entomopathogenic fungus formulations could be used to keep its population below the economic threshold in biological control or integrated pest management.

CONCLUSIONS

In our current study, the *L. psalliotae* species, which is a pathogen on other insects and sunn pest, was isolated from the sunn pest and identified from Turkey. *A. muscarium* was reported for the first time with 87.7% mortality rate on the sunn pests. This species identified in this study is thought to be one of the candidate control agents that can be used against sunn pests. But, studies are needed to determine the effectiveness of the pathogen under field conditions.

ACKNOWLEDGEMENTS

The authors would like to thank Prof. Salih Maden for his help in morphological identification of the *A. muscarium*.

REFERENCES

Abbott, W.S. (1925) A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18, 265-267. DOI: http://dx.doi.org/10.1093/jee/18.2.265a

Ali, S., Zhang, C., Wang, Z., Wang, X.M., Wu, J.H., Cuthbertson, A.G.S., Shao, Z., Qiu, B.L. (2017) Toxicological and biochemical basis of synergism between the entomopathogenic fungus *Lecanicillium muscarium* and the insecticide matrine against *Bemisia tabaci* (Gennadius). Scientific Reports, 7, 46558. DOI: https://doi.org/10.1038/srep46558

Amjad, M., Bashir, M.H., Afzal, M., Sabri, M.A., Javed, N. (2012) Effects of Commercial Pesticides against cotton whitefly (*Bemisia tabaci* Genn.) and mites (*Tetranychus urticae* Koch) on growth and conidial germination of two species of entomopathogenic fungi. Pakistan Journal of Life and Social Sciences, 10 (1), 22-27.

Asadolahpour, M., Zafari, D., Zare, R. (2008) First report of *Lecanicillium psalliotae* as an entomogenous fungus from Iran. Rostanıha, 9 (2), 290-291. Available at: https://www.sid.ir/en/journal/ViewPaper.aspx?id=137373

Danilovich, M.E., Ovruski, S.M., Fariña, J.I., Delgado, O.D. (2020) First report on the entomopathogenicity and virulence of *Akanthomyces muscarius* LY 72.14, a Yungas native fungal isolate, for *Anastrepha fraterculus* control. Biocontrol Science and Technology, 30 (11), 1212-1227. DOI: https://doi.org/10.1080/09583157.2020.1802699

Davari, A., Parker, B.L. (2018). A review of research on Sunn Pest {*Eurygaster integriceps* Puton (Hemiptera: Scutelleridae)} management published 2004–2016. Journal of Asia-Pacific Entomology, 21 (1), 352-360. DOI: https://doi.org/10.1016/j.aspen.2018.01.016

Glass, N.L., Donaldson, G.C. (1995). Development of Primer Sets Designed for Use with the PCR to Amplify Conserved Genes from Filamentous Ascomycetes. Applied and Environmental Microbiology, 61 (4), 1323-1330. DOI: https://doi.org/10.1128/aem.61.4.1323-1330.1995

Hariri, G., Williams, P.C., El-Haramein, F.J. (2000) Influence of Pentatomoid insects on the physical dough properties and two-layered flat bread baking quality of Syrian wheat. Journal of Cereal Science, 31, 111-118. DOI: https://doi.org/10.1006/jcrs.1999.0294

Hussain, M., Zouhar, M., Ryšánek, P. (2017) Comparison between biological and chemical management of sugar beet nematode, *Heterodera schachtii*. Pakistan Journal of Zoology, 49 (1), 45-51. DOI: http://dx.doi.org/10.17582/journal.pjz/2017.49.1.45.51

Hussain, M., Zouhar, M., Ryšánek, P. (2018) Suppression of *Meloidogyne incognita* by the entomopathogenic fungus *Lecanicillium muscarium*. Plant Disease, 102 (5), 977-982. DOI: https://doi.org/10.1094/PDIS-09-17-1392-RE

Iranipour, S., Nozad Bonab, Z., Michaud, J.P. (2010) Thermal requirements of *Trissolcus grandis* (Hymenoptera: Scelionidae), an egg parasitoid of sunn pest. European Journal of Entomology, 107 (1), 47-53. DOI: https://doi.org/10.14411/eje.2010.005

Kepler, R.M., Luangsa-Ard, J.J., Hywel-Jones, N.L., Quandt, C.A., Sung, G.H., Rehner, S.A., Aime, M.C., Henkel, T.W., Sanjuan, T., Zare, R., Chen, M., Li, Z., Rossman, A.Y., Spatafora, J.W., Shrestha, B. (2017) A phylogenetically-based nomenclature for *Cordycipitaceae* (*Hypocreales*). IMA fungus, 8 (2), 335-353. DOI: https://doi.org/10.5598/imafungus.2017.08.02.08

Kope, H.H., Leal, I. (2005) A new species of *Lecanicillium* isolated from the white pine weevil, Pissodes strobi. Mycotaxon, 94, 331-340.

Lodos, N., Önder, F. (1983) Süne (*Eurygaster integriceps* Put.)'nin Türkiye'de yayılışı üzerinde düşünceler. Bitki Koruma Bülteni, 23 (2), 53-60. Available at: https://www.acarindex.com/pdfs/51959

Lu, L.M., Du, D.C., Hu, X.R., Pu, Z.X., Huang, Z.D., Chen, G.Q. (2016) Biological characteristics of three *Lecanicillium* species infecting Asian citrus psyllids. Acta Agriculturae Zhejiangensis, 28 (10), 1738-1744. Available at: http://www.zjnyxb.cn/article/2016/100

Marshall, R.K., Lester, M.T., Glare, T.R., Christeller, J.T. (2003) The fungus, *Lecanicillium muscarium*, is an entomopathogen of passionvine hopper (*Scolypopa australis*). New Zealand Journal of Crop and Horticultural Science, 31 (1), 1-7. DOI: https://doi.org/10.1080/01140671.2003.9514229

Parker, B.L., Skinner, M., Costa, S.D., Gouli, S., Reid, W., El Bouhssini, M. (2003) Entomopathogenic fungi of *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae): collection and characterization for development. Biological Control, 27, 260-272. DOI: https://doi.org/10.1016/S1049-9644(03)00017-3

Pirali-Kheirabadi, K., Haddadzadeh, H., Razzaghi-Abyaneh, M., Bokaie, S., Zare, R., Ghazavi, M., Shams-Ghahfarokhi, M. (2007) Biological control of *Rhipicephalus (Boophilus) annulatus* by different strains of *Metarhizium anisopliae*, *Beauveria bassiana* and *Lecanicillium psalliotae* fungi. Parasitology Research, 100 (6), 1297-1302. DOI: https://doi.org/10.1007/s00436-006-0410-x

Razmjou, J., Davari, M., Ebadollahi, A. (2016) Effect of two plant essential oils and the entomopathogenic fungus, *Lecanicillium muscarium* (Zare & Gams) on the cotton aphid, *Aphis gossypii* Glover. Egyptian Journal of Biological Pest Control, 26 (4), 775-779.

Saber, M., Hejazi, M.J., Kamali, K., Moharramipour, S. (2005) Lethal and sublethal effects of fenitrothion and deltamethrin residues on the egg parasitoid *Trissolcus grandis* (Hymenoptera: Scelionidae). Journal of Economic Entomology, 98 (1), 35-40. DOI: http://dx.doi.org/10.1603/0022-0493-98.1.35

Senthil Kumar, C.M., Jacob, T.K., Devasahayam, S., D'Silva, S., Krishna Kumar, N.K. (2015) Isolation and characterization of a *Lecanicillium psalliotae* isolate infecting cardamom thrips (*Sciothrips cardamomi*) in India. BioControl, 60 (3), 363-373. DOI: https://doi.org/10.1007/s10526-015-9649-4

Sevim, A., Demir, I., Tanyeli, E., Demirbag, Z. (2010) Screening of entomopathogenic fungi against the European spruce bark beetle, *Dendroctonus micans* (Coleoptera: Scolytidae). Biocontrol Science and Technology, 20 (1), 3-11. DOI: https://doi.org/10.1080/09583150903305737

Wainwright, M., Betts, R.P., Teale, D.M. (1986) Antibiotic activity of oosporein from *Verticillium psalliotae*. Transactions of the British Mycological Society, 86 (1), 168-170. DOI: https://doi.org/10.1016/S0007-1536(86)80133-4

White, T.J., Bruns, T., Lee, S., Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., eds. PCR Protocols: a guide to methods and applications. New York: Academic Press, pp. 315-322. DOI: http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1

Zare, R., Gams, W. (2001) A revision of Verticillium section Prostrata. IV. The genera *Lecanicillium* and *Simplicillium*. Nova Hedwigia, 73 (1-2), 1-50. DOI: https://doi.org/10.1127/nova.hedwigia/73/2001/1

Zibaee, A., Bandani, A.R., Tork, M. (2009) Effect of the entomopathogenic fungus, *Beauveria bassiana*, and its secondary metabolite on detoxifying enzyme activities and acetylcholinesterase (AChE) of the Sunn pest, *Eurygaster integriceps* (Heteroptera: Scutellaridae). Biocontrol Science and Technology, 19 (5), 485-498. DOI: https://doi.org/10.1080/09583150902847127