

## Isolation of Some different Fungi from the Larvae of the European Cockchafer *Melolontha melolontha* L. (Col: Scarabaeidae) at Erzincan Province in Turkey

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### ABSTRACT

The European cockchafer (*Melolontha melolontha*, L., (coleoptera: scarabaeidae) is a widely distributed pest throughout turkey and damage many plant species and cause substantial losses in meadows, pasture, horticulture, forest and other production. In our studies, we focused on the isolation of entomopathogenic fungi for *M. melolontha*. The larvae were collected from the ploughing fields in 3 locations (merkez-ekşisu, geyikli village, buğdaylı village; üzümlü-çermik village; kemah) in erzincan province at the end of april 2015. The most abundant species was found *Beauveria bassiana* (53.3% of samples). Two isolates *Olecanium* sp. (13.3 %) and *Fusarium* sp. (13.3%) were also detected. No entomopathogenic fungi were isolated from 3 of the samples (20%).

**Keywords:** European Cockchafer, *Melolontha melolontha*, *Beauveria bassiana*, *Lecanicillium* sp., *Fusarium* sp. Chemical insecticides

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### 1. Introduction

The European cockchafer is widely distributed harmful pest, over the world and Turkey as well. The insect, both larvae and adult are destructive for vegetation plant. In the last 25 years, *M. melolontha* extensive and lethal damage to the roots of meadows and ornamental plants, vegetable crops and young trees, causes economic losses of cultivated plants and forest production [1-15]. Turkey and other country use of chemical insecticides to control *M. melolontha* and these insecticides have been considerably reduced due to environmental and public health concerns [7]. It seems that population density of *M. melolontha* will increase so that it becomes abundant and important pest species. Entomopathogenic organisms are the most promising control agents. There are numerous papers on entomopathogens of *M. Melolontha*, such as viruses, rickettsia, bacteria, nematodes and fungi [8-19], but a few records of pathogens causing diseases in *M. melolontha* populations in Turkey. Some research-

er tested entomopathogenic bioproduct based on *Beauveria brongniartii* on *M. melolontha* larvae. According to this study, the cumulative effect of the biological product provided a decreased density of larval populations under economic threshold level [20]. Some scientist studied on the possible joint action of microorganisms pathogenic for *M. melolontha*. For this, they determined the effects of simultaneous or consecutive contamination of the larvae of *M. melolontha* with entomopathogenic organisms, *Beauveria tenella*[21]. Successful use of entomopathogenic fungi as microbial control agents of insects will ultimately depend on how well the strains are selected. For the development of mycoinsecticides based on entomopathogenic fungi, this research was conducted in the province of Erzincan province in years 2015. Our aim was to isolate the insect pathogenic fungi found in agricultural land and urban pests.

## 2. Materials and methods

### 2.1. Information About the Research Area

The province of Erzincan (39°02'n to 40°05'n, 38°16'e to 40°45'e) covers ca. 11,900 km<sup>2</sup> of turkey and is located in the eastern part of Anatolia, which has a continental climate. Soil samples were collected from different geographical sites distributed through the Erzincan province (Merkez-ekşisu, ge-yikli village, buğdaylı village; üzümlü-çermik vil-lage; Kemah,). The definition «Mountains around and vineyards in the centre» made by the people for Erzincan indicate the geographical position of the city. The province is surrounded by mountain ranges in the south and in the north. There are very fertile lands in the river basins between these mountains. The Erzincan plain, where Erzincan province is located is covered with vineyards and has been the cradle of the great civilizations since the early ages of history. The karaparmak grapes from the Erzincan vineyards are famous.

### 2.2. Exploration of Entomopathogenic Fungi

The method by collecting insects were sick or dead due to fungus infection [22]. The infected insects that showed symptoms of dry body and the presence of conidia and fungal conidia, white or green body of the larvae were isolated. Then, the fungus-infected insects were isolated in the laboratory at a cabinet of laminar air flow that had been sterilized by 70% alcohol.

### 2.3. Isolation and Identification of Fungi from Insects

we focused on the isolation of entomopathogenic fungi for *M. melolontha* from soil. The larvae were collected from the plowing fields in 3 locations (Merkez-Ekşisu, Üzümlü-Çermik village and Kemah) in Erzincan province at the end of April 2015. Isolation of entomopathogenic fungi used methods of Herlinda [23]. The fungus-infected insects and caterpillars were sterilized with 1% sodium hypochlorite or 70% alcohol for three minutes. Then insects were rinsed with sterile water three times, and dried on top of sterile filter paper. Then, they were placed in a petri dish (diameter 9 cm) containing moist sterile paper and incubated to stimulate conidial germination. Fungi were isolated, cultured on

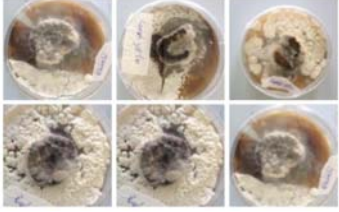
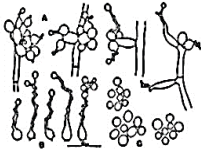


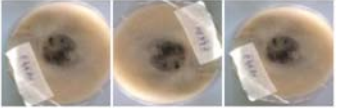

Saborroud Dextrose Agar (SDA) medium, and incubated for seven days at 25-27 C and relative humidity (RH) 80-85% 12-14 days pure culture of fungi were identified by using reference of Toledo [24].

## 3. Results and discussions

Isolation fungi from the larvae of European cockchafer; The most abundant species was found *B. bassiana* (53.3% of samples). Two isolates of *Lecanicillum sp.* (13.3 %) and *Fusarium sp.* (13.3%) were also detected. No entomopathogenic fungi were isolated from 3 of the samples (20%) (Table 1). Entomopathogens fungi cause disease in *M. melolontha* is of interest as agents for natural control of this pests. *B. bassiana* and *Lecanicillum spp* are entomopathogenic fungi on insects. Some researcher reported that *Fusarium spp.* has got the ability of pathogenic on insects [25]. Especially *B. bassiana* of these fungi is originated from soil and can infected *M. melolontha*. Because *B. bassiana* has got wide host species. The fungi species isolate from *M. melolontha* population in Erzincan Province, belonged to Ascomycota division. *Beauveria bassiana* and *Lecanicillum* spare from family Cordycipitaceae, *Fusarium* from Nectriaceae [26]. The main route of entrance of the entomopathogen is through integument and it may also infect the insect by ingestion method or through the wounds or trachea the main route of an entrance of the entomopathogen is through integument and it may also infect the insect by ingestion method or through the wounds or trachea [27]. At recent times, about 90 genera and almost above 700 species are considered as insect infecting fungi that represent about all the major classes of fungi [28,29]. There is a new interest in using entomopathogens for biological control of plant pest insects in Turkey. Despite Turkey has potential to find and develop entomopathogens fungi species, studies on entomopathogenic organisms infecting *M. melolontha* has limited. In this paper, entomopathogenic fungi infecting *M. melolontha* is presented to stimulate.

In conclusion, several side effects of chemical pesticides such as the development of resistance and the negative impact on the environment have encouraged several researchers to investigate alternative control methods on important agricultural and urban pests. In consequence, the development of biopesticides that are effective, biodegradable and no harmful side effect on the environment, turn out to be a priority of these studies. Our isolates of

**Table.** *Isoaltion fungi from the larvae of European cockchafer*

Fungus Species	Number of Isolated	Original Picture	Original Figure
<i>Beuveria bassiana</i> (Hypocreales: Cordycipitaceae)	7		
<i>Lecanicillium sp</i> (Hypocreales: Cordycipitaceae)	3		
<i>Fusarium sp.</i> (Hypocreales: Nectriaceae)	3		

this fungus species, especially *B. bassiana* and *Lecanicillium* sp are reported not only in agriculturally harmful insects but also in a variety of sources for the use of tick and mosquitoes in the human and animal health. If we want to develop an alternative biological control agent to chemical pesticides and developed good biocontrol agent against any pests, it should be noted that the first work, we have to make the isolate of entomopathogenic fungus that is the biological control agent.

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#### 5. References

- [1] N. Lodos, Türkiye Entomolojisi IV. Ege Üniversitesi Ziraat Fakültesi Yayınları, Yayın, İzmir, No: 493, 1995 (in Turkish).
- [2] T. Tóth, Damage caused by cockchafers (*Melolontha* spp.) in Hungary during the last 30 years. Proceedings: Population Dynamics, Impacts, and Integrated Management of Forest Defoliating Insects. McManus and A.M. Liebhold (editors). USDA Forest Service General Technical Report NE-247, (1998) 341.
- [3] B. Głowacka A. Sierpińska, Control of adult cockchafers *Melolontha* spp. with Mospilan 20 SP. Folia Forestalia Polonica series A 54 (2012) 109–115.
- [4] Z. Laznik, M. Vidrih, S. Trdan, The effect of different entomopathogens on white grubs (Coleoptera: Scarabaeidae) in an organic hay-producing grassland. Arch. Biol. Sci. Belgrade (2012) 64: 1235-1246.
- [5] E. Trotus, M. Naile, AA. Buburuz, Research on the flight evolution of *Melolontha melolontha* L. (Coleoptera, Scarabaeidae) species in the central of Moldavia conditions, Romania. Cercetări Agronomice în Moldova, Vol. XLVI (2013) 154.
- [6] M. Yaman, G. Alg, BG. Güner, Ö. Ertürk, S. Ünal, R. Radek, First record, occurrence and distribution of entomopathogens in populations of the European cockchafer, *Melolontha melolontha* (Coleoptera: Scarabaeidae) in Turkey. North-Western Journal of Zoology. 12 (2016) 192-195.
- [7] G. Woreta, Control of cockchafer *Melolontha* spp. grubs – a review of methods. Folia Forestalia Polonica, series. A 57 (2015) 33–41.
- [8] T. Lakatos, T. Tóth, Biological control of European cockchafer larvae (*Melolontha melolontha* L.)– Preliminary results. J. Fruit

- Ornam. Plant Res. 14 (2006) 73-78.
- [9] M. Švestka, Changes in the abundance of *Melolontha hippocastani* Fabr. and *Melolontha melolontha* (L.) (Coleoptera:Scarabaeidae) in the Czech Republic in the period 2003–2009, *J. For. Sci.* 56 (2010) 417–428.
- [10] L.J.W. De Goffau, Population development and dispersal of *Melolontha* and other Scarabaeidae in the Netherlands during the past ten years. *Bulletin OILB/SROP*, 19 (2), (1996) 9–14.
- [11] L. Mattedi, M. Varner, Presence and diffusion of the common cockchafer (*Melolontha melolontha* L.) in the areas of Mezzocorona and San Michelle a/A in Trento Province. *Bulletin OILB/SROP*, 19 (2), (1996) 15–20.
- [12] S. Keller, H. Brenner, Development of the *Melolontha* populations in the canton Thurgau, eastern Switzerland, over the last 50 years. *Bulletin OILB/SROP*. 28 (2), (2005) 31–35.
- [13] H. Malinowski, Current problems of forest protection connected with the control of cockchafers (*Melolontha* spp.). *Progress in Plant Protection/ Postępy w Ochronie Roślin*, 47 (1), (2007) 314–322.
- [14] Oltean I., Varga M., Gliga S., Florian T., Bunesco H., Bodis I., Covaci A. Monitoring *Melolontha melolontha* L. species in 2007, in the nursery from U.P. IV Bătrâna O.S. Toplița, Harghita Forest District. *Bulletin UASVM Horticulture*. 67 (1), (2010) 525.
- [15] Svestka M. Changes in the abundance of *Melolontha hippocastani* Fabr. and *Melolontha melolontha* (L.) (Coleoptera: Scarabaeidae) in the Czech Republic in the period 2003–2009. *J. of Forest Science*, 56 (9), (2010) 417–428.
- [16] B. Hurpin, PH. Robert Comparison of the activity of certain pathogens of the cockchafer *Melolontha melolontha* in plots of natural meadowland. *Journal of Invertebrate Pathology* 19: (1972) 291-298.
- [17] B. Hurpin, PH. Robert, Effects on a natural population of *Melolontha melolontha* (Col.: Scarabaeidae) of the introduction of *Rickettsiella melolonthae* and *Entomopoxvirus melolonthae*. *Entomophaga*, 22 (1977) 85-91.
- [18] W. Schweigkofler, Microbial pathogens of the cockchafer (*Melolontha* spp.). *Laimburg J.* 2 (2004) 186-192.
- [19] H. Malinowski, Possibility of forest protection against insects damaging root systems with the use of biological method based on entomopathogenic nematodes and bacteria. *Polskie Towarzystwo Lésne*, Warszawa, Poland, *Sylwan* 155 (2011) 104-111.
- [20] Fătu AC, Dinu MM, Ciorne C, Andrei AM.. Biological control of *Melolontha melolontha* L. larvae with entomopathogenic bioinsecticide based on *Beauveria brongniartii*. *Agro-Life Scientific J.* 4 (2015) 64-69.
- [21] P. Ferron, B. Hurpin, Effects of simultaneous or consecutive contamination of the larvae of *Melolontha melolontha* (Col. Scarabaeidae) with *Beauveria tenella* and *Entomopoxvirus melolonthae*. *Annales de la Societe Entomologique de France* 10 (1974) 771-779.
- [22] S. Herlinda, S.I. Mulyati, Suwandi, Selection of isolates of entomopathogenic fungi, and the bioefficacy of their liquid production against *Leptocorisa oratorius* Fabricius nymphs. *Microbiol Indones.* 2 (2008) 1-5.
- [23] S. Herlinda, M.D. Utama, Y. Pujiastuti, Suwandi, Density and viability of spores of (*Bals.*) Vuill. due to subcultures and media enriched, and its virulence against larvae of *Plutella xylostella* (Linn.). *J HPTT.* 6 (2006) 70-8.
- [24] A.V. Toledo, A.M.M. Remes Lenicov, C.C. López Lastra, Histopathology caused by the entomopathogenic fungi, and, *Metarhizium anisopliae* in the adult planthopper, , a maize virus vector. *J Insect Sci* 10:35. (2010) p 10. [online]. <http://www.insectscience.org/10.35>. [Sep 2018].
- [25] K.Chehri, Molecular identification of entomopathogenic *Fusarium* species associated with *Tribolium* species in stored grains. *J. Invertebr Pathol.* 144 (2017) 1-6.
- [26] R. A. Samson, H. C. Evans, and J.P. Latg Atlas of entomopathogenic fungi. Springer, Berlin Heidelberg New York. 1988.
- [27] J.D. Holder, O.N. keyhani, Adhesion of the entomopathogenic fungus *beauveria* (*Cordyceps*) *bassiana* to substrat. *Applied and Environmental Microbiology*, 71 (2005) 5260-5266.
- [28] E. R. Moorhouse, A. T. Gillespie, E. K. Sellers, & A. K. Charnley, Influence of fungicides and insecticides on the entomogenous fungus *Metarhizium anisopliae* a pathogen of the vine weevil, *Otiorhynchus sulcatus*. *Biocontrol Science and Technology*, 2(1) (1992) 49-58.
- [29] A.E. Hajek, & St. R.J. Leger, Interactions between fungal pathogens and insect host. *Annual Review of Entomology*, Vol. 39, (1994) pp. 293-322.