Applied Zoology
Research Article

ACTA ZOOLOGICA BULGARICA
Acta zool. bulg., 69 (4), 2017: 575-581

Efficacy of Entomopathogenic Fungi against the Alder Leaf Beetle *Agelastica alni* (L.) (Coleoptera: Chrysomelidae)

Emine Sonmez[1], Seda Kocacevik[1], Ali Sevim[2], Zihni Demirbag[1] & Ismail Demir[1]*

[1]Department of Biology, Faculty of Science, Karadeniz Technical University, 61080 Trabzon, Turkey
[2]Faculty of Engineering and Architecture, Genetic and Bioengineering, Ahi Evran University, Kirsehir, Turkey

Abstract: The alder leaf beetle, *Agelastica alni* L. (Coleoptera: Chrysomelidae) is one of the most dangerous defoliator pests of *Alnus* spp., *Corylus* spp., *Populus* spp. and *Salix* spp. worldwide. In order to discover a significant biocontrol agent against this pest, thirteen local entomopathogenic fungal isolates were screened against different growth stages of *A. alni* with dose of 1×10^7 conidia ml^{-1} under laboratory conditions. These included four isolates of *Beauveria bassiana* (Bals.) Vuill., seven isolates of *Metarhizium anisopliae* (Metsch.) Sorokin, one isolate of *Myriodontium keratinophilum* Samson and Polon and one isolate of *Isaria fumosorosea* (Wize) Brown and Smith. The *B. bassiana* isolate KTU–24 showed the highest mortality rate (100 %) at all stages of *A. alni* within 14 days after inoculation. Dose–mortality response bioassays showed that *B. bassiana* isolate (KTU–24) had the highest mortality rate (100 %) against 1st, 3rd instar larvae and adults of *A. alni* within 14 days after inoculation. These results indicate that *B. bassiana* KTU–24 seems to be a promising candidate for further studies as a biocontrol agent against *A. alni*.

Key words: Microbial control, virulence, *Beauveria bassiana*, *Agelastica alni*

Introduction

The alder leaf beetle, *Agelastica alni* L. (Coleoptera: Chrysomelidae) is widely distributed in Europe, the Caucasus, Siberia, North-Eastern Kazakhstan and USA (Belles et al. 1984, Kolk & Starzyk 1996). This insect feeds on a variety of broadleaf species including hazelnut (*Corylus* spp.) and alder leaves (*Alnus* spp.) during spring and summer. The insect also occasionally damages other species and genera such as *Betula pendula*, *Salix caprea*, *Populus* spp. and *Tilia* spp. (Medvedev 1983, Kolk & Starzyk 1996). The pest has high reproductive rate and inoculum potential, thus causing severe defoliation to host plants in their native habitats (Evans & Oszako 2007).

Insect pests are generally controlled by chemical pesticides but their excessive use induces insecticide resistance. In addition, the use of chemical pesticides also results in environmental pollution and imparts adverse effects on human health (French-Constant et al. 2004). Therefore, researchers have been looking for an alternative method to control pest populations (Wilson & Tisdall 2001). Microbial biocontrol agents (MBCAs) are eco-friendly and represent a potential substitute for the chemical pesticides. The MBCAs are natural enemies of the pest populations with no harmful effects on human health and the environment (Nicholson 2007). It is well established that MBCAs have complex mode of action and, therefore, it is very difficult for a pest to develop resistance against MBCAs. The common MBCAs are viruses, bacteria, nematodes and fungi (Weeden et al. 2007, Uygun et al. 2010, Lacey & Kaya 2007, Danismazoglu et al. 2013, Vega & Kaya 2012). However, fungal microbial biocontrol agents are the most important among all the MBCAs due to easy delivery, improved formulation, vast number of pathogenic strains, easy engineering techniques and over-expression of endogenous proteins or exogenous toxins (Butt et al. 2001, Wang

*Corresponding author: idemir@ktu.edu.tr

& St. Leger 2007, Federici et al. 2007, St. Leger & Wang 2010).

Several reports have shown that different fungal species such as *Beauveria bassiana* and *Metarhizium anisopliae* are entomopathogenic agents for many different insect species of various orders (Goettel et al. 2005, Zimmermann 2007, Sevim et al. 2010a, b, c). Fungal strains were isolated from a different location and host insect varied in performance; *Beauveria bassiana* and *Metarhizium anisopliae* were the most common entomopathogenic fungi in agricultural fields (Meyling & Eilenberg 2006, Tanyeli et al. 2010).

Over the years, several chemical (Carbaryl 5 %, Methiocarb 50 % and Malathion 200 g/L) approaches have been used to control *A. alni* in Turkey (The Ministry of Agriculture of Turkey 2010). However, recent interest in developing eco-friendly pest control approaches has motivated us to study the potential of different fungal biological agents against *A. alni*.

Materials and Methods

Collection of insects

Adults and larvae of *Agelastica alni* were collected from infested *A. glutinosa* (alder) trees in the vicinity of Trabzon, Turkey, between March and June 2013. Insect samples were either collected from undersides of leaves by a soft fine-tipped paintbrush or collected by a sweep net (flying adults). The collected samples were then placed into plastic boxes (20× 20cm) with ventilated lids and freshly collected plane leaves were used as a source of food. Healthy adults and larvae were acclimated for two days in laboratory conditions and then, healthy adults and first and third instars larvae were separated and used for bioassay.

Fungal isolates

Fungal isolates were obtained from the Department of Biology at Karadeniz Technical University, Trabzon, Turkey. Thirteen entomopathogenic fungi, including *Beauveria bassiana* (Bals.) Vuill.(four isolates), *Metarhizium anisopliae* Sensulato (7 isolates), *Myriodontium keratinophilum* Samson and Polon (one isolate) and *Isaria fumosorosea* (Wize) (one isolate), were used for the bioassays (Table 1). Some of these isolates are known to be highly virulent against different pests in laboratory conditions (Sevim et al. 2010a, c, 2013, Kocacevik et al. 2015). Fungal isolates were cultured on potato dextrose agar + 1 % yeast extract (PDAY; Merck, Darmstadt, Germany) for 4–5 weeks at 28 °C and were stored at 4 °C until required for the bioassay.

Preparation of spore suspensions

Fungal isolates were obtained from a single colony of pure cultures. One hundred µL of spore suspension (1×10^6 conidia ml^{-1}) from stock fungal cultures was plated on PDAY and incubated at 25°C for 4–5 days under a 12-h L / 12-h D photoperiod. After fungal growth, a single colony was transferred to another fresh PDAY plate and incubated at 25°C for 4–5 weeks until plates were fully overgrown. Conidial suspensions of fungal isolates were prepared by adding 15 ml of sterile Tween-80 (0.01 %) into the 4-week-old culture dishes and gently scraping the surface of the cultures with a sterile bent glass rod to dislodge the conidia from the surface of the agar plates. The conidial suspensions were filtered through two layers of sterile muslin cloth into 50ml sterile plastic tubes to remove mycelium and agar pieces. The obtained conidial suspensions were vortexed for 5 min for homogenisation. The spore concentration was determined with a Neubauer hemocytometer and adjusted to the desired concentrations.

The germination rate of freshly produced conidia obtained from the pure cultures on PDAY was assessed by inoculating 50 µl (1×10^6 conidia ml^{-1}) of the suspension on PDAY plates and then incubated for 8 h in the dark at 25 ± 1 °C. For each culture, germination was observed in three separate fields of view at magnification of × 40.

Screening tests

All selected cultures were assayed against 1st, 3rd instars larvae and adults of the *A. alni*. Thirty insects of each stage were immersed for 15 s in 10-15 ml of conidial spore suspension (1×10^7 conidia ml^{-1}). Control groups were treated with sterile 0.01 % Tween-80. Infected insects were carefully placed on the underside of the small alder leaves in a plastic box (20 × 20 cm) with a ventilated lid. All treated and untreated adults and larvae were kept in rearing boxes at 25 ± 1°C for two weeks under a 12-h L / 12-h D photoperiod. Freshly collected plane leaves of alder trees were provided every day during the experiment. The mortality of *A. alni* was recorded five days after inoculation and then continued every five days during the experimental period. Dead insects were transferred to the petri dishes lined with filter paper to encourage fungal emergence and sporulation on cadavers.

Concentration–mortality response test

The fungal strain KTU–24 was selected for concentration–mortality response tests against adults and larvae of *A. alni* for further experimentation due to its highest pathogenic effect on pest populations at

Table 1. Fungal isolates used in this study and their sources.

No	Species	Isolates	Locality	Source	Reference
1	*Metarhizium anisopliae*	KTU–60	Gümüşhane	Soil	Sevim et al. 2010a
2	*Metarhizium anisopliae*	KTU–2	Ardeşen, Rize	Soil	Sevim et al. 2010a
3	*Metarhizium anisopliae*	Gg–12	Akçaabat, Trabzon	*Gryllotlpa gryllotalpa* L. (Orthoptera: Gryllotalpidae)	Sonmez et al. 2016
4	*Metarhizium anisopliae*	KTU–51	Gümüşhane	Soil	Sevim et al. 2010a
5	*Beauveria bassiana*	KTU–24	Samsun	*Thaumetopoea pityocampa* (Den. & Schiff.) (Lepidoptera: Thaumetopoeidae)	Sevim et al. 2010c
6	*Beauveria bassiana*	KTU–7	Yomra, Trabzon	Soil	Sevim et al. 2010a
7	*Beauveria bassiana*	Gg–1	Akçaabat, Trabzon	*Gryllotlpa gryllotalpa*	Sonmez et al. 2016
8	*Isaria fumosorosea*	KTU–42	İkizdere, Rize	Soil	Sevim et al. 2010a
9	*Metarhizium anisopliae*	KTU–27	İkizdere, Rize	Soil	Sevim et al. 2010a
10	*Myriodontium keratinophilum*	Gg–11	Akçaabat, Trabzon	*Gryllotlpa gryllotalpa*	Sonmez et al. 2016
11	*Beauveria bassiana*	Mm–1	Akçaabat, Trabzon	*Melolontha melolontha*	Unpublished
12	*Metarhizium anisopliae*	KTU–40	Akçaabat, Trabzon	Soil	Sevim et al. 2010a
13	*Metarhizium anisopliae*	KTU–21	Borçka, Artvin	Soil	

the end of the screening tests.Thirty1st,3rd instars larvae and adults were treated with four different conidial concentrations (1×10^5, 1×10^6, 1×10^7 and 1×10^8 conidia ml^{-1}). After that, larvae and adults were separately put into plastic boxes (20 × 20 cm) with ventilated lids including a small alder leaf. Mortality of adults and larvae were checked every day for the next 15 days after the inoculation of conidial concentrations. Finally, mortality data were corrected using Abbott's formula (Abbott 1925) and lethal concentration (LC_{50}) value was calculated using probit analysis. All concentrations were assayed in triplicate on different days.

Statistical analysis

Data analyses were performed using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA) for analysis of variance (ANOVA). Dunnett's one-tailed t-test was used to compare test isolates against the control group with respect to mortality and mycosis (Abbott 1925). Lethal concentration at 50 % (LC_{50}) value was calculated by probit analysis using SPSS 16.0 statistical software.

Results

Screening test

Beauveria bassiana (Bals.) Vuill. (four isolates), *Metarhizium anisopliae* (Metschn.) (seven isolates), *Myriodontium keratinophilum*Samson and Polon (one isolate) and *Isaria fumosorosea* (Wize) (one isolate) caused different mortality rates. The mortality rate varied from 35 % to 100 % based on fungal strains against both 1st and 3rd instars larvae and adults of *A. alni* within 15 days with 1×10^7 conidial ml^{-1} concentration (Figs. 1–3).

All isolates showed more than 75 % mortality rate on1st instar larvae. However, these rates were different based on fungal isolates ($F=1.632$, $d.f.=12$, $p<0{,}05$; Fig. 1). Significant differences were observed between control and treatment groups ($F=26.146$, $d.f.=13$, $p<0{,}05$). The highest mortality and mycosis values were observed for *B. bassiana* KTU–24 and *M. anisopliae* Gg–12 (Fig. 1).

The fungal isolates showed different mortality rates (from 50 to 100 %) in comparison to each other on mortality of the 3rd instar larvae ($F=2.983$, $d.f.=12$, $p<0{,}05$; Fig. 2). The effects of isolates were also significantly different from the control group ($F=8.996$, $d.f.=13$, $p<0{,}05$). *Metarhizium anisopliae* (KTU–60) and *B. bassiana* (KTU–24) provided the highest mortality and mycosis value with absolute 100 % within 15 days after treatment with 1×10^7 conidial ml^{-1} concentration. The second highest mortality (93.3 %) was obtained from *M. anisopliae* (KTU–2), *M. anisopliae* (KTU–51) and *M. anisopliae* (Gg–12) within the same period. All fungal isolates produced different mycosis in comparison to each other ($F=5.227$, $d.f.=12$, $p<0{,}05$) and there was a significant difference between the control and fungal isolates except for *B. bassiana* (Mm–1) ($F=9.272$, $d.f.=13$, $p<0{,}05$). *Beauveria bassiana* (KTU–24) and *M. anisopliae* (KTU–60) also produced the highest mycosis value with 100 % (Fig. 2).

All isolates produced significantly different adult mortality values when compared with each

Table 2. Probit regression estimates for the multiple-concentration bioassays performed with the *B. bassiana* isolate KTU–24 against 1st and 3rd instars larvae and adult of *A. alni*

Bioassay	Intercept	Slope ± SE[a]	LC50 (95 % confidence interval)	$\chi 2^b$	*df*
1st instar larvae	-5.217 ± 0.948	0.904 ± 0.154	5.94×10^5 (2.47×10^5 to $1{,}19 \times 10^6$)	2.158	2
3rd instar larvae	-4.327 ± 0.805	0.691 ± 0.124	1.83×10^6 (7.12×10^6 to 4.29×10^6)	1.355	2
Adult	-5.759 ± 0.911	0.908 ± 0.141	2.21×10^6 (1.08×10^6 to 4.46×10^6)	1.188	2

[a]Slope of the concentration ± standard error response of larvae and adult of *A. alni* to *B. bassiana* isolate KTU–24.
[b]Pearson chi-square goodness-of-fit test on the probit model ($\alpha = 0.05$).

other (F=9.877, *d.f.*=12, *p<0,05*; Fig. 3) and with the control group in screening test after 15 days of application (F=17.311, *d.f.*=13, *p<0,05*). Fungal isolates *M. anisopliae* (KTU–40), *B. bassiana* (KTU–24), *M. anisopliae* (KTU–21) and *M. anisopliae* (KTU–2) produced 100 % mortality on adults of *A. alni*. All fungal isolates showed different degree of mycosis when compared to each other (F=17.702, *d.f.*=12, *p<0,05*). The highest mycosis values (100 %) were provided from *M. anisopliae* (KTU–40), *B. bassiana* (KTU–24) and *M. anisopliae* (KTU–2); all other treatments and the control groups showed differences from each other (F=31.049, *d.f.*=13, *p<0,05*; Fig. 3).

Concentration-mortality response tests

Fungal isolate of *B. bassiana* (KTU–24) was selected for concentration-mortality response tests owing to its high pathogenic effect on all stages of *A. alni*. In concentration-mortality response test, 100 % mortality values were reached with 1×10^7 conidia ml^{-1} concentration on all stages of the pest (Figs. 4–6). One hundred percent mortality on 1st and 3rd instar larvae and adults were induced by *B. bassiana* KTU–24 within 10, 11 and 11 days, respectively. The LC_{50} of *B. bassiana* (KTU–24) against the 1st instar larvae within ten days after treatment was found to be 5.94×10^5 conidia ml^{-1} (Table 2). The 3rd instar larvae and adult mortality also reached 100 % within 11 days after application of 1.83×10^6 and 2.21×10^6 conidia ml^{-1} concentration, respectively (Table 2).

Discussion

Agelastica alni L. is an important pest of hazelnut and alder trees in the Black Sea Region of Turkey. There are only a few reports regarding microbial control agents against this pest. Sezen et al. (2004) studied the effect of *Pseudomona sfluorescens* (Aa4) on various growth stages of *A. alni* populations. The highest insecticidal effects of *P. fluorescens* (Aa4) was observed on larvae and adults of *A. alni* within seven days after treatment. In another study, Sezen & Demirbag (2006) showed that two *Bacillus* species had 90 % insecticidal effect on the larvae of *A. alni*. In the present paper, we showed the insecticidal effects of 13 entomopathogenic fungi on different stages of *A. alni.* Fungal isolates had different levels of insecticidal effects: from 35 to 100 %, on *A. alni* larvae and adults. Fungal isolates of *Beauveria bassiana* and *Metarhizium anisopliae* were the most virulent against 1st and 3rd instars larvae and adults of *A. alni*. *Beauveria bassiana* (KTU–24) and *M. anisopliae* (G–12) were found as the most effective isolates with 100 % insecticidal effect on 1st instar larva (Fig. 1). On the other hand, *B. bassiana* KTU–24 and *M. anisopliae* KTU–60 were the most effective isolates with 100 % insecticidal effect on 3rd instar larva (Fig. 2). *Beauveria bassiana* KTU–24 and *M. anisopliae* KTU–40 and KTU–2 yielded 100 % mortality on adults of *A. alni* in screening tests (Fig. 3). At the end of all the screening tests we decided to continue our study with *Beauveria bassiana* KTU–24 for concentration response tests because it was the most effective fungus against all stages of the target pest.

Previously, some researchers have showed that *Beauveria* sp. is a natural fungal pathogen of many different pest species in both agriculture and forestry (Goettel et al. 1990, Kreutz et al. 2004, Sevim et al. 2010a, 2013). Different species and strains of the genus *Beauveria*, which were isolated from different geographical locations and host insects, vary in performance and it is the most common enthomopathogenic fungus in agricultural fields (Meyling & Eilenberg 2006). Scientific researchers showed that *B. bassiana* could infect many bark beetles and displayed significant potential for control of forest pests (Kreutz et al. 2004, Draganova et al. 2007, Kocacevik et al. 2015, Tanyeli et al. 2010, Sevim et al. 2010a). Quesada-Moraga et al. (2006) reported that medfly pupae was susceptible against *B. bassiana* isolates and mortality rates produced by that isolates ranged from 14 to 95.5 %. In another study, Cossentine et al. 2010 showed that *B. bassiana* had significant mortality and mycosis on adults and

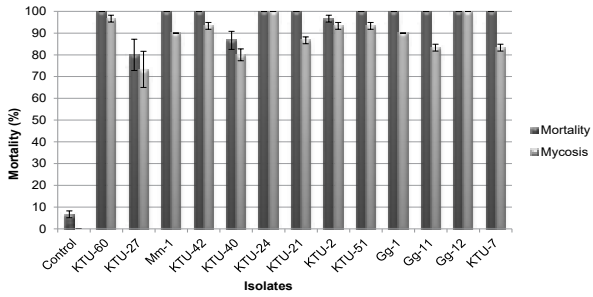

Fig. 1. Pathogenicity of fungal isolates to 1st instar larvae of *A. alni* within 14 days after application of 1×10^7 conidia ml^{-1}. Mortality data were corrected according to Abbott's formula (Abbott 1925). Different uppercase and lowercase letters represent statistically significant differences among mortality and mycosis, respectively, between treatments according to LSD multiple comparison test ($p < 0.05$). Bars show standard deviation.

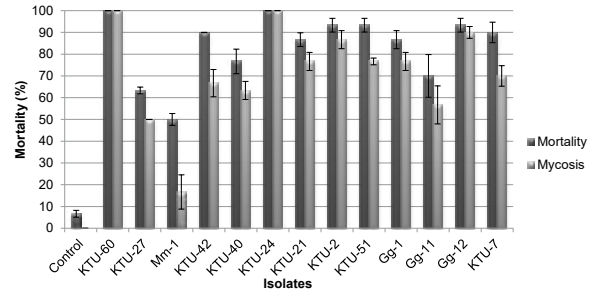

Fig. 2. Pathogenicity of fungal isolates to 3rd instar larvae of *A. alni* within 15 days after application of 1×10^7 conidia ml^{-1}. Mortality data were corrected according to Abbott's formula (*ABBOTT* 1925). Different uppercase and lowercase letters represent statistically significant differences among mortality and mycosis, respectively, between treatments according to LSD multiple comparison test ($p < 0.05$). Bars show standard deviation.

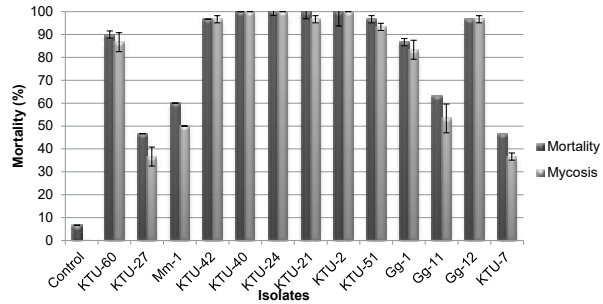

Fig. 3. Pathogenicity of fungal isolates to adults of *A. alni* within 14 days after application of 1×10^7 conidia ml^{-1}. Mortality data were corrected according to Abbott's formula (Abbott 1925). Different uppercase and lowercase letters represent statistically significant differences among mortality and mycosis, respectively, between treatments according to LSD multiple comparison test ($p < 0.05$). Bars show standard deviation.

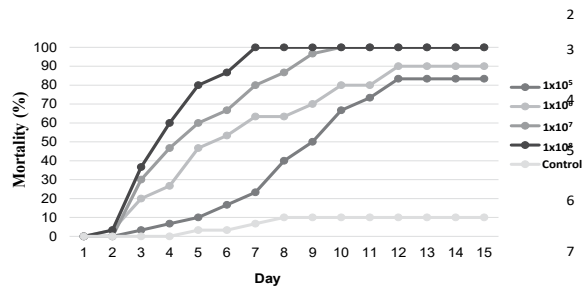

Fig. 4. Cumulative mortality of 1st instar larvae of *A. alni* after application of different concentrations of *B. bassiana* KTU–24. Concentration unit is conidia ml^{-1}.

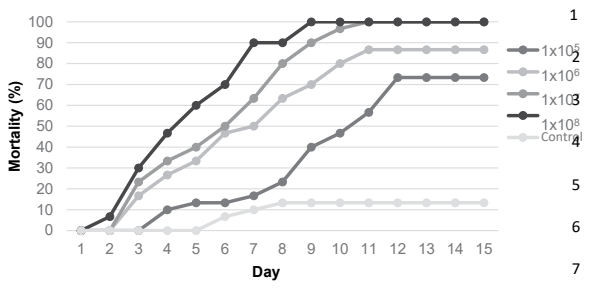

Fig. 5. Cumulative mortality of 3rd instar larvae of *A. alni* after application of different concentrations of *B. bassiana* KTU–24. Concentration unit is conidia ml^{-1}.

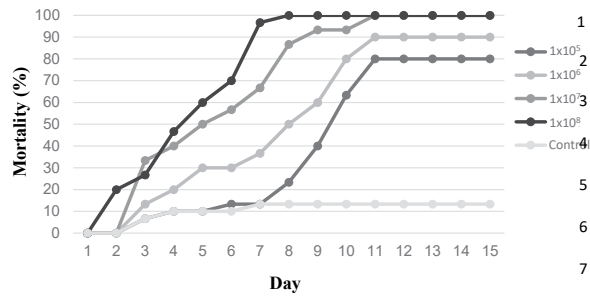

Fig. 6. Cumulative mortality of adults of *A. alni* after application of different concentrations of *B. bassiana* KTU–24. Concentration unit is conidia ml^{-1}.

pupae of another fruit fly, the western cherry fruit fly (*Rhagoletis indifferens*, Diptera: Tephritidae). *Beauveria bassiana* also has good activity against adults and nymphs of *Corythucha ciliata* (Say, 1932) (Hemiptera: Tingidae) under laboratory conditions, with insecticide effect of 73 and 66 %, respectively (Sevim et al. 2013).

Concentration-response experiments showed that increasing the concentration of *B. bassiana* KTU–24 gradually increased mortality, which reached 100 % with 1×10^7 and 1×10^8 conidia ml^{-1} concentration, and that mortalities were dose-dependent. First instar larvae were also determined to be more sensitive than 3rd instar larvae and adults in the concentration-mortality test. Older instars are generally more resistant to pathogens presumably because they have thicker cuticles but this pattern depends on the insect species and pathogen. For example, the 1st instar larvae of *Ostrinia nubilalis* are more susceptible than the 4th instar to *B. bassiana* (Feng et al. 1985). In contrast, 3rd and 4th instar diamond back moth larvae are more susceptible to fungal pathogens than 2nd instar larvae (Vandenberg et al. 1998). Some believe that the variability in larval susceptibility is linked to moulting: insects shedding exuviae soon after inoculation being less susceptible, taking longer to die or even escaping infection (Ferron 1967, Vandenberg et al. 1998).

In conclusion, in the current study, we tested 13 different entomopathogenic fungus isolates against 1st and 3rdinstars larvae and adults of *A. alni*. The results showed that *B. bassiana* KTU–24 was highly virulent against *A. alni,* and thus increase the chance to develop a microbial control agent against *A. alni*. All stages appeared to be significantly susceptible against *B. bassiana* KTU–24 with both screening and concentration-response test. However, more work needs to be done in order to determine the best formulation (dry vs wet conidia vs blastospores) method to be used against *A. alni*. Also more information is needed on the persistence of conidia in the nests and the wintering and to what extend faecal pellets that accumulate in the wintering interfere with fungal viability and transmission. It is anticipated that contamination of conidia leaf surface and the nests will result in even higher mortalities since insects would be exposed to the pathogen every time they collected to the wintering after feeding.

References

Abbott W. S. 1925. A method of computing the effectiveness of insecticides. Journal of Economic Entomology 18: 265-267.

Belles X., Baldellou M. I. & Piulachs M. D. 1984. Actividad ovicida del precocene 2 sobre la galeruca de aliso, *Agelastica alni* (L.) (Col. Chrysomelidae). Boletin de la Estacion Central de Ecologia 13: 97-99.

Butt T. M., Jackson C. & Magan N. 2001. Introduction. Fungal biological control agents: progress, problems and potential. In: Butt T. M., Jackson C. & Magan N. (Eds.): Fungi as biocontrol agents: progress, problems and potential. Wallingford: CAB International, pp. 1-8.

Cossentine J., Thistlewood H., Goettel M. & Jaronski S. 2010. Susceptibility of preimaginal western cherryfruit fly, *Rhagoletis indifferens* (Diptera: Tephritidae) to *Beauveria bassiana* (Balsamo) Vuillemin Clavipitaceae (Hypocreales). Journal of Invertebrate Pathology 30: 1-5.

Danismazoglu M., Demir I., Sevim A., Demirbag Z. & Nalcacioglu R. 2012. An investigation on the bacterial flora of *Agriotes lineatus* (Coleoptera: Elateridae) and pathogenicity of the flora members. Crop Protection 40: 1-7.

Draganova S., Takov D. & Doychev D. 2007. Bioassays with isolates of *Beauveria bassiana* (Bals.) Vuill. and *Paecilomyces farinosus* (Holm.) Brown and Smith against *Ips sexdentatus* Boerner and *Ips acuminatus* Gyll. (Coleoptera: Scolytidae). Plant Science 44: 24-28.

Evans H. F. & Oszako T. 2007. Alien invasive species and international trade. Forest Research Institute. 179 p.

Federici B. A., Park H. W., Bideshi D. K., Wirth M. C., Johnson J. J., Sakano Y. & Tang M. 2007. Developing recombinant bacteria for control of mosquito larvae. Journal of the American Mosquito Control Association 23: 164-175.

Feng Z., Carruthers R. I., Roberts D. W. & Robson D. S. 1985. Age-specific dose-mortality effects of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) on the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). Journal of Invertebrate Pathology 46: 259-264.

Ferron P. 1967. Etude en laboratoire des conditions écologiques favorisant le développement de la mycose à *Beauveria tenella* du ver blanc. Entomophaga 12: 257-293.

Ffrench-Constant R. H., Daborn P. J. & Le Goff G. 2004. The genetics and genomics of insecticide resistance. Trends in Genetics 20: 163-170.

Goettel M. S., Poprawski T. J., Vandenberg J. D., Li Z. & Roberts D. W. 1990. Safety to Non-target Invertebrates of Fungal Biocontrol Agents Safety of Microbial Insecticides. In: Laird M., Lacey L. A. & Davidson E. W. (Eds.): Safety of microbial insecticides. Boca Raton: CRC Press, pp. 209-232.

Goettel M. S., Eilenberg J. & Glare T. 2005. Entomopathogenic fungi and their role in regulation of insect populations. In: Gilbert L. I., Iatrou K. & Gill S. S. (Eds.): Comprehensive molecular insect science. Amsterdam: Elsevier, pp. 361-405.

Kolk A. & Starzyk J. R. 1996. Atlas of harmful forest insects. Warsaw, Poland.

Lacey L. A. & Kaya H. K. 2007. Field Manual of Techniques in Invertebrate Pathology: Application and Evaluation of Pathogens for Control of Insects and Other Invertebrate Pests, Second Edition. Dordrecht: Springer.

Kocacevik S., Sevim A., Eroglu M., Demirbag Z. & Demir I. 2015. Molecular characterization, virulence and horizontal transmission of *Beauveria pseudobassiana* from *Dendroc-*

tonus micans (Kug.) (Coleoptera: Curculionidae). Journal of Applied Entomology 139: 381-389.

Kreutz J., Zimmermann G. & Vaupel O. 2004. Horizontal transmission of the entomopathogenic fungus *Beauveria bassiana* among the spruce bark beetle, *Ips typographus* (Col., Scolytidae) in the laboratory and under field conditions. Biocontrol Science and Technology 14**:** 837-848.

Medvedev L. N. 1983. Chrysomelidae from Iran (Insecta: Coleoptera). Senckenbergiana – Biologica 64: 133-140.

Meyling N. V. & Eilenberg J. 2006. Occurrence and distribution of soil borne entomopathogenic fungi within a single organic agroecosystem. Agriculture, Ecosystems and Environment 113: 336-341.

Nicholson G. M. 2007. Fighting the global pest problem: Preface to the special Toxicon issue on insecticidal toxins and their potential for insect pest control. Toxicon 49: 413-422.

Quesada-Moraga E., Landa B. B., Muñoz-Ledesma J., Jiménez-Díaz R. M. & Santiago-Álvarez C. 2006. Entophytic colonization of opium poppy, *Papaver somniferum*, by an entomopathogenic *Beauveria bassiana* strain. Mycopathologia 161**:** 323-329.

Sevim A., Demir I., Hofte M., Humber R. A. & Demirbag Z. 2010a. Isolation and characterization of entomopathogenic fungi from hazelnut-growing region of Turkey. BioControl 55**:** 279-297.

Sevim A., Demir I., Tanyeli E. & Demirbag Z. 2010b. Screening of entomopathogenic fungi against the European spruce bark beetle, *Dendroctonus micans* (Coleoptera: Scolytidae). Biocontrol Science and Technology 20: 3-11.

Sevim A., Demir I. & Demirbag Z. 2010c. Molecular characterization and virulence of *Beauveria* spp.from the pine processionary moth, *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae). Mycopathologia 170: 269-277.

Sevim A., Demir I., Sönmez E., Kocacevik S. & Demirbag Z. 2013. Evaluation of entomopathogenic fungi against the sycamore lace bug, *Corythucha ciliata* (Say) (Hemiptera: Tingidae). Turkish Journal of Agriculture Forestry 37: 595-603.

Sezen K., Demir I. & Demirbag Z. 2004. Study of the bacterial flora as a biological control agent of *Agelastica alni* L. (Coleoptera: Chrysomelidae). Biologia, Bratislava 59: 327-331.

Sezen K. & Demirbag Z. 2006. Insecticidal effects of some biologicalagents on *Agelastica alni* L. (Coleoptera: Chrysomelidae). Biologia, Bratislava 61: 687-692.

Sonmez E., Sevim A., Demirbag Z. & Demir I. 2015. Isolation, characterization and virulence of entomopathogenic fungi from *Gryllotalpa gryllotalpa* (Orthoptera: Gryllotalpidae). Applied Entomology and Zoology 51: 213-223.

St. Leger R. J. & Wang C. S. 2010. Genetic engineering of fungal biocontrol agents to achieve greater efficacy against insect pests. Applied Microbiology and Biotechnology 85: 901-907.

Tanyeli E., Sevim A., Demirbag Z., Eroglu M. & Demir I. 2010. Isolation and virulence of entomopathogenic fungi against the great spruce bark beetle, *Dendroctonus micans* (Kugelann) (Coleoptera: Scolytidae). Biocontrol Science and Technology 20: 695-701.

The Ministry of Agriculture of Turkey 2010. Plant Protection Technical Recommendations Agrochemicals Chapters. Ankara: Pulat Publisher, pp. 21-22.

Uygun N., Ulusoy M. R. & Satar S. 2010. Biological control. Biological Control of Society of Turkey 1: 1-14.

Vandenberg J. D., Ramos M. & Altre J. A. 1998. Dose-response and age and temperature-related susceptibility of the diamondback moth (Lepidoptera: Plutellidae) to two isolates of *Beauveria bassiana* (Hyphomycetes: Moniliaceae). Environmental Entomology 27: 1017-1021.

Vega F. E. & Kaya H. K. 2012. Insect Pathology. Second Edition. London: Academic Press, 490 p.

Wang C. & St. Leger R. J. 2007. A scorpion neurotoxin increases the potency of a fungal insecticide. Nature Biotechnology 25: 1455-1456.

Weeden C. R., Shelton A. M. & Hoffman M. P. 2007. Biological Control: A Guide to Natural Enemies in North America.

Wilson C & Tisdall C. 2001. Why farmers continue to use pesticides despite Environmental, health and sustainability costs. Ecological Economics 39: 449-462.

Zimmermann G. 2007. Review on safety of the entomopathogenic fungus *Metarhizium anisopliae.* Biocontrol Science and Technology 17: 879-920.

Received: 16.08.2016
Accepted: 27.11.2017