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Efficacy of some Entomopathogenic fungi in controlling filbert aphid, *Myzocallis coryli* Goetze (Hemiptera: Aphididae)

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Abstract
The Filbert Aphid is one of the pests of hazelnut growing area of Turkey. This study was carried out all fungal isolates caused significant mortality against first instar nymphs of filbert aphid after 2 and 5 days of conidial treatments at 18, 22 and 25°C temperatures under laboratory conditions. The results showed that first instar nymphs of *M. coryli* were more susceptible to the entomopathogenic fungi at 25 °C than 18 °C. *M. anisopliae* was highly and statically equally effective against nymphs of *M. coryli* at 18, 22 and 25 °C (86,0 %, 95,8 %, 100 %) after 5 days of conidial treatment. The other isolates; TR-05, TR-78.07 and TR-11 weren't so effective as *M. anisopliae* on nymphs of *M. coryli* at 25°C (66,6 %, 53,6 %, 33,2 %). This study showed that isolate of *M. anisopliae* has virulent and highly potential for biological control on nymphs of *M. coryli.*

Keywords: *Myzocallis coryli*, *Metarhizium anisopliae*, Entomopathogenic Fungi, Biological Control

1. Introduction
The hazelnut is one of the oldest crops known to humans and is native to the Black Sea coast. Approximately 430,000 hectares of hazelnut trees stretch across the Black Sea region of Turkey. With annual production of 600,000 ton, Turkey is the world's leading hazelnut producer and exporter supplying 80% of the world's hazelnuts [30].

The filbert aphid, *Myzocallis coryli* (Goetze), is a serious pest of hazelnut, *Corylus avellana* (L.) in North America, Italy, Spain, and Turkey [2, 4]. It usually appears in small populations, but in the years of heavy infestation it decreases yields through direct feeding on leaves as well as the honeydew production leading to growth of dark mould on leaf blades [5, 12, 20, 24, 37, 38]. The harmful pests affect yield and quality of Turkish hazelnuts and so most of the farmers use insecticides and spray 1-2 times every year for to protect their products [32, 33]. Use of pesticides in order to aphids control is commonly technique but pesticide use have some problems such as insect resistance to insecticides, the increase in secondary pests, pest ecological replacement, decrease of pesticide effects, environmental effects, carcinogenic and mutagenic effects and may lead to adverse effects generally [34].

Biological control agents (such as bacteria, viruses, fungi and protozoa) generally are not hazardous to humans and animals; they are biodegradable and environment friendly products, effective on target organisms, and no effective on the beneficial organisms, simple genetic modification [10]. Among the different microbial agents, entomopathogenic fungi are gaining importance in pest control. More than 750 species of fungi are pathogenic to insects and many of them offer a great potential for the management of sucking pests [28]. Application of entomopathogenic fungi is considered as a priority, as its use will facilitate a decrease in the harmful side effects of chemical pesticides [1]. The entomopathogenic fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin has been effective in controlling more than 200 species of insect pests [27]. The application of *M. anisopliae* has several advantages over the conventional chemical pesticides, such as limited harm to humans, honey bees, livestock, and crops [22]. The aim of this study was to investigate the virulence efficacy of some entomopathogenic fungi in controlling Filbert Aphid (*Myzocallis coryli* Goetze).

2. Materials and Methods
2.1 Isolation of *M. anisopliae* from insect: The *Metarhizium anisopliae* were isolated from infected insects [*Xylosandrus germanus* (Coleptera: Curculionidae: Scolytinae)] in hazelnuts orchards in the provinces of Samsun, Turkey. The insects were surface disinfected with 5% sodium hypochlorite and placed in an environmental chamber on a water agar medium amended with antibacterial agents, on moistened filter paper in a sealed container and incubated at 25±1ºC for fifteen days. The insects with hyphae were then transferred to selective medium for the isolation of *M. anisopliae*. The fungus was then grown on Potato dextrose agar (Hi-Media) fortified with 1% yeast extract at 25±1ºC in dark. Single-spore isolates were obtained by serial dilution [8] and identified as *M. anisopliae.*

2.2 Isolation of the 3 other fungal cultures: Fungal cultures were isolated from infected *Palomena prasina* (Heteroptera: Pentatomidae) and *Hyphantria cunea* (Lepidoptera: Arctiidae) in hazelnuts orchards in the provinces of Düzce and Samsun, Turkey. Single-spore isolates were obtained by serial dilution [8] and identified as *Lecanicillium muscarium* (isolate TR-05 from *P. prasina*), *Simplicillium lamellicola* (isolate TR-11 from *P. prasina*) and *Isaria fumosorosea* (isolate TR-78.07 from *H. cunea*). Isolates were maintained in tubes containing 6.5% Sabouraud dextrose agar (SDA) (Merck Ltd., Darmstadt, Germany) and deposited in the fungal culture collection of the Mycology Laboratory at the Ondokuz Mayis University, Faculty of Agriculture's Department of Plant Protection in Samsun, Turkey and in the USDA-ARS Entomopathogenic Fungal Culture Collection in Ithaca, NY (ARSEF 11731, 11737 and 12177 respectively).

2.3 Conidial germination assessment: The viability of conidia of *M. anisopliae* and the 3 other isolates (TR-05, TR-11 and TR-78.07) were evaluated using a method modified from [18]. A conidial suspension was adjusted to 1×10^4 conidia/mL, and 0.2 mL was sprayed onto 9-cm-dia. Petri plates containing potato dextrose agar (PDA) (Oxoid Ltd, Basingstoke, UK). Petri plates were maintained at 25±1°C. After 24 h of incubation, percentages of germinated conidia were counted using an Olympus CX-31 compound microscope at 400x magnification. Conidia were regarded as germinated when they produced a germ tube at least half of the conidial length. Germination ratios for each fungus were calculated after examining a minimum of 200 conidia from each of 3 replicate plates.

2.4 Inoculum of entomopathogen isolates: Isolate of *M. anisopliae* and the 3 other isolates TR-05, TR-11 and TR-78.07) were grown on SDA at 25±1°C for 15 days. Conidia were harvested with sterile distilled water containing 0.03% Tween 80. Mycelia were removed by filtering conidia suspensions through 4 layers of sterile cheesecloth. Conidia were counted under a compound microscope using a Neubauer hemocytometer to calibrate a suspension of 1×10^8 conidia/mL for each isolate.

2.5 Commercial products: The effects of *M. anisopliae* were compared with those of commercially available biocontrol products [Nostalgist BL (SL; *Beauveria bassiana*, Bb-1 %1.5, $1x10^8$ kob/ml min.) and Nibortem (SL; *Verticillium lecanii* V1-1 %1.5, $1x10^8$ kob/ml min.)] at a dosage: 250 mL Nostalgist BL /100 L water and 250 mL Nibortem /100 L water. The commercial products were diluted to recommended rates for used this study.

2.6 Insect rearing: The female adults of filbert aphid were collected from hazelnut orchards in Samsun, Turkey, during early June of 2015. They were reared as a group of 10 female adults separately on hazelnut leaves in 13 cm diameter dishes and established same groups with 1000 females in growth chamber (18 ± 1 °C, 22 ± 1 °C, 25 ± 1 °C; 75 ± 5% R.H; 16:8 h L:D). Females give birth and then nymphs collected and transferred to new 9 cm diameter dishes as a group of 10 nymphs separately on hazelnut leaves. One day old nymphs were used for the virulence tests.

2.7 Bioassay: First instar nymphs of *M. coryli* were placed on hazelnut leaves in 9 cm diameter dishes containing sterile water-soaked blotters (10 nymphs per dishes). Conidial suspensions of *M. anisopliae* and the 3 other entomopathogenic fungi (TR 05, TR 11, TR 78.07) and the 2 other products (Nibortem and Nostalgist BL) were applied to the first instar nymphs of *M. coryli* (2 mL per dishes) using a Potter spray tower (Burkard, Rickmansworth, Hertz UK). Control units were treated with sterile distilled water (2 mL). Each of dishes was loosely capped to prevent escape after applications. Dishes were incubated at 18 ± 1 °C, 22 ± 1 °C and 25 ± 1 °C at 75±5% and 16:8h L:D photoperiod for 5 days. All dishes were inspected daily. Dead nymphs of *M. coryli* were counted and removed into empty dishes. Mortality of nymphs was recorded on 1-5 days after treatment. The experiment was repeated 10 replicates per treatment.

2.8 Statistical analysis: The mortality percentage of nymphs for each isolates and products were analyzed using one way ANOVA (SPSS 21 for Windows); means were separated by Duncan's mean separation test. Mortality was considered significantly different at $P < 0.001$.

3. Results and Discussion

Dose-response relationship was determined for *M. anisopliae* and the other entomopathogenic fungi applied to the first instar nymphs of *M. coryli* at 3 different temperatures in laboratory conditions by using spraying method. The accumulated mortality recorded during 1-5 days and showed that all isolates were found effective against on nymphs at different rates (Figure 1, 2, 3). According to our study, significantly different effects on mortality were observed among different isolates and commercial products at 3 different temperatures ($p < 0.001$). Nymphs in control units survived to finish of experiments without any mortality. All living nymphs in all applications and control units were fed with hazelnut leaves in dishes.

3.1 Two days after application

M. anisopliae was the most efficacious in controlling first instar nymphs of *M. coryli* at 18, 22 and 25 °C temperatures (22, 8 %, 30,6 %, 36,4 %). The other isolates; TR-05 (*L. muscarium*), TR-78.07 (*I. fumosorosea*) and TR-11 (*S. lamellicola*) weren't so effective on nymphs of *M. coryli* at 18°C (13,8 %, 11,4 %, 7,0 %), 22°C (19,4 %, 12,0 %, 7,6 %) and 25°C (19,8 %, 16,2 %, 8,8 %) temperatures. The commercial biocontrol products (Nibortem and Nostalgist BL) were less pathogenic on nymphs of *M. coryli* at 3 different temperatures and mean mortality ranged from 3,0 to 5,0 % (Table 1).

3.2 Five days after application

All fungal isolates caused highly significant mortalities after 5 days of conidial treatments. *M. anisopliae* was highly and statically equally effective against nymphs of *M. coryli* at 18, 22 and 25 °C (86,0 %, 95,8 %, 100 %). The other isolates; TR-05 (*L. muscarium*), TR-78.07 (*I. fumosorosea*) and TR-11 (*S. lamellicola*) weren't so effective on nymphs of *M. coryli* at 18°C (48,2 %, 40,4 %, 23,2 %), 22°C (61,2 %, 48,2 %, 28,8 %) and 25°C (66,6 %, 53,6 %, 33,2 %) temperatures. The commercial biocontrol products (Nibortem and Nostalgist BL) were least pathogenic on nymphs of *M. coryli* at 3 different temperatures and mean mortality ranged from 14 to 24,6 % (Table 2).

Table 1: Mortality percentages of nymphs of *M. coryli* at 3 different temperatures after 2 days of application

Isolates and commercial products	Mortality percentage of nymphs after 2 days of application						
	18°C		22°C		25°C		P*
M. anisopliae	22,8 ± 0,86	a*C**	30,6 ± 0,68	aB	36,4 ± 1,36	aA	<0,001
TR-05	13,8 ± 0,58	bB	19,4 ± 0,93	bA	19,8 ± 0,58	bA	<0,001
TR-78.07	11,4 ± 0,60	cB	12,0 ± 0,71	cB	16,2 ± 0,86	cA	0,001
TR-11	7,0 ± 1,05	d	7,6 ± 0,40	d	8,8 ± 0,58	d	0,247
Nibortem	3,8 ± 0,49	e	3,8 ± 0,49	e	5,0 ± 0,32	e	0,125
Nostalgist BL	3,0 ± 0,45	e	3,6 ± 0,51	e	4,2 ± 0,37	e	0,207
P*	<0,001		<0,001		<0,001		

*The small letters within columns indicates significant differences between means (isolates)
**The capital letters within rows indicates significant differences between means (temperatures)

Table 2: Mortality percentages of nymphs of *M. coryli* at 3 different temperatures after 5 days of application

Isolates and commercial products	Mortality percentage of nymphs after 5 days of application						
	18°C		22°C		25°C		P*
M. anisopliae	86,0 ± 1,22	a*C**	95,8 ± 0,66	aB	100 ± 0,001	aA	<0,001
TR-05	48,2 ± 0,80	bC	61,2 ± 1,46	bB	66,6 ± 0,93	bA	<0,001
TR-78.07	40,4 ± 0,68	cC	48,2 ± 0,97	cB	53,6 ± 0,93	cA	<0,001
TR-11	23,2 ± 0,58	dC	28,8 ± 1,46	dB	33,2 ± 0,97	dA	<0,001
Nibortem	16,2 ± 0,58	eC	19,6 ± 0,81	eB	24,6 ± 0,81	eA	<0,001
Nostalgist BL	14,0 ± 1,05	eB	19,4 ± 0,81	eA	22,4 ± 1,29	eA	<0,001
P*	<0,001		<0,001		<0,001		

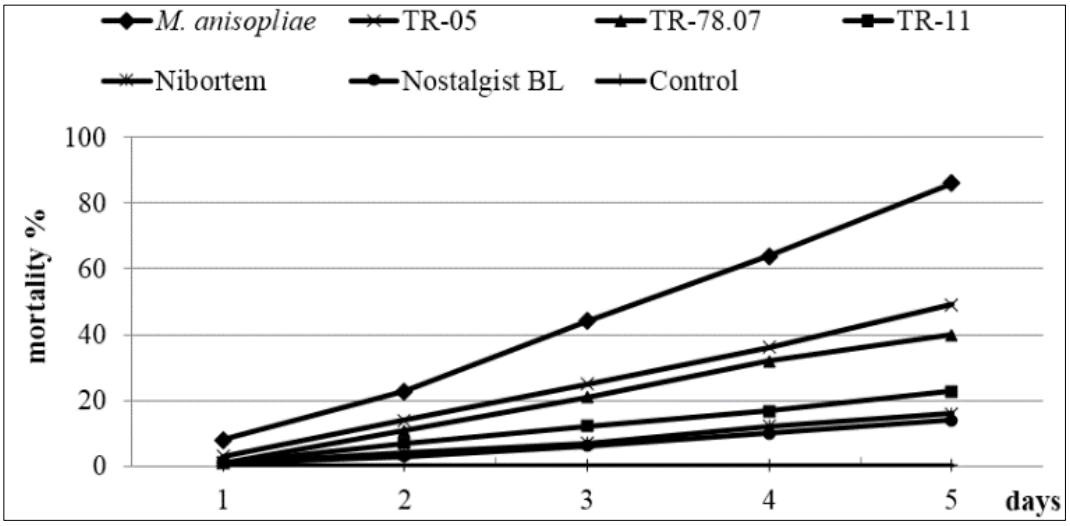

Fig 1: Cumulative mortality percentages of nymphs of *M. coryli* at 18°C

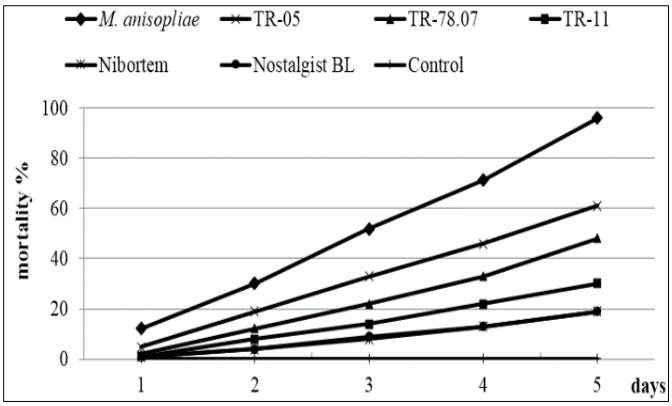

Fig 2: Cumulative mortality percentages of nymphs of *M. coryli* at 22°C

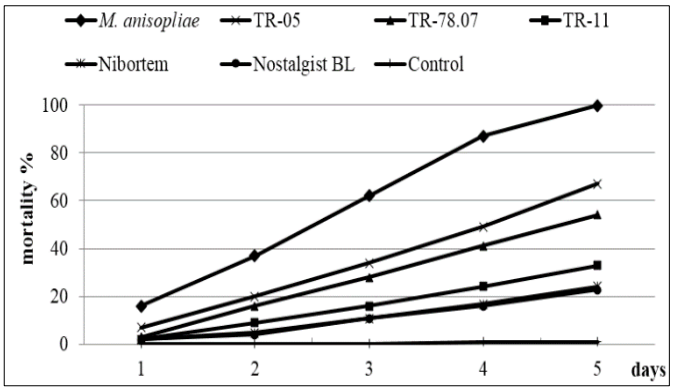

Fig 3: Cumulative mortality percentages of nymphs of *M. coryli* at 25°C

Isolate of *M. anisopliae*, isolated from *Xylosandrus germanus* (Coleptera: Curculionidae: Scolytinae), was the most efficacious in controlling nymphs of *M. coryli* at spraying method, especially at 25°C with 100 % mortality after 5 days of treatment. *L. muscarium* (TR-05) was the second most effective isolate in this study that was isolated from infected adults of *P. prasina* (Hemiptera: Pentatomidae). *I. fumosorosea* (TR-78.07) was isolated from infected pupae of *H. cunea*, and its mortality was lesser than *M. anisopliae*. *S. lamellicola* (TR-11) and 2 commercially biocontrol products weren't so effective on nymphs of *M. coryli* at spraying method.

Temperature can affect the germination and growth as well as the viability of an entomopathogenic fungus in the laboratory as well as in the field. *Metarhizium anisopliae* is a mesophilic fungus with a temperature range generally between 15 and 35°C, and the optimum for germination and growth between 25 and 30°C [23, 35, 29, 6, 14, 36, 11, 21].

Our results showed that first instar nymphs of *M. coryli* were more susceptible to the entomopathogenic fungi at 25 °C than 18 °C. The infection of *M. anisopliae* in *M. coryli* increased as temperature increased. Because temperature have significant effects on germination, radial growth and virulence of the various isolates. All the fungal isolates grew at 18, 22 and 25°C the temperatures in our study, and the most suitable temperature 25°C for all isolates. Ekesi *et al.* (1999) [11] and Dimbi *et al.* (2004) [9] reported that the optimum temperature for radial growth of most isolates of *M. anisopliae* was 25 and 30°C. Ouedraogo *et al.* (1997) [25] reported that the optimum temperature for vegetative growth of *M. anisopliae* isolates ranged between 25 and 32°C, with 25°C being the optimum for most isolates. Kuboka (2013) [17] pointed that the highest sporulation of 10^8 conidia/ml occurred at 25ºC, while the lowest sporulation 10^8 conidia/ml occurred at 15ºC, in addition at 25 and 30ºC, the all isolates induced 100% mortality to adult *F. occidentalis* in six days. Bugeme (2008) [7] pointed that the best fungal germination was observed at 25 and 30°C, while for the fungal radial growth it was 30°C on virulent to *Tetranychus evansi*.

4. Conclusion

Pesticides are currently the most common method to control aphids in Turkish hazelnut production. Because chemicals are easily available in the open market and are aggressively promoted by commercial manufacturers, they have become the most dominant feature of the hazelnut pest control landscape [3, 13, 19, 26, 31]. But, the filbert aphid is highly resistant to chemical pesticides as carbaryl [3], a number of organophosphates [16], and the synthetic pyrethroid, esfenvalerate [15].

The fungal biocontrol agents entomopathogenic fungi have been widely applied for insect control, such as aphids, and they dont cause to resistant on insects as chemical pesticides. It appears that the use of *M. anisopliae* can provide protection on hazelnuts against aphids and it can be effective as biocontrol agent on *M. coryli.* Spores of *M. anisopliae* can be developing and they can play a significant role in sustainable development of organic agriculture practices.

5. References

1. Ajamhassani M, Sendi JJ, Zibaee A, Askary H, Farsi MJ. Immunoliogical Responses of *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae) to Entomopathogenic Fungi, *Beauveria bassiana* (Bals.-Criy) and *Isaria farinosae* (Holmsk.) Fr. Journal of Plant Protection Research. 2013; 53(2):110-118.
2. Aliniazee MT. Insect and mite pests of filberts. Oregon Agricultural Experiment Station Bulletin. 1980; 643:13.
3. AliNiazee MT. Carbaryl resistance in the filbert aphid. J Econ. Entomol. 1983; 76:1002-4.
4. AliNiazee MT. Pests of Hazelnuts in North America: a review of their bionomics and ecology. Proc. Int. Congr Hazelnuts, Avellino, Italy, 1983, 6376.
5. Aliniazee MT. Insect pest management in hazelnut orchards of North America. Acta Horticulturae. 1997; 351:543-550.
6. Alves SB, Risco SH, Almeida LC. Influence of photoperiod and temperature on the development and sporulation of *Metarhizium anisopliae* (Metsch.) Sorok. Journal of Applied Entomology. 1984; 97:127-129.
7. Bugeme DM, Maniania NK, Knapp M, Boga HI. Effect of temperature on virulence of *Beauveria bassiana* and *Metarhizium anisopliae* isolates to *Tetranychus evansi*. Exp Appl Acarol. 2008; 46:275-285.
8. Dhingra OD, Sinclair JB. Basic plant pathology methods (2nd ed.). Boca Raton: CRC Press. 1995.
9. Dimbi S, Maniania NK, Lux SA, Mueke JM. Effect of constant temperatures on germination, radial growth and virulence of *Metarhizium anisopliae* to three species of African tephritid fruit flies. Biocontrol. 2004; 49:83-94.
10. Droby S, Chalutz E, Hofstein R, Wilson CL, Wisniewski ME, Fridlender B, *et al.* Pilot testing of *Pichia guilliermondiui*: a biocontrol agent of postharvest diseases of citrus fruit. Biological Control. 1993; 3:47-52.
11. Ekesi S, Maniania NK, Ampong-Nyarko K. Effect of temperature on germination, radial growth and virulence of *Metarhizium anisopliae* and *Beauveria bassiana* on *Megalurothrips sjostedti*. Biocontrol Sci Technol. 1999; 9:177-185.
12. Gantner M. Aphidofauna of hazel bushes (*Corylus* L.) on a protected plantation, an unprotected plantation and in a forest. Annales UMCS. 2000; 8:455-562.
13. Guarino L, Storti C, Vitagliano A. Protection of hazelnuts. Control of the principal diseases and insects by chemical and biological methods. *Terra Sole.* 1993; 48:614.
14. Hywel-Jones NL, Gillespie AT. Effect of temperature on spore germination in *Metarhizium anisopliae* and *Beauveria bassiana*. Mycological Research. 1990; 94:389-392.
15. Katundu JM. Status, distribution and seasonal variation of filbert aphid resistance to selected insecticides in the Willamette Valley, Oregon. MS thesis. Or. State Univ., Corvallis. 1986, 73.
16. Katundu JM, AliNiazee MT. Variable resistance of *Myzocallis coryli* to insecticide in the Willamette Valley, OR. *J Econ. Emtomol.* 1990; 83:41-47.
17. Kuboka MN. Effect of Temperature on the efficacy of *Metarhizium Anisopliae* (Metchnikoff) Sorokin in the control of western flower thrıps in french beans. 2013, 105.

18. Lazreg F, Huang Z, Ali S, Ren S. Effect of *Lecanicillium muscarium* on *Eretmocerus* sp. nr. *furuhashii* (Hymenoptera: Aphelinidae), a parasitoid of *Bemisia tabaci* (Hemiptera: Aleyrodidae). J Pest. Sci. 2009; 82:27-32.
19. Maeso J, Torell A, Barrios G. Testing of insecticides against the hazelnut weevil (*Curculio nucum* L.). Fulls Inf. Tec. No. 1988, 154-3.
20. Messing RH, Aliniazee MT. Natural enemies of *Myzocallis coryli* (*Hom. Aphididae*) in Oregon hazelnut orchards. J Entomol. Soc., 1985; 82:14-18.
21. Milner RJ, Lozano LB, Driver F, Hunter D. A comparative study of two Mexican isolates with an Australian isolate of *Metarhizium anisopliae* var. acridum-strain characterisation, temperature profile and virulence for wingless grasshopper, Phaulacridium vittatum. BioControl. 2003; 48:335-348.
22. Monique MT, Ravely CO, Daniela AL, João AP. Toxicity of the insect growth regulator lufenuron on the entomopathogenic fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin assessed by conidia germination speed parameter. Afr. J Biotechnol. 2011; 10(47):9661-9667.
23. Müller-Kögler E. Pilzkrankheiten bei Insekten. Anwendung zur biologischen Schadlingsbekampfung und Grundlagen der Insektenmykologie. Berlin: P. Parey. 1965, 444.
24. Naeem M, Compton S. Population dynamics of filbert aphid, *Myzocallis coryli* (Goetze) on hazel bushes to an agroforestry system. Pakistan J Biol. Sc., 2000; 3(2):306-308.
25. Ouedraogo A, Fargues J, Goettel MS, Lomer CJ. Effect of temperature on vegetative growth among isolates of *Metarhizium anisopliae* and M. flavoviride. Mycopathologia. 1997; 137:37-43.
26. Petanovic R, Dobrivojevic K, Boskovic R. Life-cycle of the hazelnut big-bud mite *Phytoptus avellanae* (Nal.) (Acarida: Eriophyoidea) and results of its control. Za t. Bilja. 1989; 40:433-41.
27. Pu ZL, Li ZZ. Insect Mycology. Anhui Sci. Technol. Press, Hefei, Anhui, China. 1996, 360-368.
28. Rabindra RJ, Ramanujam B. Microbial control of sucking pests using entomopathogenic fungi. Journal of Biological Control. 2007; 21(Special):21-28.
29. Roberts DW, Campbell AS. Stability of entomopathogenic fungi. Miscellaneous Publications of the Entomological Society of America. 1977; 10:19-76.
30. Spaulding AD, Tulum O, Saghaian S, Özertan G. Impact of a New Price Support Policy on the Turkish Hazelnut Industry. Acta Horticulturae, VII International Congress on Hazelnut. 2009: 845:795-800.
31. T´oth L. Data on weevils causing damage to hazel nuts and on control measures. N¨ov´enyv´edelem. 1984; 20:366-368.
32. Tuncer C, Akça I, Saruhan I. Integrated pest management in Turkish hazelnut orchards. Acta Hort. 2001; 556:419-429.
33. Tuncer C, Ecevit O. Current status of hazelnut pests in Turkey. Proceedings of the 4th International Symposium on Hazelnut. Acta Hort. 1997; 445:545-552.
34. Verkerk RHJ, Neugebauer KR, Ellis PR, Wright DJ. Aphids on cabbage: tritrophic and selective insecticide interactions. Bulletin of Entomological Research. 1998; 88:343-349.
35. Walstad JD, Anderson RF, Stambaugh WJ. Effects of environmental conditions on two species of muscardine fungi (*Beauveria bassiana* and *Metarhizium anisopliae*). Journal of Invertebrate Pathology. 1970; 16:221-226.
36. Welling M, Nachtigall G, Zimmermann G. Metarhizium spp. isolates from Madagascar: Morphology and effect of high temperature on growth and infectivity to the migratory locust, Locusta migratoria. Entomophaga. 1994; 39:351-361.
37. Wojciechowicz-Żytko E, Wojciechowicz A. Występowanie *Myzocallis coryli* Goetze (*Homoptera, Aphidodea*) na różnych odmianach leszczyny. Zesz. Nauk. AR w Krakowienr 387, sesja naukowa. 2002; 82:303-306.
38. Wojciechowicz-Żytko E. Development of *Myzocallis coryli* Goetze (*Homoptera, Aphidodea*) on the different hazel (*Corylus* L.) cultivars. J Plant Protection Res., 2003; 43(4):369-374.