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Isolation and identification of entomopathogenic fungi from coastal districts of Ordu province, Turkey

Ordu ili kıyı ilçelerinden entomopatojen fungusların izolasyonu ve tanımlanması

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ABSTRACT

A total of 250 soil samples were taken from the forest, hazelnut, kiwi, vegetable, and meadow-rangeland areas in the coastal regions of Ordu province, Turkey. Entomopathogenic fungi were isolated from these soil samples using the *Galleria*-bait method. Eighty-five fungal isolates were isolated from these soil samples, after which they were morphologically and molecularly identified. After morphological characterization, 64 out of 85 isolates were identified molecularly. Based on the molecular characterization results, twenty-three out of the 64 isolates were *Beauveria bassiana* (35.94%), 11 isolates were *Metarhizium brunneum* (17.19%), 8 isolates were *Metarhizium anisopliae* (12.5%), 6 isolates were *Metarhizium robertsii* (9.38%), 4 isolates were *Purpureocillium lilacinum* (6.25%), 4 isolates were *Clonostachys rogersoniana* (6.25%), 3 isolates were *Fusarium solani* (4.69%), 1 isolate was *Clonostachys rossmaniae* (1.56%), 1 isolate was *Aspergillus flavus* (1.56%), 1 isolate was *Cordyceps cicadae* (1.56%), 1 isolate was *Cordyceps fumosorosea* (1.56%), and 1 isolate was *Fusarium oxysporum* (1.56%). In the coastal area of Ordu province, the most common entomopathogen fungal genus is *Metarhizium* followed by *Beauveria bassiana*.

INTRODUCTION

Ordu is located in the Eastern Black Sea region of Turkey. It has a mild climate with an average temperature of 7 ℃ in January and 23.2 ℃ in July. The average annual temperature is 14.4 ℃, the highest temperature is 37.3 ℃, and the lowest is -7.2 ℃. It has a humid climate with cool winters and warm summers. The average annual precipitation is 1045.2 mm, and seasonal precipitation is observed in all months. Ordu has a coastline length of 100 km in the Black Sea. The annual drought index is very humid. The vegetation is classified as a very humid forest. Humid areas increase towards the East. The annual average relative humidity is 74.7%, the average seawater temperature is 15.4 ℃, and the average number of sunny days is 58 days. The northern part, which receives abundant rainfall, is quite rich in terms of vegetation compared to the southern (inner) part, where the continental climate is dominant. The forests, which cover the largest area with 202.893 hectares in the land distribution, constitute 34% of the land of Ordu. The hazelnut orchards, which constitute the most important product of the provincial economy, dominate the areas up to 800 m high from the coast. Beside the hazelnut orchards, there are

kiwi orchards, and field lands where corn, potatoes, beans, cabbage, and other field products are grown. There are also forests consisting of chestnut, alder, hornbeam, oak, beech, elm, and maple species. Areas over 1.000 meters form pastures and plateaus. In general, 43.9% of Ordu's land is agricultural, 33.8% is forestland, 8.4% is meadow-rangelands, and 13.9% is residential and non-agricultural land (Anonymous 2023).

It can be thought that the climatic characteristics of Ordu province may provide an advantage in biological control, which is one of the important branches of pest control, as well as agricultural production. Biological control is the use of living organisms/agents (fungi, bacteria, viruses, or insects) to suppress the population density or effect of the pest organism or to reduce its damage (Eilenberg et al. 2001). Entomopathogenic fungi (EPF), which constitute an important class of biological control agents, are used in the control of many pest groups. EPF infect their target host by cuticle penetrating and using their nutrient resources. This fungal attack causes mechanical damage to the tissues of the target host. EPF secrete enzymes, toxins, and secondary metabolites, which are host-directed during this process (Shin et al. 2013). In this way, they can directly kill the host or weaken the vital activities of the host (Kulkarni 2015).

The soil is an essential environment for EPF, and the majority of EPF species sustain in the soil (Abdullah et al. 2015, Majchrowska-Safaryan and Tkaczuk 2021). Although soil provides an excellent habitat for EPF, factors such as temperature, humidity, pH, and soil microbiota affect their survival and sustainability (Niu et al. 2019).

Abiotic factors, such as temperature and humidity, play an important role in the germination and development of fungal spores and can limit the pathogenicity of even a strong EPF under unsatisfied conditions. Mishra et al. (2015) reported that when *Beauveria bassiana* isolate was sprayed on the house flies under different temperature and humidity conditions, the highest mortality rate was observed to be 25-30 ℃ at 75-100% relative humidity, fungal growth slowed down at lower and higher temperatures, and the mortality decreased.

The presence and distribution of EPF in the soil can be affected ways differently by geographical location, habitat, soil type, and cultural practices (Vänninen 1996). Knowledge of the presence, distribution, and diversity of indigenous EPF species have importance in the biological control of insect pest populations (Meyling and Eilenberg 2006). The possibility of getting a successful mycoinsecticide that controls pest insects can enhance by choosing and testing various indigenous EPF isolates having different characteristics (Şahin and Yanar 2021). Based upon this idea, it was aimed to investigate the presence of EPF and their molecular identification in the soil samples taken from the coastal districts of the Ordu province in this study.

MATERIALS AND METHODS

Collection of soil samples

A total of 250 soil samples were collected from different sampling sites in fields, forests, and meadow-rangeland areas in Ünye, Fatsa, Perşembe, Center, and Gülyalı districts located in the coastal part of Ordu province during 2019 and 2020 (Figure 1). The areas to be sampled were determined using the random sampling method. Thus, 50 soil samples were obtained from each of the districts. Soil samples were taken from 5 different points at a depth of 0-20 cm with a shovel in ways that represent the entire field and mixed. Approximately 1 kg of soil taken from the mixture was placed in 25 x 42 cm polyethylene bags and labeled. GPS data, including altitude information, were recorded using a Magellan Explorist 310 (Magellan, Santa Clara, CA, USA) handheld GPS receiver.

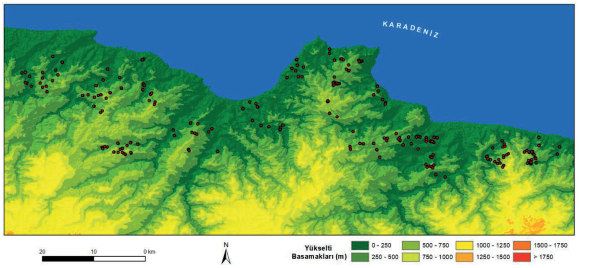

Figure 1. Distribution of soil sampling points in coastal part of Ordu province

Isolation of entomopathogenic fungi using the Galleria bait method

EPF was isolated using the "*Galleria bait method*" proposed by Zimmermann (1986). *Galleria mellonella* larvae were reared in closed double-layer glass jars with filter paper on the artificial diet by Han and Ehlers (2000) in the laboratory under 16 h light and 8 h dark conditions at 26 ℃. The larvae were soaked in 55 ℃ water for 5-10 s to reduce their silk webbing formation. Ten-fourth or fifth instar larvae of *G. mellonella* were baited in each soil and placed in a 90 mm glass Petri dish moistened with distilled water. The Petri dishes were kept at room temperature for 10-15 days. Soil moisture was kept to approximate field capacity by moistening it daily. Petri dishes were regularly turned upside down to ensure the larvae had contact with the soil. The infected insect cadavers were subjected to surface sterilization in 1% NaClO solution for 2-3 min, washed three times in sterile distilled water then the cross-sections from cadavers were placed on water agar (1.5% w/v). After mycelia had grown on the cadaver, they were transferred to PDA to get pure fungal culture. The isolates obtained were incubated at 25 ℃ for 15-30 days.

Colony morphology and spore structures of the isolates were evaluated according to the key described by Humber (1997), and isolates showing similar morphology were grouped at the genus level. Agar block smear preparation was used to examine the conidial structures of the fungi (Woo et al. 2010).

Fungal DNA extraction, polymerase chain reaction (PCR) and sequencing

Genomic DNA isolation of fungi was performed with a Turkuaz DNA purification kit (patent pending), adapted by Saygılı (2019) and Keskin et al. (2014). One hundred/one-hundred-fifty milligrams of mycelia were scraped from pure culture and taken into 1.5 ml microcentrifuge tubes. Liquid nitrogen was added to the tube and the tissues were crushed using a pipette tip. Two hundred-fifty microliters of 1X TE buffer was added and vortexed. Then, 500 µl TLB and 40 µl Proteinase K solution were added and mixed gently. The tubes were incubated at 65 °C water bath for 1 h. Seven hundred-fifty microliter of chloroform and isoamyl alcohol in a ratio 24:1 was added to the tubes and mixed thoroughly to form an emulsion. The mixed tubes were centrifuged at 12 000 rpm for 5 min. The supernatant was taken into a new tube and 500 µl of cold 2-propanol was added. After inverting the tubes for a few min, they were centrifuged at 12 000 rpm for 2 min, and the supernatant was discarded. Two microliters of RNase (10 mg/ml) and 24 µl of 5 M NaCl solution were added onto the pellets and incubated at 65 °C water bath for 30 min. The tubes were turned upside down after adding 750 µl of 96% cold ethanol to the melted pellet. Then, the DNA was precipitated by keeping the tubes at -20 °C for 10 min. The tubes were centrifuged at 12 000 rpm for 10 min after reaching room temperature then the liquids were discarded. The tubes were centrifuged at 12 000 rpm for 10 min, and the supernatant was discarded. The pellets were washed slowly with 70% cold ethanol, and they were dried at room temperature for 2 h. Genomic DNA isolation was performed by dissolving the dried pellets in 50 µl of 1X TE buffer. Resultant DNAs were electrophoresed in 1X TBE buffer on 1% agarose gel added 0.5 µg/ml ethidium bromide and checked with a UV imaging system (Vilber Lourmat CN-08). The displayed bands were recorded using the BioCapt v.11.02 program. Purified DNA was stored at -20 °C until PCR experiments. ITS (Internal Transcribed Spacer) and EF1-α (elongation factor 1-alpha) gene regions were preferred for the amplification of purified DNA. ITS amplification was achieved using the ITS5 (5'–GGAAGTAAAAGTCGTAACAAGG–3') / ITS4 (5'-TCCTCCGCTTATTGATATTGC–3') primers (White et al. 1990); EF1-α amplification was achieved using the 1567R (5'–ACHGTRCCRATACCACCSATCTT–3') and 1577F (5'–CARGAYGTBTACAAGATYGGTGG–3').primers (D'Alessandro et al. 2014). PCR reactions and conditions used were as described by Gül (2016).

The PCR products were subjected to single-direction sequence analysis by a commercial sequencing service (Atlas Biotechnologies Inc, Ankara, Turkey). Sequence chromatograms were arranged with MEGA X program and compared with NCBI BLAST (Basic Local Alignment Search Tool). Phylogenetic trees were created using the Maximum Likelihood method using BioEdit 7.2 and MEGA X programs to determine the genetic relationships between DNA sequences (Şahin and Yanar 2021).

RESULTS

The number of EPF obtained from soil samples taken from Ünye, Center, Merkez, Perşembe, and Gülyali districts of Ordu province are listed in Table 1. In total, 85 entomopathogenic fungi were isolated from 250 soil samples. The 85 isolates were identified based on morphological characteristics.

Morphological identification

The isolates were grouped to the genus level according to their colony morphology. Then, the isolate groups were classified by examining their spore structures using the agar block method under the microscope.

Beauveria spp.

This fungus group initially formed a white mycelium. Within 14-21 days, it generated white powdery spores on PDA media. The colony's color turned yellowish-white over time. Conidiophores were zigzag-shaped and transparent,

Table 1. The number of EPF obtained from soil samples taken from Ünye, Center, Merkez, Perşembe, and Gülyalı districts of Ordu province

Locality	The number of			
	Soil samples	Entomopathogenic fungi isolated	Presence of entomopathogenic fungi (%)	Isolates identified
Ünye	50	17	34	8
Fatsa	50	20	40	12
Merkez	50	14	28	10
Perşembe	50	25	50	25
Gülyalı	50	9	18	9
Total	250	85	34	64

Table 2. Species names, strains, gene regions, sampling data, GenBank accession numbers, and percent identity of the isolates identified

No	Strain no	Gene Region	Habitat	Altitude (m)	Sampling date	Species	Accession no	Percent Identity
1	ORU-11	ITS	Hazelnut Orchard	609	29.06.2019	*Beauveria bassiana*	MW410165	100%
2	ORU-21	ITS	Hazelnut Orchard	236	29.06.2019	*Beauveria bassiana*	MW410166	100%
3	ORU-23	ITS	Hazelnut Orchard	101	29.06.2019	*Beauveria bassiana*	MW410167	99.79%
4	ORU-25	ITS	Hazelnut Orchard	170	29.06.2019	*Metarhizium robertsii*	MW410168	100%
5	ORU-40	ITS	Hazelnut Orchard	580	30.06.2019	*Metarhizium robertsii*	MW410169	100%
6	ORU-50	ITS	Hazelnut Orchard	427	10.03.2020	*Beauveria bassiana*	MW410170	100%
7	ORF-3	ITS	Hazelnut Orchard	626	28.04.2019	*Beauveria bassiana*	MW410171	100%
8	ORF-8	ITS	Hazelnut Orchard	560	28.04.2019	*Beauveria bassiana*	MW410172	99.79%
9	ORF-9	ITS	Hazelnut Orchard	396	28.04.2019	*Beauveria bassiana*	MW410173	100%
10	ORF-11	ITS	Hazelnut Orchard	353	28.04.2019	*Beauveria bassiana*	MW410174	100%
11	ORF-17	ITS	Hazelnut Orchard	106	29.04.2019	*Beauveria bassiana*	MW410175	100%
12	ORF-22-a	ITS	Hazelnut Orchard	243	29.04.2019	*Beauveria bassiana*	MW410176	100%
13	ORF-23	ITS	Hazelnut Orchard	146	29.04.2019	*Beauveria bassiana*	MW410177	100%
14	ORF-25	ITS	Kiwi Orchard	6.6	29.04.2019	*Beauveria bassiana*	MW410178	100%
15	ORF-30	ITS	Hazelnut Orchard	426	7.03.2020	*Metarhizium brunneum*	MW410179	100%
16	ORF-42	ITS	Vegetable Garden	194	8.03.2020	*Beauveria bassiana*	MW410180	100%
17	ORF-43	ITS	Hazelnut Orchard	90	8.03.2020	*Beauveria bassiana*	MW410181	100%
18	ORM-8	ITS	Vegetable Garden	313	13.07.2019	*Metarhizium anisopliae*	MW410182	100%
19	ORM-14	ITS	Vegetable Garden	444	13.07.2019	*Aspergillus flavus*	MW410183	99.80%
20	ORM-21	ITS	Hazelnut Orchard	276	13.07.2019	*Purpureocillium lilacinum*	MW410184	100%
21	ORM-39	ITS	Hazelnut Orchard	175	10.03.2020	*Beauveria bassiana*	MW410185	100%
22	ORM-40	ITS	Vegetable Garden	83	10.03.2020	*Metarhizium brunneum*	MW410186	100%
23	ORM-45	ITS	Hazelnut Orchard	221	10.03.2020	*Beauveria bassiana*	MW410187	100%
24	ORM-47	ITS	Hazelnut Orchard	55	10.03.2020	*Metarhizium anisopliae*	MW410188	100%
25	ORM-48	ITS	Hazelnut Orchard	45	10.03.2020	*Clonostachys rogersoniana*	MW410189	99.57%
26	ORM-50	ITS	Hazelnut Orchard	88	10.03.2020	*Beauveria bassiana*	MW410190.1	100%
27	ORP-1	ITS	Hazelnut Orchard	243	23.11.2019	*Metarhizium brunneum*	MW410191.1	100%
28	ORP-2	ITS	Vegetable Garden	275	23.11.2019	*Metarhizium anisopliae*	MW410192.1	99.34%
29	ORP-4	ITS	Vegetable Garden	344	23.11.2019	*Metarhizium robertsii*	MW410193.1	99.78%
30	ORP-9	ITS	Vegetable Garden	289	23.11.2019	*Cordyceps cicadae*	MW410194.1	99.39%
31	ORP-13	ITS	Hazelnut Orchard	467	23.11.2019	*Metarhizium brunneum*	MW410195.1	99.78%
32	ORP-14	ITS	Hazelnut Orchard	392	23.11.2019	*Metarhizium brunneum*	MW410196.1	99.78%
33	ORP-15	ITS	Hazelnut Orchard	371	23.11.2019	*Beauveria bassiana*	MW410197.1	100%
34	ORP-16	ITS	Forestland	402	23.11.2019	*Metarhizium robertsii*	MW410198.1	98.01%
35	ORP-17	ITS	Forestland	351	23.11.2019	*Metarhizium brunneum*	MW410199.1	99.78%
36	ORP-18	ITS	Hazelnut Orchard	344	23.11.2019	*Metarhizium brunneum*	MW410200.1	97.57%
37	ORP-22	ITS	Hazelnut Orchard	472	24.11.2019	*Metarhizium brunneum*	MW410201.1	98.67%
38	ORP-24	ITS	Forestland	462	24.11.2019	*Beauveria bassiana*	MW410202.1	100%
39	ORP-26	EF1-α	Hazelnut Orchard	675	24.11.2019	*Purpureocillium lilacinum*	MW464658.1	100%
40	ORP-27	ITS	Vegetable Garden	683	24.11.2019	*Metarhizium brunneum*	MW410203.1	99.56%
41	ORP-29	ITS	Hazelnut Orchard	523	24.11.2019	*Clonostachys rogersoniana*	MW410204.1	100%
42	ORP-30	ITS	Hazelnut Orchard	456	24.11.2019	*Metarhizium anisopliae*	MW410205.1	94.03%
43	ORP-34-a	ITS	Hazelnut Orchard	221	24.11.2019	*Cordyceps fumosorosea*	MW410206.1	99.39%
44	ORP-34-b	ITS	Hazelnut Orchard	221	24.11.2019	*Metarhizium brunneum*	MW410207.1	94.69%
45	ORP-35	ITS	Forestland	30	24.11.2019	*Beauveria bassiana*	MW410208.1	100%
46	ORP-36	EF1-α	Hazelnut Orchard	53	24.11.2019	*Purpureocillium lilacinum*	MW464659.1	100%
47	ORP-37	ITS	Hazelnut Orchard	101	24.11.2019	*Metarhizium brunneum*	MW410209.1	96.46%
48	ORP-39	ITS	Forestland	33	24.11.2019	*Metarhizium anisopliae*	MW410210.1	98.67%
49	ORP-40	ITS	Forestland	23	24.11.2019	*Metarhizium anisopliae*	MW410211.1	94.91%
50	ORP-46	ITS	Hazelnut Orchard	206	9.03.2020	*Beauveria bassiana*	MW410212.1	100%
51	ORP-48	ITS	Meadow-Rangelands	151	9.03.2020	*Metarhizium robertsii*	MW410213.1	94.47%
52	ORG-1	ITS	Hazelnut Orchard	147	11.03.2020	*Beauveria bassiana*	MW410214.1	100%
53	ORG-2	ITS	Kiwi Orchard	303	11.03.2020	*Beauveria bassiana*	MW410215.1	100%
54	ORG-5	ITS	Hazelnut Orchard	296	11.03.2020	*Clonostachys rogersoniana*	MW410216.1	99.36%
55	ORG-6	ITS	Hazelnut Orchard	299	11.03.2020	*Metarhizium anisopliae*	MW410217.1	99.34%
56	ORG-21	ITS	Hazelnut Orchard	314	11.03.2020	*Metarhizium anisopliae*	MW410218.1	99.56%
57	ORG-24	ITS	Hazelnut Orchard	199	11.03.2020	*Clonostachys rossmaniae*	MW410219.1	99.36%
58	ORG-35	ITS	Hazelnut Orchard	401	12.03.2020	*Clonostachys rogersoniana*	MW410220.1	99.57%
59	ORG-42	ITS	Vegetable Garden	472	12.03.2020	*Metarhizium robertsii*	MW410221.1	96.46%
60	ORG-48	EF1-α	Kiwi Orchard	151	12.03.2020	*Purpureocillium lilacinum*	MW464660.1	100%
61	ORM-7	ITS	Hazelnut Orchard	299	13.07.2019	*Fusarium solani*	MW410222.1	100%
62	ORU-10	ITS	Hazelnut Orchard	476	29.06.2019	*Fusarium solani*	MW410223.1	100%
63	ORU-39	ITS	Hazelnut Orchard	477	30.06.2019	*Fusarium solani*	MW410224.1	100%
64	ORF-22-b	ITS	Hazelnut Orchard	243	29.04.2019	*Fusarium oxysporum*	MW410225.1	99.78%

and their base parts were swollen. Conidia were 1.5-3 µm in size, transparent, spherical, and densely clustered.

Metarhizium spp.

Mycelia, which initially started to form in white color, turned yellowish-green in a few days. The colony morphologies, spore development and colors differed according to the isolates. Spores were dark green and clustered on white mycelium. There were vertically branched conidiophores and Penicillium-like phialides. The conidia were cylindrical, average 5-7 µm long, 2-3 µm wide, and were densely clustered or chained at the ends of the phialides.

Purpureocillium spp.

Mycelia, which initially started to form in white color on PDA medium, turned into a purplish-gray color in a few days. Purple-violet powdery sporulation was seen on the mycelium over time. Conidiophores had slender phialides, and the conidia extended in the form of chains at the ends of these phialides. Conidia were transparent, spherical, and approximately 2.5 x 2 µm in size.

Clonostachys spp.

Mycelia grew as white concentric circles and formed reddish-orange sporulation on PDA medium. Reddish-orange pigment formation was also observed on the colony reverse. Conidia were clustered at the tip of the phialides of the verticillate conidiophore. Their conidial structures were elliptical and narrower at one end than the other. They were approximately 3-7 x 1-2 µm in size. The fungus generated chlamydospores which are 4-7 x 3-6 µm in size, larger than the conidia.

Fusarium spp.

Mycelia, which were initially white-colored overhead, turned reddish-orange, yellowish, or purplish depending on their species on PDA media in the following days. Chlamydospores were occasionally seen on the mycelium. The macroconidia formed on the sporodochia were pointed sickle-shaped and had 3-6 septa. Microconidia varied in shape from elliptical to cylindrical, septate to aseptate.

Aspergillus spp.

Brownish sporulation generated on white mycelium on PDA medium. The vesicles at the ends of the conidiophores had phialides, and the conidia extended in short chains at the ends of these phialides. The spherical conidia were approximately 2-3 x 2-2.5 µm in size.

Molecular identification

Twenty-five of these fungi were morphologically similar to *Fusarium* spp. The use of entomopathogenic *Fusarium* isolates in biological control is still debated because they are weak pathogens, saprophytic strains are high on cadavers, they have the potential to be plant pathogens, and they can produce mycotoxins (Teetor-Barsch and Roberts 1983). Due to these disadvantages, only 4 representatives of *Fusarium isolates*, which are thought to have different morphological characters, were selected for molecular diagnosis.

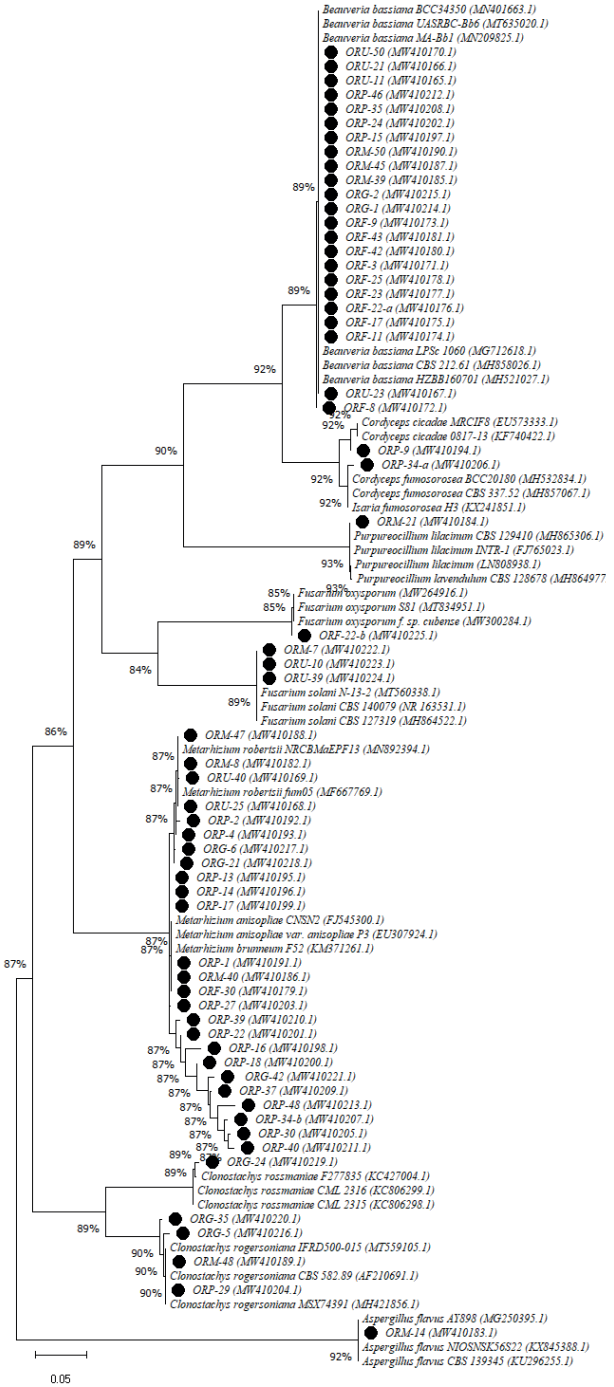

Figure 2. Phylogenetic tree of isolates identified by amplifying the ITS4-5.8S-ITS5 gene region

Bands with a size of approximately 600 bp were obtained in the PCR products obtained from the ITS gene region, and approximately 700 bp in the PCR products were obtained from the EF1-α gene region. One-way sequence analysis results were arranged with the MEGA X program and compared with other sequences registered in the GenBank database using the BLAST tool. The names of the species identified according to the percent identity as a result of the comparison and the recorded accession numbers are given in Table 2. Accordingly, 64 out of 23 isolates were *Beauveria bassiana* (35.94%), 11 isolates were *Metarhizium brunneum* (17.19%), 8 isolates were *Metarhizium anisopliae* (12.5%), 6 isolates were *Metarhizium robertsii* (9.38%), 4 isolates were *Purpureocillium lilacinum* (6.25%), 4 isolates were *Clonostachys rogersoniana* (6.25%), 3 isolates were *Fusarium solani* (4.69%), 1 isolate was *Clonostachys rossmaniae* (1.56%), 1 isolate was *Aspergillus flavus* (1.56%), 1 isolate was *Cordyceps cicadae* (1.56%), 1 isolate was *Cordyceps fumosorosea* (1.56%) and 1 isolate was *Fusarium oxysporum* (1.56%).

Phylogenetic similarities between the species identified by amplifying the ITS4-5.8S-ITS5 region were determined by reference to other species in the GenBank database, and the dendrogram is shown in Figure 2. The similarity rate between taxa is above the branches. Accordingly, it was observed that ORU-23 and ORF-8 isolates among B. bassiana species were more similar to each other than other Beauveria spp., and other B. bassiana species were also highly similar. It was determined that *Metarhizium* spp. showed more diversity among each other compared to other species. The affinities in the remaining species were among themselves at varying rates.

DISCUSSION AND CONCLUSION

EPF isolated from the soil of a region is more effective in the management of local pests in that region (Liu et al. 2021). EPF isolation is relatively affected by geographic regions. This may be related to changes in the climatic conditions of geographic areas, agricultural practices, or sampling timing (Ali-Shtayeh et al. 2002). The soil environment is usually the classical isolation zone for EPF species in the Hypocreales, and various EPF species can be found in both arable soils and more natural environments (Meyling and Eilenberg 2007). Keller et al. (2003) reported that *M. anisopliae* is common in arable soils and meadows with higher density. While Vänninen (1996) reported that *M. anisopliae* was isolated more frequently from the southern parts of Finland, and tillage did not adversely affect the isolation of this fungus. Steenberg (1995) suggested that in Danish soil, *M. anisopliae* is more common in open areas than in shady habitats (Meyling and Eilenberg 2007). Similarly, Bidochka et al. (1998) reported that *M. anisopliae* is more common in agricultural areas compared to forests in Canada, and *B. bassiana* is mostly found in shady and natural habitats such as forests. Contrary to these studies, Meyling and Eilenberg (2006) found that *B. bassiana* was also frequently seen in agricultural soil in a part of Denmark. Also Mietkiewski et al. (1997) found that *B. bassiana* was the dominant species in arable land. Gebremariam (2021) emphasized that the difference in fungal species and the number isolated between cultivated and uncultivated soils may be caused by the insect host, soil structure, shading area that protects the region from UV radiations, and any pesticide application. In the current study, based on the results of molecular identification, no clear relationship was found between the entomopathogen fungi species and the sampling habitat. While different entomopathogenic fungal species are encountered in hazelnut orchards, only *B. bassiana* and *Metarhizium* spp. was obtained from forest soil; *B. bassiana* and *P. lilacinum* were obtained from kiwi orchards, *B. bassiana*, *Metarhizium* spp, and *C. cicadae* species were obtained from vegetable fields isolate. Sevim et al. (2010) conducted an EPF survey on hazelnut-growing areas of the Black Sea region, including Ordu, and determined 3 different species of EPF, which *M. anisopliae*, *B. bassiana*, *I. fumosorosea*, and *Evlachovaea* spp. from 301 soil samples. The sampling location involved hazelnut, meadow, tea, vegetable, apple, poplar, and oak vegetation. *M. anisopliae* was the most commonly detected fungus in all vegetations, which were mainly tea and hazelnut, followed by *B. bassiana.* The fact remains that *I. fumosorose*a was only isolated in agricultural fields. Similarly, *Cordyceps* (=*Isaria*) species detected in the present study were isolated from hazelnut and vegetable vegetation. On the contrary, Vänninen (1996) asserted that *I. fumosorosea* occurred only in natural habitats and was never isolated from intensively cultivated soil. Ali-Shtayeh et al. (2002) defended that EPF isolation and diversity were not significantly affected by soil pH and chemical characteristics. In conclusion, entomopathogenic fungi species diversity is not clarified yet, so further study will be conducted to determine the interaction between soil chemical structure and entomopathogen fungi species diversity.

This study contributes to the understanding relationship between the natural distribution and vegetation of EPF and increases the number of EPF species previously described in the Ordu province of the Black Sea Region of Turkey. The European Council Farm to Fork Strategy, which was published recently, targeted removing especially more toxic synthetic pesticides up to 50% of pesticides markets by 2030. So biological control agents come forward, and their use in pest management programs will be increased for

the establishment of agricultural sustainability (European Commission 2021).

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ÖZET

Ordu ili kıyı bölgelerindeki orman, fındık, kivi, sebze ve çayır-mera alanlarından toplam 250 adet toprak örneği alınmıştır. Bu toprak örneklerinden *Galleria*-tuzak yöntemi kullanılarak entomopatojen funguslar izole edilmiştir. İzolasyonlar sonucunda 85 fungal izolat elde edilmiştir. Morfolojik karakterizasyondan sonra 85 izolattan 64'ü moleküler olarak tanımlanmıştır. Moleküler karakterizasyon sonuçlarına göre 64 izolattan 23'ü *Beauveria bassiana* (%35.94), 11 izolat *Metarhizium brunneum* (%17.19), 8 izolat *Metarhizium anisopliae* (%12.5), 6 izolat *Metarhizium robertsii* (%9.38), 4 izolat *Purpureocillium lilacinum* (%6.25), 4 izolat *Clonostachys rogersoniana* (%6.25), 3 izolat *Fusarium solani* (%4.69), 1 izolat *Clonostachys rossmaniae* (%1.56), 1 izolat *Aspergillus flavus* (%1.56), 1 izolat *Cordyceps cicadae* (%1.56), 1 izolat *Cordyceps fumosorosea* (%1.56) ve 1 izolat *Fusarium oxysporum* (%1.56) idi. Ordu ilinin kıyı kesimlerinde en yaygın entomopatojen mantar türü *Metarhizium*'dur ve bunu *Beauveria bassiana* izlemektedir.

Anahtar kelimeler: entomopatojen fungus, izolasyon, biyolojik mücadele, orman, fındık, Karadeniz

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