AN EVALUATION OF SOME ENTOMOPATHOGENIC FUNGI FOR GREEN PEACH APHID (*MYZUS PERSICAE* [SULZER]), (HOMOPTERA: APHIDIDAE) UNDER LABORATORY CONDITONS

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Abstract. This study was carried out between 2014 and 2017 in Erzincan province Turkey. Our aim was to test pathogenesis of soil isolated entomopathogenic fungi from Erzincan and the *Myzus persicae* (Sulzer), (Homoptera: Aphididae). A total of 78 entomopathogenic fungi isolates including 63 *Beauveria bassiana* and 15 *Metarhizium anisopliae* were obtained. Our study was followed by incubation for 12 days and the first cases were seen on the third day. It was recorded that *B. bassiana* isolates caused the highest mortality rate on the 3rd day (BbEMRKZ2a, 10.50%); 5th day (BbEMRKZ5b and BbEÜ3, 22.39%); 7th day (MaEM3i, 45.71); 9th day (BbEMRKZ1a and BbER4, 50.00%), and after 12 days (BbEİ5, 62.54%). Also, it was recorded that *M. anisopliae* isolates caused the highest mortality rate on the 3rd day (MaEMR1a, 5.78%), 5th day (MaET3, 23.32%), 7th day (BbER4, 37.4%), 9th day (MaEİ3 50.84%), and after 12 days (MaET3, 60.01%). In the control group, the highest mortality rate was 1.12% at the end of the incubation period.
Keywords: *biocontrol, Beauveria bassiana, Metarhizium anisopliae, Myzus persicae*

Introduction

Aphids are one of the most destructive pests in agricultural production. They cause direct physical damage by extracting carbohydrates and amino acids from plant phloem and also indirectly through spreading a variety of viruses (Milner, 1997; Dedryver et al., 2010; Kim et al., 2013). Some of their species are cosmopolitan, such as; green peach aphid (*Myzus persicae* (Sulzer)), and black bean aphid (*Aphis fabae* Scopoli, 1763). they cause the large economic yield losses over the three hundred plants at the world (Aydemir, 2008; Dedryver et al., 2010; Kim et al., 2013). Most researcher reported that chemical pesticide application is the most commonly used method of aphid control, but it cannot wipe aphids out since aphids easily develop resistance to chemical insecticides and multiply very rapidly. Moreover, the over use of pesticide has resulted in environmental pollution as well as adverse effects on the health of humans and other organisms (Dedryver et al., 2010). As a principle, the increased volume of the world-wide trade of agricultural crop production also requires environmentally friendly pest control. Biocontrol is an alternative to chemical pesticides used in the management of plant pests (Ren and Chen, 2012). A group of them are microbial control agents and called entomopathogens (Clarkson and Charnley, 1996; Butt and Copping, 2000; Hajek, 2004). Entomopathogens (bacteria, fungi, virus, nematode, etc.) are living organisms used to kill insects and to create an epidemic disease that spreads rapidly but targets only the harmful pest (Clarkson and Charnley, 1996; Butt and Copping, 2000; Hajek, 2004). Entomopathogenic fungi (EPF) are the most important microbial pathogens of insect pests and they are unlike bacteria and viruses that have to be ingested to cause diseases, fungi typically infect insects by direct penetration of the cuticle followed by multiplication in

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the hemocoel (McCoy et al., 1998; Lacey et al., 2001; St. Kılıç and Yıldırım, 2008; St. Leger et al., 2011). EPF are approximately, 60% of insect diseases are caused by pathogenic fungi (Faria and Wraight, 2007). The approach thirty of commercial mycoinsecticides are known to infect aphids, and several species frequently cause naturally epizootics in aphid populations (Gustafsson, 1965; Thoizon, 1970; Balazy, 1993; Keller, 1997; Goettel et al., 2005; Kim et al., 2013). Most aphid-pathogenic fungi are in the order of Entomophthorales (Zygomycota), however, several Hypocreales (Ascomycota) genera, such as *Beauveria*, *Verticillium*, and *Paecilomyces*, are also known to infect aphids (Miller, 1997). Most researcher reported that we can use *B. bassiana and M. anisopliae* as microbial control agents for aphids which have a wide host range and widely distributed in all regions of the world, in addition both species can be easily isolated from insects and soil (Butt, 2004; Meyling et al., 2006; Freed et al., 2011a, b). So far, a variety of strains of B. *bassiana* and *M. anisopliae* have been used for the control of aphids (Jackson et al., 1985; Steenberg and Humber, 1999; Devi et al., 2003; Kim, 2004; Shia and Feng, 2004; Quesada-Moraga et al., 2006; Kim et al., 2010). In the present study, it was aimed to determine the pathogenicity of 63 isolates of *B. bassiana*, and 15 isolates of *M. anisopliae*, an entomopathogenic fungi, taken from Erzincan province and isolated from soil and control *M. persicae* under laboratory conditions .

Materials and methods

Aphid culture

The green peach aphid (*Myzus persicae* (Sulzer)) (Homoptera: Aphididae) were collected from different fields in 2016-2017-2018 at Erzincan. The aphids were reared on common bean, *Phaseolus vulgaris* L. under laboratory conditions [(25 °C ± 2 and 70% ± 10 R.H.) (16: 8 h (L:D)] (Kim et al., 2013).

Collecting soil samples

The province of Erzincan (39°02′N to 40°05′N, 38°16E to 40°45′E) covers ca. 11,900 km^2 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, Üzümlü, Tercan, Mercan, Kemaliye, Kemah, İliç, Çayırlı, Otlukbeli, Refahiye; *Fig. 1*).

Figure 1. *Soil samples were collected from different geographical sites distributed through the Erzincan province*

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Soil samples were collected with a garden spade to a depth of 20 cm after removal of surface litter. At every site, five 500 g soil samples were collected from five randomly selected points from an area of 50 cm^2, placed in clear plastic bags (30-25 cm), sealed with a rubber band and returned to the laboratory. There were 30 samples from cultivated habitats (24 samples from field crops, 1 sample from fruit and vegetable crops, 4 samples from vegetable crops, 1 sample from sugar beet crops) and 30 samples from natural habitats (26 samples from natural pastures, 3 samples from forest, 1 sample from meadow).

Isolation and identification of fungi

Insect-associated fungi were isolated from soil samples by using 'Galleria bait method' (Zimmermann, 1986). The wax moth larvae, *Galleria mellonella* L., were reared continuously in constant darkness at 28 °C. The third or fourth instar larvae (approximately 30 days after hatching) were used as baits. Ten larvae were placed on the soil samples in each box and covered with a lid and incubated at 25 ± 1 °C for two weeks. The larvae were examined on days 7 and 14 after inoculation. Surface of dead larvae were sterilized by 3% sodium hypochlorite for 3 min and then rinsed twice with sterile distilled water. After removing free water of the larvae surface, they were placed onto PDA plates. The fungi were identified using morphological characteristics of reproductive structures with the aid of relevant taxonomic literature (De Hoog, 1972; Samson et al., 1988; Humber, 1998; Luangsa-Ard et al., 2007). As a result, we obtained 78 fungal isolates and we gave a code for every isolates. These fungal cultures consist of the 63 isolates of *B. bassiana*, and 15 isolates of *Metarhizium anisopliae.* They were isolated from soil at Erzincan province (2014-2016) (*Table 1*).

Preparation of conidial suspension

Conidia of B. bassiana and *M. anisopliae* isolates were harvested by scraping the surface of 3-week-old sporulating cultures grown on Potato Dextrose Agar (PDA). The spores were harvested 0.01% Tween 80 and drained with chrsee cloth into the sterilized glass Erlenmeyer flasks. Then it was rinsed on a rinsing device for 5 min. After that, the spores were counted in the suspensions using a hemocytometer to 3×10^7 spores/ml (Thakur and Sandhu, 2010).

Incubation of fungal spores and the treatment of aphids

The following leaf dipping technique was used as described by Krutmuang and Mekchay, 2005; Ghatwary, 2000. The discs of common bean leaves were prepared, dipped in the tested spore suspensions for 10 s, then left to dry at room temperature and provided to the aphid in Petri dishes. In order to keep the leaves alıve for 12 days, leaf stems were covered with a sterilized cotton roll in the size of 40 × 60 mm and 2 ml sterilized and distilled water containing 1% of NPK (20-20-20) fertilizer In addition to keep the humidity at 100%, 3 ml of sterilized and distilled water was added on the filter paper. To prevent the leaves from contacting the wet surface, a plastic circular sheet with a diameter of 5 cm was placed under them. After each treatment, sides of Petri dishes were covered with parafilm. Treatments were repeated three times for each fungus isolates (*Table 1*). For control treatment, the same process was followed but 3 ml of sterilized and distilled water with 0.01% of Tween 80 was used instead of fungus isolates. After application of 25 aphid nymphs, were used for each treated leaves and incubated at (25 °C ± 2 and 70% ± 10) humididy.

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Table 1. *Fungal material and their geographical origin (2014-2016) at Erzincan*

Fungi species	**No**	**Code**	**Substrate (soil)**	**Geographical origin of isolates**
Beauveria bassiana	1	BbEMRKZ1a	Vegetable field	CENTER
	2	BbEMRKZ1b		
	3	BbEMRKZ2a	Fruit garden	
	4	BbEMRKZ2b		
	5	BbEMRKZ3	Field (barley-wheat)	
	6	BbEMRKZ4a	Meadow-grassland	
	7	BbEMRKZ4b		
	8	BbEMRKZ5a	Forest	
	9	BbEMRKZ5b		
	10	BbEÜ1a	Vegetable field	Üzümlü
	11	BbEÜ1b		
	12	BbEÜ2a	Fruit garden	
	13	BbEÜ2b		
	14	BbEÜ3	Field (barley-wheat)	
	15	BbEÜ4	Meadow-grassland	
	16	BbEÜ5a	Forest	
	17	BbEÜ5b		
	18	BbEK1a	Vegetable field	**Kemah**
	19	BbEK1b		
	20	BbEK2a	Fruit garden	
	21	BbEK2b		
	22	BbEK3a	Field (barley-wheat)	
	23	BbEK3b		
	24	BbEK4	Meadow-grassland	
	25	BbEK5a	Forest	
	26	BbEK5b		
	27	BbEİ1	Vegetable field	**İliç**
	28	BbEİ2	Fruit garden	
	29	BbEİ3	Field (barley-wheat)	
	30	BbEİ4	Meadow-grassland	
	31	BbEİ5	Forest	
	32	BbEKLY1	Vegetable field	Kemaliye
	33	BbEKLY2a	Fruit garden	
	34	BbEKLY2b		
	35	BbEKLY4	Meadow-grassland	
	36	BbEKLY5	Forest	
	37	BbER1	Vegetable field	Refahiye
	38	BbER2	Fruit garden	
	39	BbER3	Field (barley-wheat)	
	40	BbER4	Meadow-grassland	
	41	BbER5a	Forest	
	42	BbER5b		
	43	BbER5c		
	44	BbEM1	Vegetable field	Mercan
	45	BbEM2	Fruit garden	

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	46	BbEM3	Field (barley-wheat)	
	47	BbEM4	Meadow-grassland	
	48	BbEM5	Forest	
	49	BbET1	Vegetable field	Tercan
	50	BbET2	Fruit garden	
	51	BbET3	Field (barley-wheat)	
	52	BbET4	Meadow-grassland	
	53	BbET5	Forest	
	54	BbEÇ1	Vegetable field	Çayırlı
	55	BbEÇ2	Fruit garden	
	56	BbEÇ3	Field (barley-wheat)	
	57	BbEÇ4	Meadow-grassland	
	58	BbEÇ5	Forest	
	59	BbEO1	Vegetable field	Otlukbeli
	60	BbEO2	Fruit garden	
	61	BbEO3	Field (barley-wheat)	
	62	BbEO4	Meadow-grassland	
	63	BbEO5	Forest	
Metarhizium anisopliae	64	MaEMR1a	Vegetable field	Merkez
	65	MaEMR2b		
	66	MaEÜ1a	Vegetable field	Üzümlü
	67	MaEÜ1b		
	68	MaEK1a	Vegetable field	Kemah
	69	MaEk1b		
	70	MaEİ3	Field (barley-wheat)	İliç
	71	MaEKLY1a	Fruit garden	Kemaliye
	72	MaEKLY1b		
	73	MaER3	Field (barley-wheat)	Refahiye
	74	MaEM1	Vegetable field	Mercan
	75	MaEM3	Field (barley-wheat)	
	76	MaET3	Field (barley-wheat)	Tercan
	77	MaEÇ3	Field (barley-wheat)	Çayırlı
	78	MaEO3	Field (barley-wheat)	Otlukbeli

Statistical analysis

In order to determine the pathogenicity of *B. bassiana* and *M. anisopliae* isolates on aphids, dead and alive insects were checked every two days after the treatment. On the first 3 days there were no death cases and so the values were taken as 0 (zero). In the case when measurement number is below 50, angle transformation is applied to adjust the value of 0% to the normal distribution. In the present study, data were adjusted for arc-sin transformation to normalize the statistical distribution.

The incection values measured at the 1-12th days were subjected to the one way analysis of variance (ANOVA; $\alpha = 0.05$) using 11.0 SPSS software for Windows (SPSS Inc., 2002). Comparison of the isolates found to be different from the controls was made using Tukey test ($\alpha = 0.05$) in ANOVA.

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Results

Pathogenicity of B. bassiana on M. persicae

The infection rates of the 63 *B. bassiana* isolates at 3×10^7 conidia/ml consatration on larval stages of *M. persicae.* Were shown at *Table 2* . Within 12 days post inoculation the first deaths were seen on the third day. At the end of the 3rd day, BbEMRKZ2a isolate had the highest mortality rate with 10.50%. On the 5th day, BbEMRKZ5b and BbEÜ3, isolates had the highest mortality rate with 22.39%, while BbEM5 and BbEO2 isolates showed the lowest mortality rate of 9.04%. On the 7th day, BbER4 isolate had the highest mortality rate with 37.4%, while, BbEM5 and BbEO2, isolates showed the lowest mortality rate of 19.84%. On the 9th day, BbEMRKZ1a and BbER4 isolates had the highest mortality rate with 50.00%, while BbEKLY2b isolate showed the lowest mortality rate of 31.64%. On the 12th day, BbEİ5 isolate had the highest mortality rate with 62.54%, while BbEMRKZ5a, BbEÜ2b, BbEKLY4, BbEM5 and BbEO2 isolates showed the lowest mortality rate of 49.17%. In the control group, the highest mortality rate was 1.12% at the end of the incubation period.

Table 2. *Corrected percentage mortality of B. bassiana isolates (at spore concentration: 3×10^7 conidia/ml) on M. persicae (%Mean ± StDev) 12 days post inoculation*

Isolation of *B. bassiana*	3rd day	5th day	7th day	9th day	12th day
	Mean ± StDev	Mean ± StDev	Mean ± StDev	Mean ± StDev	Mean ± StDev
BbEMRKZ1a	4.79^{A}±0.84	$18.88^{A\ B}$±0.67	35.75^{A-C}±0.43	50.00^{A}±0.19	$61.67^{A\ B}$±0.02
BbEMRKZ1b	4.08^{A}±0.84	$21.46^{A\ B}$±0.53	$31.62^{A\ -\ E}$±0.17	$40.80^{A-\ F}$±0.28	$60.86^{A\ B}$±0.15
BbEMRKZ2a	10.64^{A}±2.16	$18.32^{A\ B}$±0.03	$31.59^{A-\ E}$±0.31	$40.82^{A-\ F}$±0.15	$55.02^{A\ B}$±0.25
BbEMRKZ2b	3.24^{A}±0.84	$18.12^{A\ B}$±0.48	$27.42^{A-\ E}$±0.24	$40.82^{A-\ F}$±0.09	$55.00^{A\ B}$±0.06
BbEMRKZ3	3.24^{A}±1.69	$19.63^{A\ B}$±0.91	32.39^{A-E}±0.49	45.00^{A-E}±0.06	$52.50^{A\ B}$±0.06
BbEMRKZ4a	6.61^{A}±1.34	$19.96^{A\ B}$±0.10	$31.62^{A-\ E}$±0.17	$40.82^{A-\ F}$±0.15	$55.83^{A\ B}$±0.02
BbEMRKZ4b	3.24^{A}±1.34	$16.60^{A\ B}$±0.14	$32.43^{A-\ E}$±0.29	$42.49^{A-\ F}$±0.06	$51.67^{A\ B}$±0.02
BbEMRKZ5a	8.30^{A}±0.84	$17.45^{A\ B}$±0.11	33.31^{A-E}±0.10	$40.83^{A-\ F}$±0.02	49.17^{B}±0.02
BbEMRKZ5b	7.79^{A}±2.54	$22.39^{A\ B}$±0.28	34.99^{A-D}±0.07	$44.16^{A-\ F}$±0.02	$61.68^{A\ B}$±0.09
BbEÜ1a	6.25^{A}±1.34	$16.54^{A\ B}$±0.27	$26.61^{A-\ E}$±0.19	$39.99^{A-\ F}$±0.07	$53.34^{A\ B}$±0.08
BbEÜ1b	6.61^{A}±1.34	$19.96^{A\ B}$±0.10	31.62^{A-E}±0.17	$40.82^{A-\ F}$±0.15	$55.83^{A\ B}$±0.02
BbEÜ2a	3.24^{A}±1.34	$16.60^{A\ B}$±0.14	$32.43^{A-\ E}$±0.29	$42.49^{A-\ F}$±0.06	$51.67^{A\ B}$±0.02
BbEÜ2b	8.30^{A}±0.84	$17.45^{A\ B}$±0.11	$33.31^{A-\ E}$±0.10	40.83^{A-F}±0.02	49.17^{B}±0.02
BbEÜ3	7.79^{A}±2.54	$22.39^{A\ B}$±0.28	$34.99^{A-\ D}$±0.07	$44.16^{A-\ F}$±0.02	$61.68^{A\ B}$±0.09
BbEÜ4	4.79^{A}±1.34	$19.15^{A\ B}$±0.03	$29.14^{A-\ E}$±0.10	$41.65^{A-\ F}$±0.15	$53.34^{A\ B}$±0.08
BbEÜ5a	8.75^{A}±2.54	$20.75^{A\ B}$±0.22	$34.13^{A-\ E}$±0.16	42.49^{A-F}±0.06	$60.03^{A\ B}$±0.26
BbEÜ5b	5.44^{A}±1.34	$17.31^{A\ B}$±0.44	$26.61^{A\ -\ E}$±0.19	$39.15^{A-\ F}$±0.09	$54.17^{A\ B}$±0.15
BbEK1a	3.24^{A}±1.34	$19.63^{A\ B}$±0.91	$33.26^{A-\ E}$±0.37	45.00^{A-E}±0.06	$53.33^{A\ B}$±0.02
BbEK1b	4.79^{A}±1.34	$19.15^{A\ B}$±0.03	$29.14^{A-\ E}$±0.10	41.65^{A-F}±0.15	$53.34^{A\ B}$±0.08
BbEK2a	5.55^{A}±0.00	$19.87^{A\ B}$±0.31	$35.83^{A\ -C}$±0.02	$40.83^{A-\ F}$±0.02	$55.84^{A\ B}$±0.08
BbEK2b	4.79^{A}±0.15	$16.60^{A\ B}$±0.14	$31.62^{A-\ E}$±0.17	41.66^{A-F}±0.09	$50.00^{A\ B}$±0.06
BbEK3a	7.37^{A}±0.00	$20.75^{A\ B}$±0.22	32.48^{A-E}±0.07	42.49^{A-F}±0.06	$53.35^{A\ B}$±0.34
BbEK3b	6.64^{A}±2.54	$20.75^{A\ B}$±0.22	$33.26^{A-\ E}$±0.31	$43.33^{A-\ F}$±0.02	$58.39^{A\ B}$±0.60
BbEK4	4.79^{A}±0.84	$19.96^{A\ B}$±0.10	30.83^{A-E}±0.02	$41.65^{A-\ F}$±0.15	$55.00^{A\ B}$±0.06
BbEK5a	3.24^{A}±1.69	$19.63^{A\ B}$±0.91	32.39^{A-E}±0.49	$45.00^{A-\ E}$±0.06	$52.50^{A\ B}$±0.06
BbEK5b	6.61^{A}±1.34	$19.96^{A\ B}$±0.10	31.62^{A-E}±0.17	$40.82^{A-\ F}$±0.15	$55.83^{A\ B}$±0.02

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BbEİ1	3.88^{A}±0.84	17.37$^{A\ B}$±0.31	34.99^{A-D}±0.07	40.83^{A-F}±0.02	53.34$^{A\ B}$±0.15
BbEİ2	5.78^{A}±1.34	15.71$^{A\ B}$±0.27	29.94^{A-E}±0.22	39.96$^{A-\ F}$±0.26	50.83$^{A\ B}$±0.08
BbEİ3	4.79^{A}±0.84	17.45$^{A\ B}$±0.11	28.89^{A-E}±0.96	44.15$^{A\ -\ F}$±0.15	55.85$^{A\ B}$±0.15
BbEİ4	3.24^{A}±0.84	19.15$^{A\ B}$±0.03	29.98^{A-E}±0.07	38.33^{A-F}±0.02	60.86$^{A\ B}$±0.15
BbEİ5	3.24^{A}±0.84	16.21$^{A\ B}$±1.04	33.30^{A-E}±0.16	45.00^{A-E}±0.06	62.54^{A}±0.27
BbEKLY1	2.50^{A}±0.84	13.01$^{A\ B}$±0.67	30.72^{A-E}±0.46	39.96$^{A-\ F}$±0.26	53.33$^{A\ B}$±0.02
BbEKLY2a	3.24^{A}±0.84	16.60$^{A\ B}$±0.14	25.77$^{A-\ E}$±0.19	43.33$^{A-\ F}$±0.02	52.50$^{A\ B}$±0.06
BbEKLY2b	1.12^{A}±0.00	11.09$^{A\ B}$±1.15	21.35^{C-E}±0.85	31.64^{F}±0.10	50.00$^{A\ B}$±0.06
BbEKLY4	1.12^{A}±0.00	9.91$^{A\ B}$±0.18	21.62^{B-E}±0.12	34.99$^{C\ -F}$±0.07	49.17^{B}±0.02
BbEKLY5	0.56^{A}±0.84	11.64$^{A\ B}$±0.05	23.32^{B-E}±0.03	35.83$^{B-\ F}$±0.02	50.83$^{A\ B}$±0.08
BbER1	2.24^{A}±1.34	9.91$^{A\ B}$±0.18	23.32^{B-E}±0.03	31.66^{F}±0.02	52.52$^{A\ B}$±0.44
BbER2	2.78^{A}±0.84	14.15$^{A\ B}$±0.04	27.43^{A-E}±0.23	38.33$^{A-\ F}$±0.02	54.17$^{A\ B}$±0.02
BbER3	3.24^{A}±0.84	21.66$^{A\ B}$±0.03	36.66^{A-C}±0.02	45.83^{A-D}±0.02	58.36$^{A\ B}$±0.28
BbER4	3.88^{A}±0.84	19.69$^{A\ B}$±0.75	37.41$^{A\ B}$±0.48	50.00^{A}±0.19	60.01$^{A\ B}$±0.07
BbER5a	4.08^{A}±0.84	21.46$^{A\ B}$±0.53	31.62$^{A\ -E}$±0.17	40.80$^{A-\ F}$±0.28	60.86$^{A\ B}$±0.15
BbER5b	10.64^{A}±2.16	18.32$^{A\ B}$±0.03	31.59$^{A-\ E}$±0.31	40.82^{A-F}±0.15	55.02$^{A\ B}$±0.25
BbER5c	3.24^{A}±0.84	18.12$^{A\ B}$±0.48	27.42$^{A-\ E}$±0.24	40.82$^{A-\ F}$±0.09	55.00$^{A\ B}$±0.06
BbEM1	3.24^{A}±1.69	19.63$^{A\ B}$±0.91	32.39$^{A-\ E}$±0.49	45.00^{A-E}±0.06	52.50$^{A\ B}$±0.06
BbEM2	3.88^{A}±0.84	12.30$^{A\ B}$±0.40	29.12^{A-E}±0.18	39.96$^{A-\ F}$±0.26	54.17$^{A\ B}$±0.02
BbEM3	2.50^{A}±0.84	18.27$^{A\ B}$±0.14	30.72^{A-E}±0.46	44.16$^{A-\ F}$±0.02	53.33$^{A\ B}$±0.02
BbEM4	3.24^{A}±0.84	13.73$^{A\ B}$±0.89	24.04$^{A-\ E}$±0.36	35.75$^{B-\ F}$±0.43	50.00$^{A\ B}$±0.06
BbEM5	0.28^{A}±0.00	9.04^{B}±0.23	19.84$^{D\ E}$±0.40	33.30^{D-F}±0.16	49.17^{B}±0.02
BbET1	1.63^{A}±0.84	10.81$^{A\ B}$±0.05	21.66^{B-E}±0.03	35.83$^{B-\ F}$±0.02	50.00$^{A\ B}$±0.06
BbET2	1.12^{A}±0.84	14.84$^{A\ B}$±0.35	28.30$^{A-\ E}$±0.10	40.80$^{A-\ F}$±0.28	52.50$^{A\ B}$±0.00
BbET3	4.79^{A}±1.34	21.52$^{A\ B}$±0.39	33.24$^{A\ -E}$±0.44	45.00^{A-E}±0.06	53.34$^{A\ B}$±0.08
BbET4	6.61^{A}±1.69	21.66$^{A\ B}$±0.03	32.45$^{A-\ E}$±0.22	39.16$^{A-\ F}$±0.02	56.67$^{A\ B}$±0.02
BbET5	3.24^{A}±1.34	16.60$^{A\ B}$±0.14	32.43$^{A-\ E}$±0.29	42.49^{A-F}±0.06	51.67$^{A\ B}$±0.02
BbEÇ1	4.79^{A}±0.84	14.15$^{A\ B}$±0.04	27.28$^{A-\ E}$±0.72	38.32$^{A-\ F}$±0.09	50.83$^{A\ B}$±0.08
BbEÇ2	8.07^{A}±0.47	20.75$^{A\ B}$±0.22	35.77$^{A-\ C}$±0.30	45.83^{A-D}±0.02	58.34$^{A\ B}$±0.09
BbEÇ3	0.28^{A}±0.84	13.91$^{A\ B}$±0.54	27.86$^{A-\ E}$±1.79	39.10$^{A-\ F}$±0.54	54.24$^{A\ B}$±0.92
BbEÇ4	0.00^{A}±0.00	10.81$^{A\ B}$±0.05	23.32^{B-E}±0.03	34.99$^{C\ -F}$±0.07	51.67$^{A\ B}$±0.02
BbEÇ5	0.28^{A}±0.00	12.91$^{A\ B}$±0.87	24.16$^{A-\ E}$±0.03	35.71$^{B-\ F}$±0.69	53.35$^{A\ B}$±0.40
BbEO1	3.24^{A}±0.84	13.73$^{A\ B}$±0.89	24.04$^{A\ -E}$±0.36	35.75^{B-F}±0.43	50.00$^{A\ B}$±0.06
BbEO2	0.28^{A}±0.00	9.04^{B}±0.23	19.84$^{D\ E}$±0.40	33.30$^{D\ -F}$±0.16	49.17^{B}±0.02
BbEO3	1.63^{A}±0.84	10.81$^{A\ B}$±0.05	21.66$^{B-\ E}$±0.03	35.83^{B-F}±0.02	50.00$^{A\ B}$±0.06
BbEO4	1.12^{A}±0.84	14.84$^{A\ B}$±0.35	28.30$^{A-\ E}$±0.10	40.80$^{A-\ F}$±0.28	52.50$^{A\ B}$±0.00
BbEO5	4.79^{A}±1.34	21.52$^{A\ B}$±0.39	33.24^{A-E}±0.44	45.00^{A-E}±0.06	53.34$^{A\ B}$±0.08
control	0.28^{A}±0.84	0.28^{C}±0.84	0.28^{F}±0.84	1.12^{G}±0.84	1.12^{C}±0.84

Means within columns with the same letter are not statistically different (Tukey's test at $p \leq 0.05$)

Pathogenicity of M. anisopliae on M. persicae

The infection rates of the 63 *B. bassiana* isolates at 3×10^7 conidia/ml on larval stages of *M. persicae* were showed in *Table 3.* Within 12 days post inoculation the first deaths were observed 3rd. day. At the end of the 3rd day, MaEMR1a, isolate had the highest mortality rate with 5.78%. On the 5th day, MaET3 isolate had the highest

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mortality rate with 23.32%, while MaEMR2b, MaEÜ1a, MaEKLY1a MaEÇ3 MaEO3 isolates showed the lowest mortality rate of 9.91%. On the 7th day, MaEM3 isolates had the highest mortality rate with 45.71%, while MaER3 isolates showed the lowest mortality rate of 22.08%. On the 9th day, MaEİ3 isolate had the highest mortality rate with 50.84%, while MaER3 isolate showed the lowest mortality rate of 32.48%. On the 12th day, MaET3isolates had the highest mortality rate with 60.01%, while MaEKLY1a and MaEKLY1b isolates showed the lowest mortality rate of 49.17%. In the control group, the highest mortality rate was 1.12% at the end of the incubation period.

Table 3. *Corrected percentage mortality of M. anisopliae isolates (at spore concentration: 3 × 10^{7}conidia/ml) on M. persicae (%Mean ± StDev) 12 days post inoculation*

Isolates of *M. anisopliae*	3rd day	5th day	7th day	9th day	12th day
	Means ± StDev.	Means ± StDev.	Means ± StDev.	Means ± StDev.	Means ± StDev.
MaEMR1a	$5.78^{A} \pm 1.69$	$18.12^{A} \pm 0.48$	$27.28^{A-E} \pm 0.72$	$34.89^{C-F} \pm 0.49$	$50.82^{A\,B} \pm 0.90$
MaEMR2b	$1.63^{A} \pm 0.84$	$9.91^{A\,B} \pm 0.18$	$19.06^{E} \pm 0.24$	$34.13^{C\,D\,E\,F} \pm 0.16$	$51.67^{A\,B} \pm 0.08$
MaEÜ1a	$0.28^{A} \pm 0.00$	$9.91^{A\,B} \pm 0.18$	$23.29^{B-E} \pm 0.12$	$41.58^{A-F} \pm 0.68$	$53.35^{A\,B} \pm 0.40$
MaEÜ1b	$0.00^{A} \pm 0.00$	$11.47^{AB} \pm 0.38$	$24.16^{A-E} \pm 0.03$	$46.66^{A-C} \pm 0.08$	$58.35^{A\,B} \pm 0.15$
MaEK1a	$0.00^{A} \pm 0.00$	$19.06^{AB} \pm 0.24$	$34.99^{A-D} \pm 0.07$	$50.83^{A} \pm 0.08$	$58.34^{A\,B} \pm 0.02$
MaEk1b	$0.00^{A} \pm 0.00$	$18.91^{A} \pm 0.59$	$37.49^{A} \pm 0.07$	$50.83^{A} \pm 0.02$	$60.00^{A\,B} \pm 0.00$
MaEİ3	$0.00^{A} \pm 0.00$	$18.27^{AB} \pm 0.14$	$33.26^{A-E} \pm 0.31$	$50.84^{A} \pm 0.15$	$59.17^{A\,B} \pm 0.02$
MaEKLY1a	$0.00^{A} \pm 0.00$	$9.91^{A\,B} \pm 0.18$	$21.62^{B-E} \pm 0.12$	$34.99^{C-F} \pm 0.07$	$49.17^{B} \pm 0.02$
MaEKLY1b	$0.56^{A} \pm 0.84$	$11.64^{A-} \pm 0.05$	$22.50^{B-E} \pm 0.00$	$35.83^{B-F} \pm 0.02$	$49.17^{B} \pm 0.08$
MaER3	$0.56^{A} \pm 0.84$	$11.64^{AB} \pm 0.05$	$24.16^{A-E} \pm 0.03$	$32.48^{E\,F} \pm 0.07$	$50.00^{A\,B} \pm 0.06$
MaEM1	$2.24^{A} \pm 1.34$	$11.47^{A} \pm 0.38$	$24.16^{A-E} \pm 0.03$	$35.82^{B-F} \pm 0.09$	$56.67^{A\,B} \pm 0.09$
MaEM3	$3.88^{A} \pm 0.84$	$19.06^{AB} \pm 0.24$	$34.99^{A-D} \pm 0.07$	$43.32^{A-F} \pm 0.09$	$55.86^{A\,B} \pm 0.34$
MaET3	$4.79^{A} \pm 0.00$	$23.32^{A} \pm 0.03$	$39.99^{A} \pm 0.07$	$48.33^{A\,B} \pm 0.15$	$60.01^{A\,B} \pm 0.07$
MaEÇ3	$1.63^{A} \pm 0.84$	$9.91^{A\,B} \pm 0.18$	$19.06^{E} \pm 0.24$	$34.13^{C-F} \pm 0.16$	$51.67^{A\,B} \pm 0.08$
MaEO3	$0.28^{A} \pm 0.00$	$9.91^{A\,B} \pm 0.18$	$23.29^{B-E} \pm 0.12$	$41.58^{A-F} \pm 0.68$	$53.35^{A\,B} \pm 0.40$
Control	$0.28^{A} \pm 0.84$	$0.28^{C} \pm 0.84$	$0.28^{F} \pm 0.84$	$1.12^{G} \pm 0.84$	$1.12^{C} \pm 0.84$

Means within columns with the same letter are not statistically different (Tukey's test at $p \leq 0.05$)

It is observed that the death rates have increased as time progressed for all isolates of both species. As the entomopathogenic fungi feed in the host and complete their development, secondary metabolites are secreted to kill the host therefore, the host is dying more rapidly. At the same time, the fungus develops inside the host and due to the breakdown the integument of the intestine hyphae appear on the host body surface.

Discussion

Cosmopolitan species such as *Beauveria bassiana* and *Metarhizium anisopliae* from entomopathogenic fungi have a large spectrum of arthropod hosts. They originally are isolated from soil. Most aphid-pathogenic fungi are in the order of Entomophthorales (Zygomycota), however, several Hypocreales (Ascomycota) genera, such as *Beauveria*,

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Verticillium, and *Paecilomyces*, are also known to infect aphids (Miller, 1997). *B. bassiana* ısolates were isolated from all types of area but *M. anisopliae* were obtained only cultural area (*Table 1*). ***B. bassiana*** isolates can be used successfully as a commercial. But an analysis of the results showed that there was a significant difference in the fungal virulence against *M. persicae* (Tukey's test at $p \leq 0.05$). Our *B. bassiana* isolates had the highest mortality rate with 63.35%. Also, *M. anisopliae* had the highest mortality rate with 65.84%. The results of our research overlap with previous research (Vu et al., 2007; Kim et al., 2013). Adult *M. persicae* were tested with isolates by using bait and direct contact methods. Results showed that the adult of *M. persicae* showed differences in susceptibility in the two species of *B. bassiana* and *M. anisopliae*. Our results show us that the isolate that kills as soon as possible and the isolate which has the highest mortality rate at the end of the 12- day incubation period are different from each other. Also, the virulence of the isolates of both entomopathogenic fungi are different and however, they did not show stability, different isolates were found to have different rates of mortality during this 12-day incubation period (*Tables 2* and *3*).

Conclusion

Entomopathogenic fungi are used succesfully in the control of pest insects and especially in the integrated pest management system (IPM) nowadays. It is important to know the virulence of the newly developed mycoinsecticides as well as their mortality rates. At the end of the study, we obtained a total of 78 isolates including 63 *Beauveria bassiana* isolates and 15 *Metarhizium anisopliae* as entomopathogenic fungi.

Based on the study;the isolates of both species were pathogenic to M. persicaehe and and BbEİ5 isolates had the highest mortality rate with 62.54%. the *B. bassiana* ısolates BbEKLY4 and BbEO2 were found the lowest mortality rate of 49.17%. Also in the case of *M. anisopliae*; MaEk1b and MaET3 isolates had the highest mortality rate while MaEMR1a isolates found the lowest mortality rate. Our results indicated that all of these isolates BbEİ5, MaEk1b and MaET3 have a broad host range and can be used as biocontrol agents for *M. persicae,* and as biological control agents.

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