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Isolation Entomopathogenic Fungi at Erzincan Province

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Abstract: The first rule to develop new microbial control agents that may be alternnative to chemical pesticides in agricultural pests control are to isolate entomopathogenic fungi (EPF). EPF are originate from soil and microorganisms which are friendly to human and environment. In order to develop the new mycoinsecticides we have carried out a project between 2014-2016. Our aim was to isolate the insect pathogenic fungi found in agricultural land and invertebtrata. Soil samples were collected from agricultural areas (vegetable fied, fruit field, sugarbeet and barly-weat field) and other fieds (pasture, grassland and forest area). As a result we obtained 78 fungal isolates and fungal culture consit of the 63 isolates of ***Beauveria bassiana*** (Balsamo) Vull. and 15 isolates of ***Metharhizium anisopliae*** (Metschnikoff) Sorokin. Factors affecting the occurence and distrubution of entomopathogenic fungi in 60 soil samples collected from cultivated and natural (forest, pasture) areas at Erzincan were examined. EPF were isolated from 18 of the 60 soil samples. We found however, an association between *M. anisopliae* and soil has been found from cultivated habitats. *M. anisopliae* found only cultivated habitats. We also isolated totatly 44 entomopathogenic fungi ısolates from insect that 29 ısolates of them is *B. bassiana* and 15 isolates from them is *Metharzium anisopliae*.

Key words: Isolation, Beauveria bassiana, *Metharhizium anisopliae*, soil, insects, Erzincan

1. Introduction

Entomopathogenic fungi (EPF) cause lethal infections and regulate arthropods population in nature by epizootics (Burges, 1981; Carruthers and Soper, 1987; McCoy et al., 1988). They are host specific with a very low risk of attacking non-target organisms or beneficial insects. Many reseacher reported to infect a very wide range of insects and mite pests, which are of great concern in agriculture worldwide (Roberts and Humber, 1981). In agriculture, horticulture and forestry EPF are considered as primary candidates for origin of micological biopesticides. EPF are important natural and commercial control agents for many insects and other arthropods, which significantly reduces host insect populations (Burges, 1981; Carruthers and Soper, 1987; McCoy et al., 1988). The main route of entrance of the entomopathogen is through integument and it may also

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infect the insect by ingestion method or through the wounds or trachea (Holder et al., 2005). The divisions of fungi are Ascomycota, Zygomycota and Deuteromycota (Samson et al., 1988), and the divisions Oomycota and Chytridiomycota were also included in the previous classification of fungi. At the 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 (Moorhouse et al., 1992; Hajek and St. Leger, 1994). Most researcher reported that *B. bassiana and M. anisopliae* as microbial control agents for agricultural pests which have a wide hostrange and widely distributed in all regions of the world, in addition both two species can be easily isolated from insects and soil (Ansari et al., 2008; Meyling et al., 2006; Freed et al., 2011a, b). Soil-inhabiting entomopathogenic fungi are an important and widespread component of most terrestrial ecosystems. Many species belonging to the Hypocreales (Ascomycota) inhabit the soil for a significant part of their life cycle when they are outside of their insect host. Among them, ***Beauveria*** spp., and ***Metarhizium anisopliae*** are especially common. Isolation of indigenous entomopathogenic fungi is essential to provide an insight into naturally occurring fungal biodiversity. An understanding of the parameters that determine the diversity and distribution of EPF species in the soil would help to identify those species best suited to a particular environment and improve biological control efficacy. Many more researchers have been studied; the effects of factors of insect pathogenic fungi such as geographical location, climatic conditions, habitat type, cropping system, and soil properties on the occurrence and distribution. These factors include geographical location and habitat type (cultivated or natural), sub-habitat type (cropping system in cultivated soils or type of pristine natural habitat) and soil composition. Successful use of entmopathogenic fungi as a bioinsecticides or micoinsecticides will exatly depend on how well the isolates are selected. For development of micoinsecticides based on EPF, screening for more efficient isolates are still necessary. In this paper, we isolated some EPF from soil, insects and gave code for every isolate and made a EPF collection. We identified EPF and examined the soil properties in research aria at Erzincan province.

2. Materials and Methods

1. Exploration of Entomopathogenic Fungi at Research Area

Entomopathogenic fungi exploration was done by using two methods to obtain many species or strains of the fungi. The first method of fungus exploration was to use insect as bait following method of Hashim and Azwana (2003). The insect used was *Galeria mellonella*. The second method by collecting insects were sick or dead due to fungus infection (Herlinda 2008). 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 or purified. Then, the

fungus-infected insects were isolated in the laboratory at a cabinet of laminar air flow that had been sterilized with 70% alcohol.

2. Collectting Soil Samples

The province of Erzincan (39°02′N to 40°05′N, 38°16E to 40°45′E) covers ca. 11,900 km2 of Turkey and is located in the eastern part of Anatolia, which has a continental climate. Soil samples were collected from different georafical sites distrubuted throught the Erzincan provience (Merkez, Üzümlü, Tercan, Mercan, Kemaliye, Kemah, İliç, Çayırlı, Otlukbeli, Refahiye) (Figüre 1).

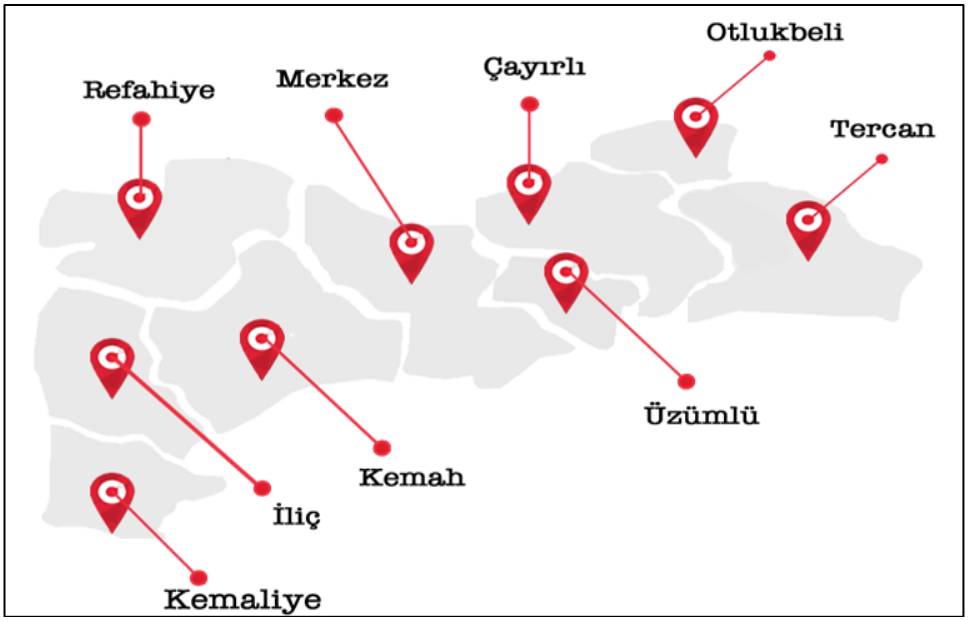

Figure 1. Soil samples were collected from different georafical sites distrubuted throught the Erzincan province

Soil samples were collected with a garden spade to 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 50cm2, placed in clear plastic bags (30-25 cm), sealed with a rubber band and returned to the laboratory. There were 30 samples from cultuvated habitats (24 samples field crops, 1 samples from fruit and vegatable crops, 4 samples from vegatable crops, 1 samples from sugarbet crops) and 30 samples from natural habitats (26 samples from natural pastutes, 3 samples from forest, 1 samples from medow).

3. Soil Analysis Methods

For each sample, soil pH was measured in water at a 1:2.5 solution ratio (Thomas 1996). Organic matter was determined by dichromate oxidation (Walkley & Black 1934) and particle soil distribution was determined using the pipette method (Gee & Bauder 1986).

4. Isolation and Identification of Fungi from Soil

Insect-associated fungi were isolated from soil samples by using 'Galleriabaitmethod' (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 boxeses and covered with a lid and incubated at 25±2°C for two weeks. The larvae were examined on days 7 and 14 days after inoculation. Surface of dead larvae were sterilized by 3% sodiumhypochlorite 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 relevant taxonomic literature (De Hoog, 1972; Samson et al., 1988).

5. Isolation and Identification of Fungi from Insects

Isolation of entomopathogenic fungi used methods of Herlinda (2006). 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 80-85%. Then, a pure culture fungus was identified by using reference of Toledo (2010).

3. Results and Discussion

In this research we made by taking climatic and soil characteristics of Erzincan province and found two species with EPF characteristics. We have examined some of the characteristics of the soil where these species live and have identified the soil types they prefer to live.

1. Climate Data for Erzincan Province (1960-2016)

Climate is so important for entomopathogenic fungi. Erzincan has got Erzincan has a continental climate with freezing, snowy winters and hot and dry summers (Table 1).

Table.1 Erzincan has a continental climate with freezing, snowy winters and hot and dry summers. Spring is the wettest season whilst late summer is the driest (General Directorate of State Meteorological Affairs, 2018).

Climate data for Erzincan (1960-2016)													
Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Year
Record High °C	14.0	17.2	25.2	30.0	33.8	35.2	40.6	40.2	36.6	30.8	22.5	19.0	40.6
Average High °C	1.7	3.7	9.9	16.7	22.1	26.9	31.4	31.7	27.3	20.0	11.5	4.6	17.29
Daily Mean °C	−2.9	−1.2	4.4	10.7	15.6	20.0	24.0	23.7	18.9	12.1	5.2	0.1	10.88
Average Low °C	−6.9	−5.5	−0.5	5.0	8.8	12.3	15.6	15.2	10.7	5.9	0.6	−3.5	4.81
Record Low °C	−26.7	−25.2	−22.4	−8.2	−0.4	2.0	5.0	6.4	0.6	−6.2	−17.4	−25.0	−26.7
Average Precipitation Mm	28.1	30.3	41.2	53.8	54.5	30.2	11.4	6.8	14.3	43.4	39.3	28.8	382.1
Average Precipitation Days	9.6	9.3	11.6	14.1	14.5	9.2	3.3	2.6	4.3	8.8	8.8	10.1	106.2
Mean Monthly Sunshine Hours	89.9	106.4	158.1	171	226.3	288	328.6	310	261	192.2	126	74.4	2,331.9

2. Some Soil Properties of Erzincan Province at Research Area

Some properties of the soil at research area from which EPF (*Beauveria bassina* and *Metharzium anisopliae)* is isolated are given in the table (Table 2).

Table 2. Some properties of the soil reseach area at erzincan province.

Location	Soil of Research Area	Water saturation (%)	Tecture (Richard L.A 1954)	EC (dS-1)	Total salt (%)	pH	Lime (Çaglar, K.Ö, 1949)	P_2O_5 Kg da^{-1} (Olsen, 1954)	K_2O Kg da^{-1} (Jakson, M,L., 1958)	Organic Matter (%)
Merkez	VegetableField	57	CL	0,391	0,014	7,77	13,31	7,39	52,61	1,62
	FruitGarden	61	CL	0,615	0,024	7,71	7,48	5,44	71,31	1,4
	Field (Barly-Weat)	59	CL	1.082	0,041	7,56	10,1	22,67	116	1,73
	Meadow-Grasland	62	CL	0,98	0,039	7,62	18,64	15,28	119,56	1,57
	Forest	66	CL	1.089	0,046	7,75	12,58	9,49	68,49	1,07
Üzümlü	VegetableField	43	CL	0,609	0,017	7,65	35,27	2,35	83,03	0,97
	FruitGarden	78		2	0,076	6,95	1,5	5,71	196,71	3,62
	Field (Barly-Weat)	65	CL	0,791	0,033	7,6	1,53	4,73	149,86	2,28
	Meadow-Grasland	61	CL	1	0,033	7,56	20,31	2,26	55,13	1,94
	Forest	47	CL	0,781	0,023	7,44	10,03	2,94	38,51	1,77
Kemah	VegetableField	56	CL	2	0,078	6,97	19,44	16,71	123,18	3,05
	FruitGarden	50	CL	0,95	0,03	7,55	11,78	2,37	68,49	0,71
	Field (Barly-Weat)	56	CL	1	0,046	7,42	14,41	9,47	92,44	2,12
	Meadow-Grasland	55	CL	1,04	0,037	7,45	33,08	5,38	52,61	0,86
	Forest	56	CL	1	0,035	7,66	19,07	4,71	52,61	0,67
İliç	VegetableField	56	CL	1,02	0,037	7,54	14,26	3,58	50,13	0,25
	FruitGarden	68	CL	1	0,059	7,49	2,95	3,9	112,48	1,22
	Field (Barly-Weat)	61	CL	1,014	0,04	7,59	5,73	3,84	83,08	0,57
	Meadow-Grasland	65	CL	2	0,066	7,22	14,33	8,13	80,07	0,91
	Forest	61	CL	1,098	0,043	7,47	5,94	4,32	63	0,83
Kemaliye	VegetableField	56	CL	1	0,052	7,38	10,32	9,86	170,41	0,46
	FruitGarden	61	CL	0,901	0,035	7,55	12,8	4,15	57,7	0,84
	Field (Barly-Weat)	60	CL	1	0,04	7,48	12,51	4,08	68,49	1,19
	Meadow-Grasland	61	CL	1,058	0,041	7,43	9,74	4,1	80,07	1,23
	Forest	75	CL	1	0,063	7,21	6,31	5,61	119,56	2,92
Refahiye	VegetableField	61	CL	0,642	0,025	7,02	5,51	12,62	89,27	2,63
	FruitGarden	63	CL	2	0,08	6,85	1,35	17,02	89,27	3,49
	Field	61	CL	0,701	0,027	6,69	0,91	7,22	55,13	1,79

	(Barly-Weat)									
	Meadow-Grasland	60	CL	1	0,038	7,58	21,26	12,77	126,84	0,6
	Forest	55	CL	0,566	0,02	6,85	1,5	8,33	77,1	2,77
Mercan	VegetableField	66	CL	1	0,036	7,51	24,84	14,42	138,13	1,41
	FruitGarden	61	CL	0,876	0,034	7,51	20,6	12,2	92,44	1,27
	Field (Barly-Weat)	59	CL	1	0,033	7,51	8,79	13,04	116	1,84
	Meadow-Grasland	57	CL	0,744	0,027	7,56	7,84	8,42	83,08	1,67
	Forest	58	CL	1	0,027	7,55	4,12	8,64	47,71	1,28
Tercan	VegetableField	56	CL	0,947	0,034	7,39	38	5,57	71,31	0,64
	FruitGarden	54	CL	1	0,024	7,43	38,73	1,63	63	0,91
	Field (Barly-Weat)	53	CL	1,065	0,036	7,36	35,92	5	50,13	0,55
	Meadow-Grasland	55	CL	1	0,029	7,4	35,37	7,62	43,01	1,05
	Forest	55	CL	0,938	0,033	7,46	28,99	3,53	63	0,78
Çayırlı	VegetableField	55	CL	1	0,022	7,61	3,68	9,98	50,13	1,24
	FruitGarden	66	CL	1,041	0,044	7,61	11,85	8,21	86,15	1,08
	Field (Barly-Weat)	68	CL	1	0,032	7,71	13,68	7,65	71,31	1
	Meadow-Grasland	60	CL	0,735	0,028	7,61	11,85	11,67	92,44	1,22
	Forest	56	CL	1	0,03	7,51	10,69	10,06	52,61	1,5
Otlukbeli	VegetableField	61	CL	0,921	0,036	7,65	12,87	9,56	63	0,91
	FruitGarden	60	CL	1	0,025	7,46	13,24	12,22	52,61	0,76
	Field (Barly-Weat)	57	CL	0,568	0,021	7,56	17,69	7,68	68,49	1,76
	Meadow-Grasland	77	CL	1	0,063	7,23	23,6	23,76	138,13	2,22
	Forest	61	CL	0,817	0,032	7,56	17,69	9,45	83,08	0,95

3. Isolation Entomopathogenic Fungi from Soil

We idendified two species as a entomopathogenic fungi (Table 3). They are so cosmopolit species and they have got wide spectrum as a biocontrol agents. As a result we obtained 78 fungal ısolats and we gave a code for every isolates. These fungal culture consist of the 63 isolates of *B. bassiana,* and 15 isolates of *Metarhizium anisopliae.* They were isolated from soil at Erzincan provience.

Table 3. Fungal material and their geografical origin (2014-2016)

Fungi Species	No	Code	Substrate (Soil)	Geografical Orijin of Isolates	Country
Beauveria bassiana	1	BbEMRKZ1a	VegetableField	ERİNCAN-MERKEZ	TURKEY
	2	BbEMRKZ1b			
	3	BbEMRKZ2a	FruitGarden	ERİNCAN-MERKEZ	
	4	BbEMRKZ2b			
	5	BbEMRKZ3	Field (Barly-Weat)	ERİNCAN-MERKEZ	
	6	BbEMRKZ4a	Meadow-Grasland	ERİNCAN-MERKEZ	
	7	BbEMRKZ4b			
	8	BbEMRKZ5a	Forest	ERİNCAN-MERKEZ	
	9	BbEMRKZ5b			
	10	BbEÜ1a	VegetableField	ERİNCAN-Üzümlü	
	11	BbEÜ1b			
	12	BbEÜ2a	Fruit-Garden	ERİNCAN-Üzümlü	
	13	BbEÜ2b			
	14	BbEÜ3	Field (Barly-Weat	ERİNCAN-Üzümlü	
	15	BbEÜ4	Meadowr-Grasland	ERİNCAN-Üzümlü	
	16	BbEÜ5a	Forest	ERİNCAN-Üzümlü	
	17	BbEÜ5b			
	18	BbEK1a	VegetableField	ERİNCAN-Kemah	
	19	BbEK1b			
	20	BbEK2a	FruitGarden	ERİNCAN-Kemah	
	21	BbEK2b			
	22	BbEK3a			
	23	BbEK3b	Field(Barly-Weat	ERİNCAN-Kemah	
	24	BbEK4	Meadowr-Grasland	ERİNCAN -Kemah	
	25	BbEK5a	Forest	ERİNCAN-Kemah	
	26	BbEK5b			
	27	BbEİ1	VegetableField	ERİNCAN-İliç	
	28	BbEİ2	FruitGarden	ERİNCAN-İliç	
	29	BbEİ3	Field(Barly-Weat	ERİNCAN-İliç	
	30	BbEİ4	Meadowr-Grasland	ERİNCAN-İliç	
	31	BbEİ5	Forest	ERİNCAN-İliç	
	32	BbEKLY1	VegetableField	ERİNCAN-Kemaliye	
	33	BbEKLY2a	FruitGarden	ERİNCAN-Kemaliye	
	34	BbEKLY2b			
	35	BbEKLY4	Meadowr-Grasland	ERİNCAN-Kemaliye	
	36	BbEKLY5	Forest	ERİNCAN-Kemaliye	
	37	BbER1	VegetableField	ERİNCAN-Refahiye	

	38	BbER2	FruitGarden	ERİNCAN-Refahiye
	39	BbER3	Field(Barly-Weat	ERİNCAN-Refahiye
	40	BbER4	Meadowr-Grasland	ERİNCAN -Refahiye
	41	BbER5a		
	42	BbER5b	Forest	ERİNCAN-Refahiye
	43	BbER5c		
	44	BbEM1	VegetableField	ERİNCAN -Mercan
	45	BbEM2	FruitGarden	ERİNCAN-Mercan
	46	BbEM3	Field(Barly-Weat	ERİNCAN-Mercan
	47	BbEM4	Meadowr-Grasland	ERİNCAN-Mercan
	48	BbEM5	Forest	ERİNCAN-Mercan
	49	BbET1	VegetableField	ERİNCAN-Tercan
	50	BbET2	FruitGarden	ERİNCAN-Tercan
	51	BbET3	Field(Barly-Weat	ERİNCAN-Tercan
	52	BbET4	Meadowr-Grasland	ERİNCAN-Tercan
	53	BbET5	Forest	ERİNCAN-Tercan
	54	BbEÇ1	VegetableField	ERİNCAN-Çayırlı
	55	BbEÇ2	FruitGarden	ERİNCAN-Çayırlı
	56	BbEÇ3	Field(Barly-Weat	ERİNCAN-Çayırlı
	57	BbEÇ4	Meadowr-Grasland	ERİNCAN-Çayırlı
	58	BbEÇ5	Forest	ERİNCAN-Çayırlı
	59	BbEO1	VegetableField	ERİNCAN-Otlukbeli
	60	BbEO2	FruitGarden	ERİNCAN-Otlukbeli
	61	BbEO3	Field(Barly-Weat	ERİNCAN-Otlukbeli
	62	BbEO4	Meadowr-Grasland	ERİNCAN-Otlukbeli
	63	BbEO5	Forest	ERİNCAN-Otlukbeli
Metharzium anisopliae	64	MaEMR1a	VegetableField	ERİNCAN-Merkez
	65	MaEMR2b		
	66	MaEÜ1a	VegetableField	ERİNCAN-Üzümlü
	67	MaEÜ1b		
	68	MaEK1a	VegetableField	ERİNCAN-Kemah
	69	MaEk1b		
	70	MaEİ3	Field(Barly-Weat	ERİNCAN-İliç
	71	MaEKLY1a	FruitGarden	ERİNCAN-Kemaliye
	72	MaEKLY1b		
	73	MaER3	Field(Barly-Weat	ERİNCAN-Refahiye
	74	MaEM1	VegetableField	ERİNCAN-Mercan
	75	MaEM3	Field(Barly-Weat	ERİNCAN-Mercan
	76	MaET3	Field(Barly-Weat	ERİNCAN-Tercan
	77	MaEÇ3	Field(Barly-Weat	ERİNCAN-Çayırlı
	78	MaEO3	Field(Barly-Weat	ERİNCAN-Otlukbeli

Bb: *Beauveria bassiana* (Balsamo) Vull. Ma: *Metharhizium anisopliae* (Metschnikoff) Sorokin

4. Isolation Entomopathogenic Fungi from Insects

In this study We isolated and idendified two species (*B. bassiana and M. anisopliae*) as a entomopathogenic fungi at different insect species. They are totaly 44 entomopathogenic fungi ısolates (Table 4). If a fungi spescies is a good biological control candidate you may ısolate disease insects. Our main thinking is this and we collected dead and disease insects from research are and isoltated entomopathogenic fungi from them.

Table 4. Isolation Entomopathogenic Fungi from Insects (2014-2016)

Fungus species	No	Code	Insects species	Geografical Orijin of Isolates	Country
Beuveria bassiana	1	Insect-Bb -1	*Melolontha melolontha* (larvae)	ERZINCAN -Merkez	TURKEY
	2	Insect-Bb2	*Musca domestica* (adult)		
	3	Insect-Bb-3	*Arctia villica*(Larvae)		
	4	Insect-Bb-4	*Agriotes spp* (larvae)		
	5	Insect-Bb-5	*Polyphulla fullo*(adult)		
	6	Insect-Bb -6	*Melolontha melolontha*(larvae)		
	7	Insect-Bb -7	*Leptinotarsa decemlineata (adult)*		
	8	Insect-Bb -8	*Grillotlpa grıllotalpa* (adult)		
	9	Insect-Bb -9	*Bemicia tabaci* (adult)		
	10	Insect-Bb -10	*Eurygaster spp.* (adult)	Erzincan-Üzümlü	
	11	Insect-Bb -11	*Aelia spp.* (adult)	Erzincan-Üzümlü	
	12	Insect-Bb -12	*Polyphulla fullo* (larvae)		
	13	Insect-Bb -13	*Arctia villica* (larvae)		
	14	Insect-Bb -14	*Melolontha melolontha*(larvae)		
	15	Insect-Bb -15	*Melolontha melolontha*(larvae)		
	16	Insect-Bb -16	*Melolontha melolontha*(larvae)		
	17	Insect-Bb -17	*Pierris bressicae* (larvae)		
	18	Insect-Bb -18	*Agrotis sp.* (larvae)		
	19	Insect-Bb -19	*Apis fabe* (adult)		
	20	Insect-Bb -20	*Apiss gossypii* (adult)		
	21	Insect-Bb -21	*Melolontha melolontha*(larvae)		
	22	Insect-Bb -22	*Leptinotarsa decemlineata (adult)*		
	23	Insect-Bb -23	*Polyphulla fullo*(larvae)		
	24	Insect-Bb -24	*Eurygaster spp.* (adult)		
	25	Insect-Bb -25	*Aelia spp.* (adult)		
	26	Insect-Bb -26	*Agriotes spp.* (larvae)		
	27	Insect-Bb -27	*Leptinotarsa decemlineata (larvae)*		
	28	Insect-Bb -28	*Grillotlpa grıllotalpa* (adult)		
	29	Insect-Bb -29	*Eurodoma ornatum* (adult)		

Matherzium anisopliae	30	Insect-Ma-1	*Grillotlpa grıllotalpa* (adult)	Erzincan-Merkez	
	31	Insect-Ma-2	*Polyphulla fullo* (larvae)	Erzincan-Üzümlü	
	32	Insect-Ma-3	*Pieris brassicae* (larvae)		
	33	Insect-Ma-4	*Tuta absoluta* (larva)		
	34	Insect-Ma-5	*Polyphulla fullo* (larva)		
	35	Insect-Ma-6	*Omophlus caucasicus* (adult)		
	36	Insect-Ma-7	*Myzus persicae* (adult)		
	37	Insect-Ma-8	*Leptinotarsa decemlineata* (larvae)		
	38	Insect-Ma-9	*Apis fabe* (adult)		
	39	Insect-Ma-10	*Apiss gossypii* (adult)		
	40	Insect-Ma-11	*Myzus persicae* (adult)		
	41	Insect-Ma-12	*Leptinotarsa decemlineata (adult)*	Erzincan-Merkez	
	42	Insect-Ma-13	*Agrotis sp.* (larvae)	Erzincan-Üzümlü	
	43	Insect-Ma-14	*Melolontha melolontha*(larvae)	Erzincan-Merkez	
	44	Insect-Ma-15	*Melolontha melolontha*(larvae)	Erzincan-Üzümlü	

Bb: *Beauveria bassiana* (Balsamo) Vull. Ma: *Metharhizium anisopliae* (Metschnikoff) Sorokin.

4. Conclusion

In this study, exploration methods that were able to find entomopathogenic fungi were deeping insect bait in the soil and collecting the infected insects from the fields. The fungi were easier to be found from the soil compared to infected insects from the fields. As a result we obtained 78 fungal isolates and fungal culture consit of the 63 isolates of *B. bassiana* (Balsamo) Vull. and 15 isolates of *M. anisopliae* (Metschnikoff) Sorokin. Factors affecting the occurence and distrubution of entomopathogenic fungi in 60 soil samples collected from cultivated and natural (forest, pasture) areas at Erzincan were examined. EPF were isolated from 18 of the 60 soil samples. The other from the infected insects often were contaminated by air fungi. Hashim and Azwana (2003) reported that the conidia in the soil tended to be more persistent, they could be easily trapped using insect bait. We isolated totatly 44 entomopathogenic fungi ısolates from insect that 29 ısolates of them is *B. bassiana* and 15 isolates from them is *Metharzium anisopliae.*

Ecological factors are very important in terms of EPF life is the biological control agent. Temperature values mean rainfall values from meteorological factors and soil characteristics (soil saturation, soil structure, organic matter, nitrogen, phosphorus, potassium, pH) are among the most important factors affecting EPF survival. We found that soils with high clay content, organic matter, water saturation improved persistence of the fungal conidia. Our result overlap Fuxa and Richter (2004) and they said that future research using clay will be required to formulate the fungus conidia to increase efficacy as biological control. We found however, an

association between *M. anisopliae* and soil has been found from cultivated habitats. *M. anisopliae* found only cultivated habitats.

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