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Original article

Anastomosis grouping and phylogenetic analysis of Rhizoctonia isolates on wheat in Türkiye

Türkiye'de buğdaydaki Rhizoctonia izolatlarının anastomosis gruplandırması ve filogenetik analizi

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ABSTRACT

This study aims to determine the species and evaluate the genetic diversity of the pathogenic and nonpathogenic *Rhizoctonia* spp. and anastomosis groups (AG) from wheat plants and rhizosphere soils in Turkey. *Rhizoctonia* species were isolated from plants and rhizosphere soils in wheat fields in 5 provinces in the Central Anatolian Region of Türkiye. As a result of the isolations, a total of 88 multinucleate (MN) and binucleate (BN) *Rhizoctonia* isolates were obtained. Identifications of the isolates were determined by rDNA-ITS sequence analyses. The identified isolates belonged to MN Waitea *circinata* var. *zeae*, *W. circinata* var. *oryzae*, *W. circinata* var. *circinata*, MN *Rhizoctonia solani* AG 2-1, AG 2-2, AG 3, AG 4-HGII, AG 4-HGIII, AG 5, AG 8, AG 11 and BN AG A, AG DI, AG E, AG G, AG H, AG I, AG I-like and AG K. The most isolated group was *W. circinata* var. *circinata*. In the pathogenicity studies, the most virulent group was determined as *R. solani* AG 4. Among the binucleate isolates, groups other than *R. cerealis* AG DI were not found to be pathogenic. Neighbor-joining phylogenetic trees of isolates were constructed from rDNA-ITS sequences. As a result of this study, the regional distribution of MN and BN *Rhizoctonia* AG isolates in important wheat production areas in the Central Anatolia Region, Türkiye was determined. In addition, this study is the first comprehensive study in which the genetic diversity of *Rhizoctonia* AGs isolates obtained from wheat and rhizosphere soils in the region was evaluated with a molecular approach.

INTRODUCTION

Wheat is one of the most used crop plants in human nutrition in the world and is one of the main nutrients in the world. It has an important place not only in terms of its use in the flour and bakery products industry (flour, bread, bulgur, semolina, pasta, biscuits, starch, etc.), which is the sub-branch of the food industry but also in terms of its use in the livestock sector such as bran and straw. According to strategists, wheat is the most important geoeconomic power of the 21st century (Koca 1999). *Rhizoctonia* genus includes many species with highly pathogenic, weakly pathogenic, endophyte, saprophytic, and mycorrhizal characters (González et al. 2006). It is one of the main causes of root rot

disease, which is a problem in wheat fields in Türkiye. The species in this genus are divided into many groupwith the number of nuclei in the hyphae cells [multinucleate (MN), binucleate (BN), uninucleate (UN)] and the anastomosis fusions they form together. *Rhizoctonia solani* Kühn is divided into 13 anastomosis groups (AGs) designated as AG 1-13, as the AG BI group has been integrated into AG 2 (Carling et al. 2002), and 20 subgroups (AG 1IA, AG 1IB, AG 1IC, AG 1ID, AG 1IE, AG 2-1, AG 2-2IIB, AG 2-2IV, AG 2-2LP, AG 2-3, AG 3PT, AG 3TB, AG 3TM, AG 4-HGI, AG 4-HGII, AG 4-HGIII, AG 6-HGI, AG-6GV, AG 9TP and AG 9TX) (Priyatmojo et al. 2001). Anastomosis groups can be diagnosed by anastomosis reactions between hyphae and molecular methods, while molecular diagnosis is required for further subgroups. *Waitea circinata* var. *oryzae* and *W. circinata* var. *zeae* have also anastomosis groups called WAG O and WAG Z, respectively (Sneh et al. 1996). Okubara et al. (2008) reported that three different genotypes of *W. circinata* var. *oryzae* (*R. oryzae* genotype I, II, and III). Binucleate *Rhizoctonia* species are divided into 19 different anastomosis groups (AG A, AG B, AG C, AG D, AG E, AG F, AG G, AG H, AG I, AG K, AG L, AG O, AG P, AG Q, AG R, AG S, AG U, AG V, AG W) (Dong et al. 2017, Erper et al. 2021, Hyakumachi et al. 2005, Misawa and Kurose 2018, Ogoshi et al. 1983, Sharon et al. 2008, Yang et al. 2015, Zhao et al. 2019).

In previous studies in the world, *Rhizoctonia solani* AG 1IB, AG 2-1, AG 2-2, AG 3, AG 4, AG 5, AG 6, AG 8, AG 11, *W. circinata* var. *circinata*, *W. circinata* var. *zeae*, *W. circinata* var. *oryzae* and *R. cerealis* AG D were determined to cause disease in wheat (Meyer et al. 1997, Roberts and Sivasithamparam 1986, Sneh et al. 1996, Tomaso-Peterson and Trevathan 2007). Of these groups, *W. circinata* var. *circinat*a and *W. circinata* var. *zeae*, AG 2-1, AG 3, AG 4-HGII, AG 5, AG 8, AG 11, BN AG I and AG K have been reported on wheat in Türkiye in some previous studies (Demirci 1998, Ünal and Dolar 2012, Ünal et al. 2015).

The classical identification of *Rhizoctonia* AGs is based on the number of nuclei in hyphae cells and the ability of the hyphae to anastomose with known tester isolates (Sneh et al. 1996). Although the anastomosis method is an accurate, valid, and still used method, it is not sufficient for the detection of advanced subgroups. Molecular identification is required for the detection of advanced subgroups. When the studies conducted in the world are examined, various molecular markers have been used for the characterization and grouping of *Rhizoctonia species*. The genetic diversity of *Rhizoctonia* isolates has been studied using RAPD-PCR, SSR-PCR, rDNA-RFLP, rDNA-ITS sequence analysis, universally primed-,PCR, and rep-PCR (Sharon et al. 2006). Currently, the rDNA-ITS sequence analysis seems to be the most appropriate method for the classification of *Rhizoctonia* spp. and sequence analysis of the ITS-5.8S rDNA region has been used as a suitable molecular tool the for identification of *R. solani* subgroups (Carling et al. 2002, Hyakumachi et al. 1998, Priyatmojo et al. 2001, Salazar et al. 2000a, 2000b, Toda et al. 2000). Similarly, the rDNA-ITS sequence analysis most accurately divided subgroups within AG 1 (Kuninaga et al. 1996, Toda et al. 2004), AG 3 (Kuninaga et al. 2000), AG 4 (Boysen et al. 1996), and AGs 6 (Pope and Carter 2001). Fewer studies were reported on rDNA-ITS sequence analyses of Binucleat isolates than on Multinucleate isolates (González et al. 2002, Hyakumachi et al. 2005, Ma et al. 2003, Otero et al. 2002, Sharon et al. 2007, 2008).

This study aims to determine the pathogenic and non-pathogenic *Rhizoctonia* anastomosis groups and subgroups in wheat roots and rhizosphere soils in Türkiye and to reveal the genetic diversity among them.

MATERIALS AND METHODS

Collection of plants and soils and isolation of Rhizoctonia spp.

Wheat fields in 58 districts within the borders of Ankara, Konya, Yozgat, Eskişehir, and Kırıkkale provinces in the Central Anatolian Region of Türkiye were examined and 330 wheat roots and 330 rhizosphere soils were collected. In the isolations from the plants, tissue pieces of the diseased root and root collar were dried on sterile blotting paper after 1-minute surface disinfection in 1% sodium hypochlorite. Then, it was placed on acidic water agar prepared by adding 3 ml of lactic acid (10%) per liter to 1.5% water agar medium. After 3-4 days, the hyphae tips of Rhizoctonia-like fungi were removed with a sterile loop and transferred to Potato Dextrose Agar (PDA; Merck, Germany). For the isolation of *Rhizoctonia* species from the soil, wheat straws sterilized by autoclaving in heat-resistant bottles were used. Soil samples were filled into pots in a greenhouse and watered until the field capacity. Sterile wheat stalks, approximately 4 cm long, were placed vertically in the soil, 4 per pot, and covered with a clean, opaque nylon bag for 3 days and left uncovered for 4 days. Then, wheat stalks were taken from the pots, washed, and transferred to Petri plates containing acidic water agar (Ogoshi et al. 1990).

Determination of anastomosis groups

The hyphae of the isolates obtained as a result of isolations from wheat and rhizosphere soil were first stained with Safranin O solution and the number of nuclei in each hyphae septa was determined (Bandoni 1979). All isolates were grouped by considering colony morphology, color compared with tester isolates, and number of nuclei. Anastomosis group determination studies were performed according to Kronland and Stanghellini (1988) using tester isolates.

Tester isolates were obtained from Türkiye (MN and BN *Rhizoctonia* spp.), Italy (*R. solani*), Japan (*MN Rhizoctonia* spp.), Poland (*Rhizoctonia* AG DI, II, III) and USA (*W. circinata* var. *oryzae* genotype I, II, III).

Pathogenicity tests

Pathogenicity tests were performed in pots using the Kate A-1 wheat variety, which is known to be susceptible to the disease (Arslan and Baykal 2002). The trials were done in a greenhouse (12 hours photoperiod at 24 ± 2 °C and 55-65% relative humidity) with plastic pots of 10 cm diameter. Inoculums were prepared by inoculating each fungal isolate into moistened sterile wheat grains in heat-resistant glass bottles. Eight wheat grains infested fungi were placed on the soil surface filling the 40 cm^3 of vermiculite and 30 cm^3 of silt loam in the pots. A clean nylon cover was covered over the pots and incubated for 4 days. Controls were created from inoculum-free pots. Trials consisted of 5 replications. At the end of the 4th day, eight Kate A-1 wheat seeds were sown in the soil, covered with 12 cm^3 of sterile soil, and irrigated with 15 ml of distilled water (Paulitz et al. 2003). After 20 days, the plant roots were washed and evaluated according to a 0 to 5 scale: 0= no disease, 1= 1-10%, 2= 11-30%, 3= 31-50%, 4= 51-80%, 5= the entire hypocotyl infected (Ichielevich-Auster et al. 1985). The scale rates were transformed into disease severity rates using the Townsend and Heuberger (1943) formula. At the end of the study, reisolation of fungi from plants was carried out.

Molecular identification and genetic diversity

DNA was isolated from the Qiagen DNeasy ® Plant Mini Kit. PCR studies were performed using ITS 1 and ITS 4 primers (White et al. 1990) according to Cobos and Martin (2008). PCR products were sequenced by a private biotechnology company. Nucleotide sequences were performed by BLAST analysis and compared with the other sequences in GenBank. Sequences in this study were registered with their accession numbers to GenBank at NCBI. Phylogenetic trees were constructed using ClustalW alignments (Thompson et al. 1994), The Tamura 3 Parameter model for MN isolates, and the Kimura 2-parameter model (Kimura 1980) for BN isolates in the Mega 7 Program (Kumar et al. 2016). Bootstrap analysis was performed with 500 copies.

RESULTS

The isolates obtained as a result of isolations from 330 wheat root and 330 rhizosphere soil samples were classically identified with the help of the number of nuclei in each hyphae septum and hyphal anastomosis reaction tests with known test isolates. As a consequence of the classical identification studies total of 88 *Rhizoctonia* isolates were identified including 36 *R. solani*, 30 *W. circinata*, and 22 BN *Rhizoctonia* spp. All isolates were also diagnosed molecularly by rDNA-ITS sequencing analysis to support anastomosis-based diagnoses andidentify advanced subgroups. The resulting sequences were checked to the sequences,in GenBank, and species, anastomosis groups and subgroups of 66 MN and 22 BN *Rhizoctonia* isolates were determined. MN *Rhizoctonia* spp. was determined as *R. solani* AG 2-1, AG 2-2, AG 3, AG 4-HGII, AG 4-HGIII, AG 5, AG 8, AG 11, *W. circinata* var. *circinata*, *W. circinata* var. *zeae* and *W. circinata* var. *oryzae*, BN *Rhizoctonia* spp. as AG A, AG DI, AG E, AG G, AG H, AG I, AG I-like and AG K (Table 1).

Table 1. Number of isolates belonging to *Rhizoctonia* species and anastomosis groups.

Anastomosis groups	Provinces					Total
	Ankara	Konya	Yozgat	Eskişehir	Kırıkkale	
AG 2	-	5	-	-	-	5
AG 3	3	-	-	-	-	3
AG 4-HGII	5	2	1	2	-	10
AG 4-HGIII	1	-	-	-	1	2
AG 5	3	5	3	-	1	12
AG 8	1	1	-	1	-	3
AG 11	1	-	-	-	-	1
W. circinata var. circinata	5	1	8	-	-	14
W. circinata var. zeae	4	1	2	1	-	8
W. circinata var. oryzae	3	-	1	2	2	8
AG A	-	1	-	-	-	1
AG DI	3	3	1	-	-	7
AG E	1	-	-	-	-	1
AG G	1	-	-	-	-	1
AG H	1	-	-	-	-	1
AG I	1	-	-	-	-	1
AG I-like	2	1	-	1	5	9
AG K	1	-	-	-	-	1
Total	36	20	16	7	9	88

All isolates generated amplicons at ≈650 bp during amplification with primers ITS1 and ITS4. Sequences were registered with GenBank at NCBI and accession numbers were got. The majority of the MN *Rhizoctonia* isolates had the highest (96-100%) ITS sequence identity with *Rhizoctonia* isolates in GenBank. Isolates 0612, 6651, 20105, 26102, 7121, and 0663 showed 96-98% similarity to DQ356414 (*R. oryzae* genotype I) from the USA (Okubara et al. 2008). In this study, it was observed that colony morphologies of 0612, 6651, 26105, 7121 and 0663 isolates different from the other *Waitea* isolates and the other *Rhizoctonia oryzae* pathogens in wheat. It was detected that they were not pathogen on wheat (Table 2).

Isolates 0612, 6651, 26105, 26102, 7121 and 0663 showed 96-98% similarity to DQ356414 (*R. oryzae* genotype I) from USA (Okubara et al. 2008). In this study, it was observed that colony morphologies of 0612, 6651, 26105, 7121 and 0663 isolates different from the other *Waitea* isolates and the other *R. oryzae* (WAG O) pathogens in wheat. It was detected that they were not pathogen on wheat (Table 3).

The majority of the BN *Rhizoctonia* isolates had the highest (83-100%) ITS sequence identity with *Rhizoctonia* isolates in GenBank. The phylogenetic neighboor-joining tree belonging to BN *Rhizoctonia* isolates consisted of seven small clusters which composed AG A, AG DI, AG E, AG G, AG H, AG I and AG K (Figure 3). Isolates 7107, 7118,

Table 2. Anastomosis group, geographic origin, source of isolation, percentage of sequence similarity with Genbank isolates and disease severity of *Rhizoctonia* solani isolates used in this study

Isolate number	Subgroup	Origin	Source of isolation	Disease severity [a] (%)	Accesion number		Similarity (%)
4246	AG 2-1	Konya	Plant	Non-pathogen	KC590548	JQ676880	99
4278		Konya	Plant	Non-pathogen	KC590570	EU730809	99
4248	AG 2-2	Konya	Plant	Non-pathogen	KC590550	EU730809	99
4269		Konya	Soil	Non-pathogen	KC590564	EU730809	99
2636		Eskişehir	Soil	Non-pathogen	KC590538	EU730809	100
0601		Ankara	Soil	Non-pathogen	KC590579	MW999160	99
0642	AG 3	Ankara	Soil	Non-pathogen	KC590544	MW999167	100
0676		Ankara	Plant	Non-pathogen	KC590568	MW999167	96
0689		Ankara	Plant	90	KC590607	MZ379606	100
6684		Yozgat-	Plant	98	KC590602	MZ379607	99
2666		Eskişehir	Plant	84	KC590595	MZ379606	99
2633		Eskişehir	Plant	90	KC590535	MZ379606	99
4230	AG 4HGII	Konya	Soil	58	KC590533	MZ379606	99
4274		Konya	Soil	78	KC590566	MZ379606	99
0617		Ankara	Soil	59	KC590589	MZ379598	100
0667		Ankara	Soil	75	KC590563	MZ379606	100
0682		Ankara	Soil	88	KC590571	MZ379606	99
0687		Ankara	Soil	86	KC590605	MZ379602	99
0640	AG 4 HGIII	Ankara	Plant	87	KC590542	KR006070	100
7108		Kırıkkale	Plant	100	KC590521	KR006070	100
0690		Ankara	Plant	Non-pathogen	KC590609	AF478452	98
4275		Konya	Plant	58	KC590567	AF478452	97
4250		Konya	Plant	Non-pathogen	KC590552	AF478452	99
4234		Konya	Plant	Non-pathogen	KC590536	AF478452	99
0639		Ankara	Plant	Non-pathogen	KC590541	AF478452	98
4268		Konya	Soil	58	KC590596	AF478452	98
4235	AG 5	Konya	Soil	56	KC590537	AF478452	97
0665		Ankara	Soil	70	KC590594	AF478452	98
6643		Yozgat	Soil	Non-pathogen	KC590545	JX162013	98
6649		Yozgat	Soil	Non-pathogen	KC590551	AF478452	99
6658		Yozgat	Plant	Non-pathogen	KC590558	AF478452	98
7155		Kırıkkale	Soil	Non-pathogen	KC590608	AF478452	99
0610		Ankara	Plant	76	KC590583	AB000011	97
26111	AG 8	Eskişehir	Plant	73	KC590576	AB000011	99
4254		Konya	Soil	72	KC590555	AB000011	99
0673	AG 11	Ankara	Soil	Non-pathogen	KC590598	AF153802	98
Control				0			

[a] Roots and hypocotyl symptoms were evaluated on the following scale: 0=no disease, 1= 1-10%, 2= 11-30%, 3= 31-50%, 4= 51-80%, 5= the entire hypocotyl infected.

Table 3. Anastomosis group, geographic origin, source of isolation, percentage of sequence similarity with genbank isolates and disease severity of *Waitea circinata* isolates used in this study

Isolate number	Subgroup	Origin	Source of isolation	Disease severity a (%)	Accesion number	The highest similar isolate in the Gen Bank	Similarity (%)
6656	*W. cir. var. circinata*	Yozgat	Plant	68	KC590556	HM807352	100
6657		Yozgat	Plant	75	KC590557	HM807352	99
0641		Ankara	Soil	86	KC590543	HQ166066	100
0611		Ankara	Soil	Non-pathogen	KC590584	FJ755887	99
0637		Ankara	Soil	50	KC590539	HM807352	100
0681		Ankara	Soil	76	KC590600	FJ154894	99
6659		Yozgat	Soil	70	KC590559	HM807352	100
6677		Yozgat	Soil	75	KC590569	HM807352	100
6629		Yozgat	Soil	95	KC590532	HM807352	100
0638		Ankara	Plant	81	KC590540	HM807352	100
6685		Yozgat	Soil	Non-pathogen	KC590603	FJ755887	100
6688		Yozgat	Soil	Non-pathogen	KC590606	FJ154894	99
4225		Konya	Soil	99	KC590530	FJ755878	97
6686		Yozgat	Soil	83	KC590604	JX631228	89
0670	*W. cir. var zeae*	Ankara	Soil	Non-pathogen	KC590565	JX631228.1	97
4226		Konya	Plant	98	KC590515	JQ350856	97
06115		Ankara	Plant	79	KC590518	JQ350862	95
0631		Ankara	Soil	78	KC590517	KC620582	96
0614		Ankara	Soil	72	KC590587	KJ623715	96
6628		Yozgat	Soil	75	KC590514	JQ350860	96
6622		Yozgat	Plant	88	KC590516	KC620580	97
26110		Eskişehir	Soil	84	KC590513	KC709579	96
0612	*W. cir. var. oryzae*	Ankara	Soil	Non-pathogen	KC590585	DQ356414	98
6651		Yozgat	Soil	Non-pathogen	KC590553	DQ356414	96
26105		Eskişehir	Soil	Non-pathogen	KC590575	DQ356414	96
26102		Eskişehir	Soil	Non-pathogen	KC590574	DQ356414	96
7121		Kırıkkale	Plant	Non-pathogen	KC590527	DQ356414	96
0662		Ankara	Soil	Non-pathogen	KC590562	KX468809	97
0663		Ankara	Soil	Non-pathogen	KC590592	DQ356414	98
7103		Kırıkkale	Soil	Non-pathogen	KC590580	EU693449.1	99
Control				0			

[a] Roots and hypocotyl symptoms were evaluated on the following scale: 0=no disease, 1= 1-10%, 2= 11-30%, 3= 31-50%, 4= 51-80%, 5= the entire hypocotyl infected.

7120, 7105, 0661, 2671, 4264 and 7106 were named as AG I-like, because as a result of the blast analysis, these isolates matched with the DQ356409.1, DQ356407 and JQ247570 Accession numbers AG I-like isolates (Okubara et al. 2008, Schroeder and Paulitz 2012) at the highest rate in Genbank (Table 4).

Three phylogenetic trees were constructed by bootstrap neighbor-joining analysis of nucleotide sequences to evaluate genetic variability among isolates belonging to *R. solani*, *Waitea* spp. and BN *Rhizoctonia* spp. The phylogenetic neighboor-joining tree belonging to MN *R. solani* isolates clearly demonstrated that the isolates were grouped into eight distinct clusters (Figure 1). It was observed that this eight clusters constituted small clusters between each other including different AGs. when the eight clusters examined, it was observed that small clusters which different anastomosis groups generated between each other. The small clusters which in the tree of *R. solani* isolates belonged to AGs 2-1, AG 2-2, AG 3, AG 4-HGII, AG 4-HGIII, AG 5, AG 8 and AG 11. The small clusters which in the tree of *W. circinata* isolates belonged to *W. circinata* var. *circinata*, *W. circinata* var. zeae (*R. zeae*) and *W. circinata* var. *oryzae* (Figure 2).

In pathogenicity studies, AG 4 was determined as the most virulent group with disease severity values that vary between 58-100% (Figure 4). The most virulent isolate was 7108 (AG 4-HGIII) with 100% diseases severity value. While the majority of MN *Rhizoctonia* isolates were pathogenic, the majority of BN *Rhizoctonia* isolates were found to be nonpathogenic. Among binucleate isolates; the groups other than *R. cerealis* AG DI were not found to be pathogen. While some isolates belonging to *R. solani* AG 5 was found as weak pathogen, some of them were not found as a pathogen. It was observed that MN *R. solani* AG 2, AG 3, AG 11, *W. circinata* var. *oryzae* genotype I was not a pathogen. Although isolates

Table 4. Anastomosis group, geographic origin, source of isolation, percentage of sequence similarity with genbank isolates and disease severity of binucleate *Rhizoctonia* isolates used in this study

Isolate number	Subgroup	Origin	Source of isolation	Disease severity a (%)	Accesion number	The highest similar isolate in the Gen Bank	Similarity (%)
4252	AG A	Konya	Plant	Non-pathogen	KC590554	MF070679.1	100
4224		Konya	Plant	80	KC590529	MZ569567.1	99
4227		Konya	Plant	62	KC590531	KJ012010	88
0645		Ankara	Plant	61	KC590547	MZ569568.1	92
0653	AG DI	Ankara	Plant	83	KC590591	M Z569567.1	83
0623		Ankara	Plant	70	KC590528	KJ012006.1	99
6632		Yozgat	Plant	50	KC590534	MZ569498.1	99
4247		Konya	Soil	65	KC590549	KY379507.1	97
06100	AG E	Ankara	Soil	Non-pathogen	KC590572	KX831960.1	99
0615	AG G	Ankara	Soil	Non-pathogen	KC590522	AB196658.1	98
0660	AG H	Ankara	Soil	Non-pathogen	KC590560	MZ396073.1	95
0616	AG I	Ankara	Soil	Non-pathogen	KC590588	AB196650.1	100
06101		Ankara	Soil	Non-pathogen	KC590573	JQ247570	96
7107		Kırıkkale	Plant	Non-pathogen	KC590525	DQ356409.1	96
7118		Kırıkkale	Plant	Non-pathogen	KC590523	MT487892.1	85
7120		Kırıkkale	Plant	Non-pathogen	KC590526	AJ242882.1	96
7105	AG I-like	Kırıkkale	Plant	Non-pathogen	KC590524	AJ242884.1	94
0661		Ankara	Soil	Non-pathogen	KC590561	JQ247570	95
2671		Eskişehir	Soil	Non-pathogen	KC590611	DQ356407	97
4264		Konya	Plant	Non-pathogen	KC590593	KC989057.1	97
7106		Kırıkkale	Plant	Non-pathogen	KC590581	MN898129	92
0680	AG K	Ankara	Soil	Non-pathogen	KC590599	MN160708.1	90
Control				0			

[a] Roots and hypocotyl symptoms were evaluated on the following scale: 0=no disease, 1= 1-10%, 2= 11-30%, 3= 31-50%, 4= 51-80%, 5= the entire hypocotyl infected

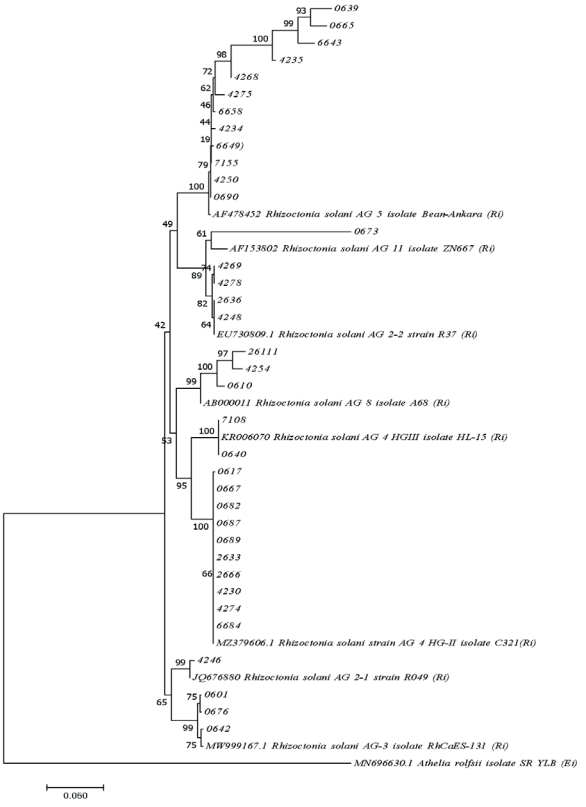

Figure 1. Phylogenetic tree of Rhizoctonia solani isolates AGs based on neighbour-joining method using MEGA 7

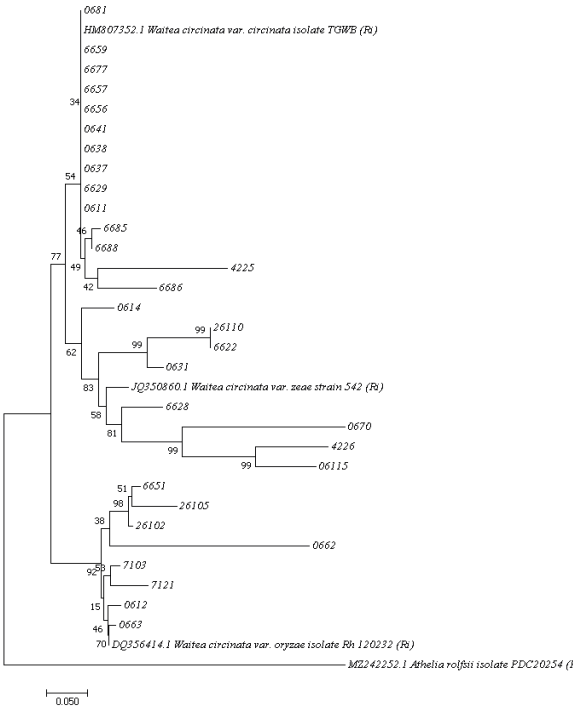

Figure 2. Phylogenetic tree of Waitea circinata isolates based on neighbour-joining method using MEGA 7

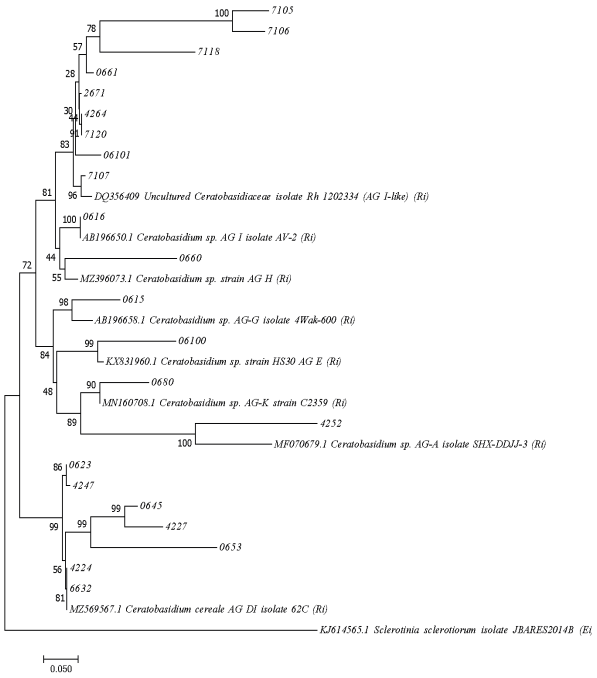

Figure 4. Phylogenetic tree of binucleate Rhizoctonia spp. isolates AGs based on neighbour-joining method using MEGA 7

Figure 3. Brown lesions caused by *Rhizoctonia solani* AG 4 anastomosis group on wheat root and crown root

belonging to pathogen *R. solani* and *R. cerealis* AG D isolates caused brown lesions at different severity in wheat root and hypocotyls as a result of pathogenicity studies, the isolates in AG groups belonging to *Waitea* spp. Caused besides light brown lesions they mostly caused symptoms such as a decrease and shortening in root formation, weak germination, or damping-off.

DISCUSSION AND CONCLUSION

In this study that was performed including different regions of Türkiye, it was determined that different anastomosis groups were causing and not causing disease on wheat. While BN isolates that were isolated from the plants consisted of AG A and DI groups, BN isolates that were isolated from the soil consisted of AG DI, AG I, AG E, AG G, AG K, and AG H. Isolates belonging to AG I-like group were isolated from both plant and soil. While MN *R. solani* AGs were not very different in terms of the place (plant or soil) where the groups were isolated, *Waitea* species were generally isolated from the soil, BN *Rhizoctonia* AGs were generally isolated from the plants. But, in some studies carried out around the world, BN *Rhizoctonia* species were mostly isolated from soil (Chen and Chuang 1997, Juan-Abgona et al. 1996). Previously, several studies were carried out in Türkiye for determining anastomosis groups on wheat, *R. solani* AG 2-1, AG 3, AG 4, AG 5, AG 8, AG 11, W. c. var. *circinata* and binucleate AG I and AG K were determined (Demirci 1998, Ünal and Dolar 2012). In this study, all isolates belonging to *R. solani* AG 4 group that was isolated from five different provinces constituted the most virulent group by causing dark brown and severe lesions in the root and hypocotyls. When examining the studies that were carried out on wheat in the world, AG 4 was the most virulent group in parallel with our study (Sneh et al. 1996). It was observed that there were differences in virulence between different AG groups belonging to the same species in this study. For example, some of the AG 5 isolates were found to be pathogenic while others were non-pathogenic. It was observed that this situation corresponded to the studies that were carried out on this subject in the world. While the rate of disease severity of twelve AG 5 isolates obtained as a result of pathogenicity tests that were made was 0% in 6 isolates, this rate changed between 56-70% in the others. When examining the studies performed in the world, while the isolates belonging to AG 5 group were reasonably virulent in some studies, it is seen that they are not pathogen or have mycorrhizal characteristic in some studies. In the study performed by Tomaso-Peterson and Trevathan (2007) and Xia and Li (1989), while they determined AG 5 as a pathogen in wheat, Demirci (1998) found it as reasonably virulent.

Waitea circinata var. *circinata* isolates that were determined

in this study showed different pathogenic characteristics as in AG 5. While 3 of the isolates that were isolated were not pathogen, it was determined that one of them (0637) was a weak pathogen, and the others were found virulent. They were also determined as a pathogen in wheat and barley. In the study performed on wheat by Demirci (1998) in Türkiye, *W. circinata* var. *circinata* was found reasonably virulent on wheat. In this study, nonpathogen and different levels of virulent *W. circinata* var. *circinata* isolates were obtained. All of the non pathogen isolates were isolated from the soil. *W. circinata* var. zeae species have been determined to significantly affect wheat emergence in wheat fields in the USA and Iran (Kuznia and Windels 1994, Telmadarrehei et al. 2011). In this study, as a result of pathogenicity studies, similar to the results of Kuznia and Windels, a decrease in the germination of *W. circinata* var. zeae isolates in wheat, stunting in plants, a decrease in the number of seminal roots, and superficial discoloration of hypocotyls and roots were observed. Due to the severe symptoms, it should be considered a potential threat to wheat cultivation in Türkiye. Okubara et al. (2008), *R. oryzae* isolates were divided into three genotypes based on their morphology, colony development, and genetic structure, and they were named *R. oryzae* genotype I, *R. oryzae* genotype II and *R. oryzae* genotype III. Okubara et al. (2008) have also shown differences between these genotypes in their study. *R. oryzae* genotype III is a species of *R. oryzae* (*W. circinata* var. *oryzae*) that has been widely known for years and is known as AG WAG O and it was determined as pathogen in many products including wheat in many studies that were performed in the world (Mazzola et al. 1996, Paulitz et al. 2003). Okubara et al. (2008) stated that genotype III is pathogen in wheat and barley but they did not give any information about the pathogenity of genotype I. In our study, six *W. circinata* var. *oryzae* isolates detected in this study took place in the same group with *R. oryzae* genotype I isolates of Okubara et al. (2008). Seven out of eight isolates of *W. circinata* var. *oryzae* isolates were isolated from the soil and none of them was found as pathogen on wheat. The results obtained in this study support the studies of Okubara et al. (2008). In the present study, all binucleate isolates except for *R. cerealis* AG DI were not found to be pathogen. These groups use in the studies of biological control in the world (Cardoso and Echandi 1987, Gutierrez and Torres 1990).

With the present study, *Rhizoctonia* species, AGs, and subgroups in wheat fields in the Central Anatolia Region of Türkiye were matched with isolates from the same group from other countries in Genbank, and their virulence status was determined. Afterward, the relationship status of these groups was revealed. With future studies, it is necessary to focus on the development of pathogenic and non-pathogenic *Rhizoctonia* species and anastomosis groups, and resistant lines and varieties against pathogenic groups in other wheat production areas of Türkiye. Studies to be carried out on biological control with pathogenic groups will provide great benefits to the producer in this field.

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ÖZET

Bu çalışmanın amacı, Türkiye'deki buğday bitkileri ve rizosfer topraklarından patojenik ve patojenik olmayan *Rhizoctonia* tür ve anastomosis gruplarının (AG) türlerini belirlemek ve genetik çeşitliliklerini değerlendirmektir. Türkiye'nin Orta Anadolu Bölgesi'ndeki 5 ildeki buğday tarlalarının bitki ve rizosfer topraklarından *Rhizoctonia* türleri izole edilmiştir. İzolasyonlar sonucunda, toplam 88 adet çok çekirdekli (MN) ve iki çekirdekli (BN) *Rhizoctonia* izolatı elde edilmiştir. İzolatların teşhislerinde rDNA-ITS dizi analizi yöntemi kullanılmıştır ve MN Waitea circinata var. zeae, *W. circinata* var. *oryzae*, *W. circinata* var. *circinata*, MN *Rhizoctonia solani* AG 2-1, AG 2-2, AG 3, AG 4-HGII, AG 4-HGIII, AG 5, AG 8, AG 11 ve BN AG A, AG DI, AG E, AG G, AG H, AG I, AG I-benzeri ve AG K'ya ait oldukları belirlenmiştir. En çok izole edilen grup *W. circinata* var. *circinata* olmuştur. Patojenite çalışmalarında, en virulent grubun *R. solani* AG 4 olduğu saptanmıştır. BN izolatlar arasında, *R. cerealis* AG DI dışındaki diğer grupların patojen olmadığı tespit edilmiştir. İzolatların rDNA-ITS dizilerinden neighbor-joining filogenetik ağaçları oluşturulmuştur. Bu çalışmanın sonucunda, Türkiye'nin Orta Anadolu Bölgesi'ndeki önemli buğday üretim alanlarında MN ve BN *Rhizoctonia* AG izolatlarının bölgedeki dağılımı belirlenmiştir. Ayrıca, bu çalışma, bölgeden elde edilen *Rhizoctonia* AG izolatlarının genetik çeşitliliğinin moleküler bir yaklaşımla değerlendirildiği ilk kapsamlı çalışmadır.

Anahtar kelimeler: akrabalık ilişkileri, anastomosis grup, buğday, *Rhizoctonia*, toprak

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Original article

Determination of plant parasitic nematodes on some oil rose growing areas of Isparta province in Türkiye

Türkiye'de Isparta ili yağ gülü yetiştirilen bazı alanlarında bitki paraziti nematodların belirlenmesi

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ABSTRACT

The study was carried out to investigate plant parasitic nematodes in oil rose growing areas in Isparta province in July-August 2022. Soil samples were collected from 4 districts and the study was carried out with 65 samples. Plant parasitic nematodes were extracted by the modified Baermenn funnel technique. A total of 12 genera of plant parasitic nematodes were determined in the study. These are *Tylenchus* spp., *Aphelenchus* spp., *Pratylenchus* spp., *Ditylenchus* spp., *Dorylaimus* spp., *Paratylenchus* spp., *Longidorus* spp., *Xiphinema* spp., *Meloidogyne* spp., *Helicotylenchus* spp., *Tylenchorhynchus* spp., and *Merlinius* spp. The *Pratylenchus* (52.3%), *Paratylenchus* (38.5%), and *Helicotylenchus* (41.5%) seemed to be the most prevailing genera. The least common genus is *Meloidogyne* (7.6%). *Meloidogyne* species were identified molecularly by using species-specific primers from second-stage juveniles. The 5 samples taken from cultivated oil rose fields were infected and detected with *Meloidogyne* hapla Chitwood, 1949. Densities of *M. hapla* varied between 60-480/100 g soil. This is the first report of *M. hapla* on oil rose (*Rosa damascena* Mill.) in Türkiye.

INTRODUCTION

Rosa damascena Mill. (Rosaceae) is an important essential oil plant. Bulgaria and Türkiye are the largest rose processing countries in the world. Approximately 15.000 tons of rose flowers are produced annually worldwide; about 8.500 tons of this production is made in Türkiye (Ersan and Başayiğit 2022). Rose oil is a very valuable product used in the perfumery, cosmetics, food industry, and pharmacy. Türkiye produces more than half of the world's rose oil and 44.4% of the world's rose concrete (Izgi 2022). 81.8% of Türkiye's oil rose production is made in Isparta is famous for its rose oil, on the other hand, it also has a privileged position due to profit per unit area, employment opportunities, and exportation. About 84% of rose production in Isparta is provided by Gönen, Keçiborlu, and Merkez districts (Arıcı et al. 2022, Gul et al. 2015). Approximately 5 tons of concrete, 2 tons of absolute, and 1.5 tons of rose oil are produced annually in 20 distillation and extraction facilities operating in Isparta province. Over 15 million Euros of foreign currency enters the economy of Isparta annually from the export of these products (Arıcı et al. 2022, Baydar 2016).

Many nematode species are agricultural pests that cause large yield losses (Stirling and Stirling 2003). Plant parasitic nematodes infect plants and caused plant nutrient deficiency and may develop symptoms such as root galls, lesions, excessive branching, blunt root formation, or root rot (Agrios 1997, Ogallo

et al. 1997). In addition, plant parasitic nematodes can cause the sensitivity in the plant to secondary microorganisms (Göze Özdemir et al. 2022). Rose plants are suspected to be infected with numerous plant parasitic nematodes, *Meloidogyne* spp., and *Pratylenchus* spp. are stated as economically important parasites of rose varieties (Fox 2001). *Pratylenchus* spp. migrate and feed within the roots, resulting in lesions initially appearing as spots along the root surface. Later, these areas may coalesce to become large areas of necrotic tissue (Castillo and Vovlas 2007). *Meloidogyne* spp. are sedentary endoparasites with a broad host range, highly pathogenic, and can destroy the host resistance (Jones et al. 2013). *Meloidogyne* spp. affect the supply of water and nutrients to plants, thereby adversely affecting growth (Portillo et al. 2013). Root-knot nematodes have been reported in rose-growing areas around the world (Oloo et al. 2009, Wang et al. 1999). Growers in commercial hydroponic rose culture in the Netherlands reported up to 40% production losses because of root-knot nematodes (García Victoria and Amsing 2005). *Rosa indica* L. and *Rosa multiflora* Thunb. are frequently attacked by *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949, *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949, and *Meloidogyne hapla* Chitwood, 1949 (Tylenchida: Meloidogynidae) (Wang et al. 2004). While *Meloidogyne hapla* may reproduce either by meiotic parthenogenesis or by *amphimixis*, *M. incognita*, *M. javanica*, and *M. arenaria* reproduce by obligate mitotic parthenogenesis (Triantaphyllou 1985).

To increase the yield and quality of roses, first of all, pests and diseases must be identified and controlled in the growing areas. Akgül and Ökten (1997) reported that 22 species of Tylenchida were determined in oil rose areas of Isparta province. However, it has been observed that no study has been done recently. Therefore, this study aimed to determine the plant parasitic nematodes in oil roses in Isparta province between June and August 2022.

MATERIALS AND METHODS

The main material of the research consists of soil samples taken from the areas where rose production is made in Isparta and plant parasitic nematodes obtained.

Soil sampling

Table 1. Sampling locations and numbers in the oil rose fields of Isparta province, Türkiye

District	Village/Town	Sampling number
Atabey	İslamköy	5
	Harmanören	5
	Merkez	3
Gönen	Güneykent	10
	Gümüşgün	5
	İğdecik	4
Keçiborlu	Merkez	2
	Kılıç	6
	Senir	9
Central	Merkez	2
	Aliköy	2
	Deregümü	3
	Kayıköy	2
	Yakaören	5
	Gelincik	2

Sampling was made at different time intervals between June and August of 2022. A total of 65 samples were taken from 15 different areas in Isparta. Sampling locations and sample numbers are shown in Table 1. Soil samples were taken from a depth of 0-30 cm with a shovel to represent the field from different points of the field, which showed stunting and yellowness in the oil rose. Approximately 1 kg of soil samples were taken from each field, placed in polyethylene bags, labeled, and brought to the laboratory in an ice box.

Nematode identification

Plant parasitic nematodes were extracted from 100 g of dry soil from each sample using a modified Baermann funnel technique (Hooper 1986, Whitehead and Hemming 1965). Nematodes were counted according to genera under the light microscope at 20x magnification.

Molecular identification

DNA isolation from root-knot nematode larvae obtained from soil samples was carried out using the "DNAeasy Tissue and Blood Kit" (Qiagen, Hilden, Germany). Species-specific

Table 2. Species-specific primers of root-knot nematodes for molecular identification

Species	Name of primer	Primer sequences (5-3)	Fragment (bp)	Reference
M. arenaria	FAR	TCGGCGATAGAGGTAAATGAC	420	Zijlstra et al. 2000
	RAR	TCGGCGATAGACACTACAACT		
M. javanica	FJAV	GGTGCGCGATTGAACTGAGC	670	Zijlstra et al. 2000
	RJAV	CAGGCCCTTCAGTGGAACTATAC		
M. incognita	INCK14R	CCCGCTACACCCTCAACTTC	399	Randig et al. 2002
	INCK14F	GGGATGTGTAAATGCTCCTG		
M. hapla	JMVhapla	GGATGGCGTGCTTTCAAC	440	Wishart et al. 2002
	JMV1	TTTCCCCTTATGATGTTTACCC		
	JMV2	AAAAATCCCCTCGAAAAATCCACC		

PCR primers were used for molecular identification which was conducted by thermocycler (Veriti Thermal Cycler, Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) (Table 2). PCR amplifications were performed in a total volume of 25 µl reaction mixtures, each containing 10 ng DNA (5 µl), PCR buffer (2.5 µl), 2 mM MgCI2 (1 µl), 0.2 mM dNTP (1 µl), 10 mM Primer F (1 µl), 10 mM Primer R (1 µl), 1 unit Taq DNA polymerase (GenEon, San Antonio, TX, USA) (0.25 µl) and ddH2O (13.25 µl).

PCR products were separated using agarose electrophoresis in 2% gel (Agarose Type I, Sigma-Aldrich, St. Louis, MO, USA) staining with ethidium bromide, then visualized and photographed under UV light using a gel documentation system.

Plant parasitic nematode community analyses

The occurrence, absolute, and relative frequency of plant parasitic nematodes in the study of the genus in oil rose areas in Isparta province were calculated using the formulas below (Evlice 2021, İmren 2018, Norton 1978).

$$\text{The prevalence rate of genus} = \frac{\text{Number of infected samples in district}}{\text{Total number of samples surveyed}} \text{X } 100$$

$$\text{Absolute frequency} = \frac{\text{Number of samples containing a genus}}{\text{Number of samples collected}} \text{X } 100$$

$$\text{Relative frequency} = \frac{\text{Relative frequency}}{\text{Sum of frequency of all genus}} \text{X } 100$$

RESULTS

It was determined that there are 12 genera of plant parasitic nematodes where oil rose is produced in Isparta province. In the study, *Tylenchus* spp., *Aphelenchus* spp., *Pratylenchus* spp., *Ditylenchus* spp., *Dorylaimus* spp., *Paratylenchus* spp., *Longidorus* spp., *Xiphinema* spp., *Meloidogyne* spp., *Helicotylenchus* spp., *Tylenchorhynchus* spp., and *Merlinius* spp. were detected. The prevalence rate of presence is shown in Table 3. The highest prevalence was found in the genus *Pratylenchus* with 52.3%, while *Helicotylenchus* spp. was ranked second with 41.5%. The least common genus was *Meloidogyne* (7.6%), and it was detected in only 5 areas. The prevalence of *Longidorus* spp. and *Xiphinema* spp. were determined as 10.8% and 9.2%, respectively (Table 3).

Table 3. The prevalence rate of plant parasitic nematodes in oil rose areas in Isparta province

Plant parasitic nematode	% Prevalence
Pratylenchus spp.	52.3
Helicotylenchus spp.	41.5
Paratylenchus spp.	38.5
Aphelenchus spp.	24.6
Tylenchus spp.	20
Ditylenchus spp.	20
Merlinius spp.	20
Dorylaimus spp.	13.8
Tylenchorhynchus spp.	10.8
Longidorus spp.	10.8
Xiphinema spp.	9.2
Meloidogyne spp.	7.6

The absolute and relative frequency at the genus level in the districts in Isparta is given in Table 4. The absolute frequency was recorded in *Meloidogyne* spp. with 15.3% in Atabey (İslamköy), 9.5% in Gönen (Güneykent), and 5.8% (Senir) in Keçiborlu district. *Meloidogyne* spp. was not found in the samples taken from the villages of the central district of Isparta. The lowest relative frequency in the Atabey district was also found in *Meloidogyne* spp. and *Xiphinema* spp. with 5.6%. *Paratylenchus* spp. (53.8%) was determined as the most common genus after *Pratylenchus* spp. (69.2%) and *Helicotylenchus* spp. (61.5%). in the Atabey district. While the lowest relative frequency in Gönen district was also found in *Longidorus* spp. and *Tylenchorhynchus* spp. with 1.7%, the highest was recorded in *Tylenchus* spp. (15.9%), *Pratylenchus* spp. (14.1%) and *Helicotylenchus* spp. (14.1%). In the Keçiborlu district, the highest absolute and relative frequency was determined in *Helicotylenchus* spp. with 41.1% and 15.2%, respectively. *Tylenchorhynchus* spp. was not found in central samples. The relative frequency of *Longidorus* spp. (2.6%) and *Tylenchus* spp. (2.6%) in the central was recorded as the lowest whereas the highest was in *Pratylenchus* spp. with 31.8% (Table 4).

As a result of the molecular identification, *Meloidogyne hapla* Chitwood, 1949 was identified in the five samples (Figure 1). The densities of Güneykent2 and İslamköy1 samples were found to be 480 larvae/100 g soil and 320 larvae/100 g soil, respectively (Figure 2).

Figure 1. PCR products amplified using primers JMV/JMV1/JMV2 (S1:Senir, IS1: İslamköy1, IS2: İslamköy2, G1: Güneykent1, G2: Güneykent2)

Table 4. Population analyses of plant parasitic nematodes on oil rose in Isparta

Plant parasitic nematode	Atabey		Gönen		Keçiborlu		Central	
	AF[1]	RF	AF	RF	AF	RF	AF	RF
Tylenchus spp.	30.7	11.4	42.8	15.9	17.6	6.5	7.1	2.6
Aphelenchus spp.	38.4	14.3	14.2	5.2	23.5	8.7	56.0	21.1
Pratylenchus spp.	69.2	25.7	38.0	14.1	29.4	10.9	85.7	31.8
Ditylenchus spp.	30.7	11.4	9.5	3.5	23.5	8.7	28.5	10.5
Dorylaimus spp.	23.1	8.5	9.5	3.5	11.7	4.3	14.2	5.2
Paratylenchus spp.	53.8	19.9	33.3	12.3	35.2	13.0	21.4	7.9
Longidorus spp.	23.1	8.5	4.7	1.7	11.7	4.3	14.2	5.2
Xiphinema spp.	15.3	5.6	9.5	3.5	11.7	4.3	7.1	2.6
Meloidogyne spp.	15.3	5.6	9.5	3.5	5.8	2.1	0	0
Helicotylenchus spp.	61.5	22.8	38.0	14.1	41.1	15.2	28.5	10.5
Tylenchorhynchus spp.	23.0	8.5	4.7	1.7	17.6	6.5	0	0
Merlinius spp.	30.7	11.4	14.2	5.2	17.6	6.5	21.4	7.9

1 AF: Absolute Frequency, RF: Relative Frequency

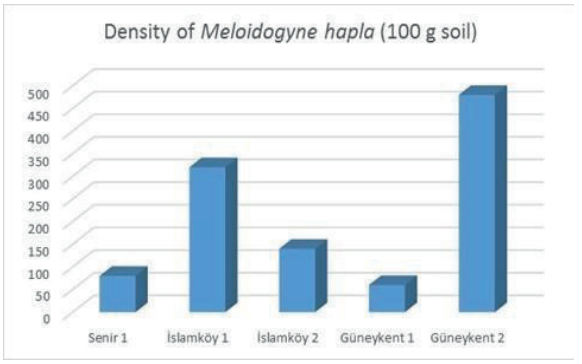

Figure 2. Soil density of *Meloidogyne hapla* in oil rose areas in Isparta province

DISCUSSION

In the present study, the 12 genera belonging to plant parasitic nematodes were identified in oil rose areas of Isparta, Türkiye. *Pratylenchus* spp. and *Helicotylenchus* spp. was determined as major genus and *Meloidogyne* spp. was found as a minor genus in the study. In a previous study, 22 species of Tylenchida were determined and three species (*Pratylenchus neglectus* (Rensch, 1924) Filipjev and Schuurmans-Stekhoven, *Ditylenchus clarus* Thorne and Malek, 1968 and *Filenchus plattensis* Thorne and Malek, 1968) were reported as the most common species in the rose growing areas of Isparta (Akgül and Ökten 1997). However, *Filenchus* spp. was not found in this study. In Florida, at least 30 species of plant parasitic nematodes have been recovered from the soil around rose roots (Lehman 1982). Nour El-Deen et al. (2015) reported the genera *Meloidogyne*, *Rotylenchulus*, *Xiphinema*, and *Pratylenchus* in their study on Taify rose planted areas in Saudi Arabia. The percentage of these nematodes was found 30%, 29.3%, 16.5%, and 11.3%, respectively. However, in this study, *Rotylenchus* spp. was not detected. Red raspberry is in the Rosaceae family like rose, Mokrini et al. (2019) reported that the most common plant-parasitic nematodes (PPN) were *Pratylenchus* spp., *Meloidogyne* spp. and *Helicotylenchus* spp. in raspberry in Morocco's Souss-Massa region. *Pratylenchus* spp., *Helicotylenchus* spp., *Tylenchorhynchus* spp., *Criconemoides xenoplax*, and *Ditylenchus dipsaci* were associated with red raspberry disease (Kroese et al. 2016, Poiras et al. 2014). In a study conducted by Magnusson and Tangvik (2018) in Norway, in raspberry (*Rubus idaeus* L.) orchards, *Tylenchus davainei* Bastian, 1865, *Cephalenchus leptus* Siddiqi, 1963, *Tylenchorhynchus dubius* (Buetschli, 1873) Filipjev, 1936, *Pratylenchus crenatus* Loof, 1960, *Pratylenchus penetrans* (Cobb, 1917) Filipjev and Schuurmans Stekhoven, 1941, *Pratylenchus fallax* Seinhorst 1968, *Helicotylenchus canadiensis* Wassem, 1961, *Helicotylenchus variocaudatus* Yuen, 1964, *Rotylenchus fallorobustus* Sher, 1965, *Paratrichodorus pachydermus* Seinhorst, 1954 and *Longidorus elongatus* (de Man, 1876) Micoletzky, 1922 species were identified. It has been reported that one of the biggest threats to the production of red raspberries is the root lesion nematode *P. penetrans* (Rudolph et al. 2017, Zasada and Moore 2014, Zasada and Walters 2016). *Raspberry* (*R. idaeus*) and blackberry (*Rubus fruticosus* L.) orchards in Türkiye, a total of 34 species, including 18 genera, were identified and the most encountered species were found *P. penetrans*, *P. neglectus*, *Filenchus filiformis* Bütschli, 1873, *Filenchus anguilonius* (Wu, 1969) Lownsberry & Lownsbery, 1985, *Helicotylenchus digonicus* Perry, 1959 and *Aphelenchus avenae* Bastian, 1865 (Çalışkan 2019).

In the present study, it was determined that Atabey (IS1, IS2), Gönen (G1, G2), and Keçiborlu (S1) district was infected with root-knot nematode, *M. hapla*. This is the first report of *M. hapla* on oil rose in Türkiye. *Meloidogyne hapla* was described first time on *Solanum tuberosum* from the USA by Chitwood (1949) (Evans et al. 1993). This specie has a wide host range, which is more common in temperate

regions and affects more than 550 crops and weed species (Grandison 1983, Jepson 1987). In addition, *M. hapla* eggs and juveniles can survive field temperatures below 0 °C (Karssen et al. 2013). Unlike thermophilic species such as *M. arenaria*, *M. incognita*, and *M. javanica*, which often cause large, coalesced galls that can cover the entire root system, the galls formed by *M. hapla* are usually smaller and more discrete (Nyoike et al. 2012). It was determined on pepino, kiwifruit, tomatoes, pepper, potatoes, strawberry, and eggplant in Türkiye (Akyazi et al. 2012, 2017, Evlice et al. 2022, Özarslandan et al. 2010, 2021, Uysal et al. 2017). It is considered that *Meloidogyne hapla* is one of the main plant parasitic nematode specie restricting rose cultivation (*Rosaceae* spp.) and is reported to have a worldwide distribution (Fox 2001, Meressa et al. 2014, Oloo et al. 2009, Wang et al. 2004). *Meloidogyne hapla* and *P. penetrans* are the most widespread nematodes in strawberries in the Rosaceae worldwide (Nyoike et al. 2012, Samaliev and Mohamedova 2011). In addition, Göze Özdemir (2022) detected *M. incognita* and *M. arenaria* infestation in lavender fields in Isparta province.

To the author's knowledge, *Pratylenchus* spp. was detected as a widespread nematode in an oil rose field in Isparta, Türkiye. Finding the northern nematode, *M. hapla* is important in Isparta. This nematode is a polyphagous and quarantine pest. However, there is no information about the effect of the amount of essential oil. Thus, it is important to determine the effect of the amount of essential oil in the continuation of the study. In addition, resistant cultivars against *M. hapla* should be determined by doing reaction studies with different rootstocks and cultivars.

ÖZET

Çalışma, Isparta ilinde yağ gülü yetiştirilen alanlarda bitki paraziti nematodların araştırılması amacıyla Temmuz-Ağustos 2022 tarihlerinde gerçekleştirilmiştir. Dört ilçeden toprak örneği alınmıştır ve çalışma 65 örnek ile yürütülmüştür. Çalışmada toplam 12 cinse ait bitki paraziti nematodlar tespit edilmiştir. Bunlar *Tylenchus* spp., *Aphelenchus* spp., *Pratylenchus* spp., *Ditylenchus* spp., *Dorylaimus* spp., *Paratylenchus* spp., *Longidorus* spp., *Xiphinema* spp., *Meloidogyne* spp., *Helicotylenchus* spp., *Tylenchorhynchus* spp. ve *Merlinius* spp.'dir. *Pratylenchus* (%52.3), *Paratylenchus* (%38.5) ve *Helicotylenchus* (%41.5) en yaygın cinsler olarak görülmüştür. En az yaygın olan cins ise *Meloidogyne*'dir (%7.6). Kök ur nematod türlerinin moleküler tanımlaması, larvalardan türe özgü primerler ile belirlenmiştir. Yağ gülü ekimi yapılan tarlalardan alınan 5 örnekte bulaşıklık saptanmış ve *Meloidogyne* hapla Chitwood, 1949 tespit edilmiştir. *Meloidogyne hapla*'nın yoğunlukları 60-480/100 g toprak arasında değişmektedir. Bu, *M. hapla*'nın Türkiye'de yağ gülü (*Rosa damascena* Mill.) üzerinde tespit edildiği ilk rapordur.

Anahtar kelimeler: yağlık gül, Rosaceae, kök lezyon nematodu, kök ur nematodu

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Original article

Determination of harmful and beneficial predator insect species and the distribution and density of *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae) in wheat-cultivated areas of Siirt province

Siirt ili buğday ekiliş alanlarında bulunan zararlı ve predatör türler ve *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae)'in yayılışı ve yoğunluğunun belirlenmesi

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ABSTRACT

Wheat is nowadays the most important source of food on earth. Many pest species have been identified that affect wheat yield and quality negatively. This study was conducted to identify harmful and beneficial insect (predator) species and of the distribution of Sunn pest in wheat cultivation areas in the districts (Siirt province Merkez, Kurtalan, Eruh, Tillo, Şirvan, Pervari, and Baykan) of Siirt Province, Türkiye in 2018 and 2019. Samplings were taken periodically for 1-2 weeks from April to July. The sweep nets, frame, pitfall trap, and visual control methods were used for sampling. At the end of the study, 42 species belonging to 6 orders and 25 families were determined. The distributions of the species were recorded as 27 species belonging to 12 families in Coleoptera, 10 species belonging to 7 families in Hemiptera, 2 species belonging to 2 families in Neuroptera, and 1 species belonging to each family in Hymenoptera, Orthoptera and Diptera. The most common and abundant species: the main pest *Eurygaster integriceps* Puton, 1881 (Hemiptera: Scutelleridae), the secondary pests *Aelia acuminata* (Linnaeus, 1758), *Dolycoris baccarum* (Linnaeus, 1758) (Hemiptera: Pentatomidae), *Cephus pygmaeus* (Linnaeus, 1767) (Hymenoptera: Cephidae) *Gryllus bimaculatus* De Geer, 1773, (Orthoptera: Gryllidae) were determined. In the study, a total of 16 species were identified, which is the first record for the local fauna of Siirt province. The highest average density of *E. integriceps* (2 adult + nymph / m^2) was found in the Garzan and Gozpinari locations of the Kurtalan district. Besides, among the beneficial species, general predators *Chrysoperla carnea* (Stephens, 1836) and *Coccinella septempunctata* (Linnaeus, 1758) are remark as the most common species

INTRODUCTION

Wheat is a cereal of the Gramineae or Poaceae family. Among cultivated plants, wheat takes place at the top, in terms of cultivation area and production amount worldwide (Anonymous 2021). Since wheat covers numerous production areas, it provides a very attractive and abundant source for diseases and pests. Drought and related yield losses occur due to climate change. Moreover, product losses caused by diseases, pests, and weeds in wheat are at levels that cannot be recently ignored. In this context, it is observed that various pesticides are used to increase the amount of product obtained per unit area in wheat. Random use of non-selective pesticides significantly affects biodiversity and deteriorates the natural balance. Pesticides enter water sources and may cause damage to non-target organisms such as plants and animals, including soil microorganisms, insects, fish, birds, and wildlife. On the other hand, it goes into the body of animals and causes them a longer existence in the food chain (Rani et al. 2021). For all of these reasons, there is a need for research on alternative methods to reduce the use of chemical control and the development of integrated control programs that are emphasized (Atlıhan and Özgökçe 2003).

In terms of wheat cultivation area, Siirt covers 36.1% of the total grain and other crop production areas of the province with 338.040 decares (Anonymous 2020). The yield obtained from the production in this area is below the average of Turkey and the region. The low yield in production may be due to various reasons such as climate, soil, disease, and pests. Generally, there are few studies on the detection of harmful and natural enemy species in wheat fields in the world (Afonina et al. 2001, Gallo and Peker 1999, Zhang et al. 2022, Zhao et al. 2013). In our country, in the study conducted by Özgökçe et al. (2022), 66 species belonging to 7 orders and 39 families were found in 165 different localities in Van province and its districts. Kıvan and Dirik (2016) identified 45 species from nine families belonging to the Heteroptera suborder at the end of the study they carried out in the wheat fields in Edirne province. In another study, 109 species belonging to 65 families and 95 genera belonging to the orders Odonata, Orthoptera, Hemiptera, Homoptera, Thysanoptera, Neuroptera, Coleoptera, Diptera, Lepidoptera, and Hymenoptera were identified in the wheat agro-ecosystem in the Adana (Sayan 2010). On the other hand Bulu (1995), 74 species were found in irrigated wheat fields and 78 species in non-irrigated fields in irrigated and dry wheat fields in the Çukurova region in 1993 and 1994.

Sunn pest, *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae) is the most serious pest of cereals (Parker et al. 2011, Sanaey and Mirak 2012). Sunn pest has infested more than 15 million hectares in total, including Syria, Iraq, Iran, Turkey, Afghanistan, and Lebanon, as well as Central Asia, the Caucasus, Bulgaria, and Romania (Salis et al. 2013). Many studies have reported that sunn pest affects wheat quality and yield negatively (Dizlek and İslamoğlu 2010, Gözüaçık and Yiğit 2020, Özgen et al. 2005). If there is not done effective control against sunn pests, losses may reach 100% (Kıvan and Kılıç 2006). Moreover, some studies have drawn attention to aphid species as potential pests apart from Sunn pests in wheat fields (Lodos 1982, Özder and Toros 1999).

We must determine harmful species and their natural enemies to implement integrated pest programs for sustainable agriculture. Moreover, which must reveal the population density and distribution of the economically important species. In the region where this study was conducted, there is no study on pests, which is one of the important factors that negatively affect crop growth. There has been no previous research on the presence, density, and prevalence of wheat-harmful species in the surroundings of Siirt province. The harmful species and their natural enemies have not been studied sufficiently in that region.

The aim of the study was to determine harmful and predator insect species found in wheat fields in Siirt province. Furthermore, the population density of the sunn pest, which is the main pest of the wheat plant, and the harmful species that have the potential to cause yield losses were determined.

MATERIALS AND METHODS

The research was carried out from April to July in Siirt and its surrounding wheat production areas (Siirt province Merkez, Tillo, Şirvan, Eruh, Kurtalan, Pervari, and Baykan) in 2020-2021. Sampling was carried out in 3 wheat fields to represent each district by going out to the field once every 15 days to the extent possible. Sampling was completed at 21 locations. The harmful and predator insects were collected by visual inspection, pitfall traps, and sweep nets from the sampling area. Depending on the size of the field, 100-200 sweep netting was made by walking along the field edges and diagonals in the samplings made using the sweep net. To determine the population densities of the Sunn pest and Aelia species, which are the main pests of wheat, were applied 1/2x1/2 = 1/4 m^2 size of the wireframes. How many counts will be made in a surveyed wheat field is related to the width of the field and is determined by adhering to the measurements given below (Polat 2003) (Table 1).

Table 1. Number of frames discarded according to field sizes (Polat 2003)

Parcel Area (da)	Number of frames (pcs)
1-15	8-12
16-50	13-16
51-200	17-24
201-800	25-32
800-<	33-42

The counts of the Sunn pests remaining in the frames were made by starting from the edge of the field and proceeding toward the center of the field at intervals of at least 20 steps. The Sunn pest species made of counts are labeled and prepared for identification in the laboratory.

Then, the population density per m^2 was calculated by evaluating the census results. Moreover, in the sampling of the species that are active by walking in the field, sufficient (3-10) pitfall traps were randomly placed in the pits opened at the soil level, depending on the size of the field. In addition to these, the stems and shoots found in all soil parts of the plant were visually inspected. Smaller insects found in these parts were transferred to Eppendorf cups and falcon tubes containing 70% and 96% alcohol, with brushes or plant parts.

The pest and predator species obtained in the study were identified to species, genus, or family level using a stereo microscope (SZ61; Olympus) by Assoc. Prof. Dr. Cevdet KAPLAN Assis. Prof. Dr. Mustafa Cemal ÇİFTÇİ and Assis. Prof. Dr. Halil DİLMEN. Some insect samples were identified using the literature at the species level and the museum material available in the Department of Plant Protection, Faculty of Agriculture, Siirt University.

RESULTS AND DISCUSSION

The total list as a result of the identification and classification of the species obtained as a result of the studies is given in Table 2. In this work, 42 species belonging to 6 orders and 25 families were identified in 21 different locations. The distributions of the species were recorded as 27 species belonging to 13 families in the order Coleoptera, 10 species belonging to 7 families in the order Hemiptera, 2 species belonging to 2 families in the order Neuroptera and 1 species belonging to each family in the orders Hymenoptera, Orthoptera and Diptera (Table 2). In similar studies, Özgökçe et al. (2022) detected 66 species in the Van wheat agricultural ecosystem, while Sayan (2010) detected 109 species in the wheat fields of Adana province. However, in this study, a total of 16 species were identified, which is the first record for the local fauna of Siirt province.

From Coleoptera species, *Cantharis lateralis* (Linnaeus, 1758), *Cantharis annularis* (Ménétriés, 1836), (Coleoptera: Cantharidae), *Haplomalachius flabellatus* (Frivaldzsky, 1835), *Clanoptilus geniculatus* (Germar, 1823), (Coleoptera: Melyridae) and *Calamosternus granarius* (Linnaeus, 1758), (Coleoptera: Scarabaeidae), *Tachyporus hypnorum* (Fabricius, 1775), (Coleoptera: Staphylinidae), *Meligethes aeneus* (Fabricius), (Coleoptera: Nitidulidae), *Brachypterolus pulicarius* (Linnaeus, 1758), (Coleoptera: Kateretidae) and *Cryptocephalus parvulus* (Müller, 1776), *Labidostomis mesopotamica* (Heyden, 1886), *Chaetocnema tibialis* (Illiger, 1807), (Coleoptera: Chrysomelidae) and *Sitona lineatus* (Linnaeus, 1758) (Coleoptera: Curculionidae) are the first records for the fauna of the Siirt province.

Furthermore, *Cercopis vulnerata* (Rossi, 1807), (Hemiptera: Cercopidae), *Carpocoris pudicus* (Poda, 1761), *Carpocoris fuscispinus* (Boheman, 1850), (Hemiptera: Pentatomidae) and *Libelloides macaronius* (Scopoli, 1763), (Neuroptera: Ascalaphidae) are the first records for the fauna of the Siirt province.

The most common harmful species found were *Eurygaster integriceps*, *Aelia acuminata*, and *Cephus pygmaeus* in the study areas (Table 2). *Aelia acuminata* (7 adults / 100 sweep nets) and *C. pygmaeus* (10 adults / 100 sweep nets) were found most intensely in the Kurtalan district. It has been determined that some insect species mentioned in Table 2 are common pests in wheat fields, both in our observations and in previous studies. It has been observed that *E. integriceps*, *A. acuminata*, and *C. pygmaeus* species from these species cause significant damage from region to region and farmers apply chemical pesticides against these species.

E. integriceps, one of the important species of Sunn, belonging to the *Eurygaster* genus belonging to the Scutelleridae family of the Hemiptera order, which causes economic losses in wheat fields in many regions of the world and in Turkey, was found in 7 different districts of Siirt province and at 21 different sampling points (Table 2 and Figure 1). The Sunn pest was most intensely recorded in Kurtalan and Merkez districts. As a result of all samplings, 833 overwintered adults in the Kurtalan district and 494 overwintered adults in the Merkez district were determined. The lowest density was found in Tillo (248 overwintered adults) and Pervari (201 overwintered adults). The Sunn pest density reached its highest level with 170 individuals on 01.06.2021 in the Kurtalan district. On these dates, overwintered adult and nymph density in Kurtalan, Merkez, Şirvan, Eruh, Tillo, Baykan, and Pervari districts were 170,105, 98, 72, 56, and 49 individuals, respectively.

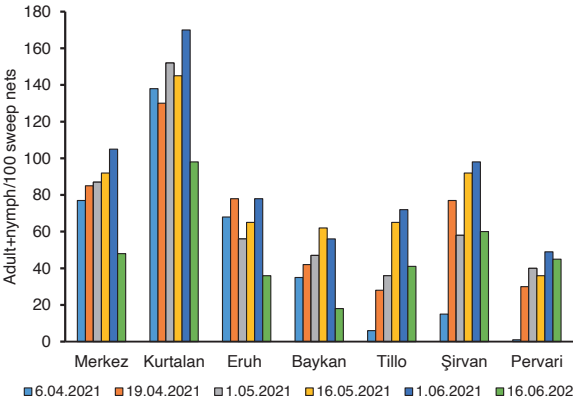

Figure 1. Locations and densities of Eurygaster integriceps (adult+nymph/100 sweep nets)

Table 2. Predator and pest species were seen in wheat cultivation areas in Siirt province

Order	Family	Species	Locations						
			1	2	3	4	5	6	7
		Predator insect species							
Coleoptera	Coccinellidae	*Coccinella septempunctata*	+	+	+	+	+	+	+
		Oenopia conglobata	-	+	-	-	-	+	-
		Scymnus bivulnerus	+	+	+	-	-	+	-
	Carabidae	*Scarites* sp.	+	+	+	+	+	+	+
Neuroptera	Ascalaphidae	*Libelloides macaronius**	+	+	-	-	-	+	-
	Chrysopidae	*Chrysoperla carnea*	+	+	+	+	+	+	+
Hemiptera	Nabidae	*Nabis* sp.	+	+	+	-	-	+	-
	Reduvidae	*Triatoma* sp.	-	+	+	-	-	-	-
		Pest insect species							
Coleoptera	Scarabaeidae	*Tropinota hirta*	+	+	+	+	+	+	+
		Anisoplia spp.	+	+	+	-	+	+	+
		*Calamosternus granarius**	+	+	+	+	+	+	+
	Melyridae	*Clanoptilus geniculatus**	+	+	-	-	+	-	-
		*Haplomalachius flabellatus**	+	+	+	+	+	+	+
	Cantharidae	*Occathemus tarsalis*	+	+	+	+	+	+	+
		*Cantharis lateralis**	+	+	+	+	+	+	+
		*Cantharis annularis**	+	+	+	+	+	+	+
	Carabidae	*Microlestes sahlbergii*	+	+	+	+	+	+	+
		Bembidion properans	+	+	-	-	-	+	+
		Notiophilus substriatus	+	+	+	+	+	+	+
	Staphylinidae	*Tachyporus hypnorum**	+	+	-	-	-	-	-
	Tenebrionidae	*Omophlus caucasicus caucasicus*	+	+	+	+	+	+	+
	Nitidulidae	*Meligethes aeneus**	+	+	-	-	-	-	-
	Kateretidae	*Brachypterolus pulicarius**	-	+	-	-	-	-	-
	Bruchidae	*Bruchus* sp.	+	+	+	+	+	+	+
	Chrysomelidae	*Cryptocephalus parvulus**	+	+	-	-	+	-	-
		Oulema melanopus	+	+	+	-	-	+	-
		*Labidostomis mesopotamica**	+	+	+	+	+	+	+
		*Chaetocnema tibialis**	+	+	+	+	+	+	+
	Curculionidae	*Protapion apricans*	+	+	+	+	+	+	+
		*Sitona lineatus**	+	+	+	+	+	+	+
		Tychius reitteri	+	+	+	+	+	+	+
Hymenoptera	Cephidae	*Cephus pygmaeus*	+	+	+	+	+	+	+
Orthoptera	Gryllidae	*Melanogryllus* sp.	+	+	+	+	+	+	+
Diptera	Tabanidae	*Tabanus* sp.	+	+	+	+	+	+	+
Hemiptera	Cercopidae	*Cercopis vulnerata**	+	+	+	-	+	+	+
	Pentatomidae	*Dolycoris baccarum*	+	+	+	+	+	+	+
		Aelia acuminata	+	+	+	+	+	+	+
		*Carpocoris fuscispinus**	+	+	+	+	+	+	+
		*Carpocoris pudicus**	+	+	+	+	+	+	+
	Scutelleridae	*Eurygaster integriceps*	+	+	+	+	+	+	+
	Miridae	*Stenodema* sp.	+	+	-	+	-	+	-
	Cicadellidae	*Aphrodes* sp.	+	+	+	+	+	+	+

*Species of which there is no record that they are wheat pests in the literature. 1: Merkez, 2: Kurtalan, 3: Eruh, 4: Tillo, 5: Şirvan, 6: Pervari, 7: Baykan

The population densities of adult Sunn pests in Siirt province were recorded as a result of field samplings made as of 06.04.2021 when the landing of *E. integriceps* from the wintering was completed. Generally, when the population densities and environments of E. integriceps are examined in Table 3; the average of the Sunn pest population densities in all districts was found to be below the Economic Damage Threshold (EDT). An average of 2 individuals/m^2 was found in the Garzan and Gözpınarı locations of the Kurtalan district, where the Sunn pest is most concentrated. This location was followed by Merkez Aktaş village with an average of 1.66 individuals /m^2 and Baykan Tütenocak village with an average of 1.26 individuals /m^2 in terms of density. On the other hand, other locations were generally determined at an average of 1 individual/m^2 or less. *E. integriceps*, *A. acuminata*, and *C. pygmaeus*, are shown among the most important pests of wheat in many reports. In the study, it is a pleasing situation that they do not reach the population

Table 3. Sunn pest population densities and averages in Siirt province and districts

District	Locations	Wheat phenology	Sunn density in m² (number of individuals/m²)	District average (number of individuals/m²)	General average (number of individuals/m²)
Merkez	Merkez	Spike period-Milk stage	0.66	0.88	0.74
	Kezer		0.33		
	Aktaş		1.66		
Kurtalan	Çayırlı	Spike period-Milk stage	0.4	1.42	
	Garzan		2		
	Gözpınar		1.86		
Eruh	Çizmeli	Spike period-Milk stage	0.73	0,79	
	Bayramlı		0.66		
	Bilgili		1		
Tillo	Sinep	Spike period-Milk stage	0.4	0.40	
	Hatrant		0.4		
	Tasbalta		0.4		
Şirvan	Taşlı	Spike period-Milk stage	0.7	0.69	
	İncekaya		1.06		
	Tatlıpayam		0.33		
Pervari	Ekindüzü	Spike period-Milk stage	0.4	0.37	
	Köprüçay		0.4		
	Güleçler		0.33		
Baykan	Tütenocak	Spike period-Milk stage	1.26	0.66	
	Çaykaya		0.4		
	Dedebakırı		0.33		

level that requires control and are generally not subject to complaints by producers and agricultural organizations.

The degree and form of damage caused by Sunn pest to wheat; vary depending on the pest's density, biological periods, phenological status, product variety, and climatic conditions (temperature, precipitation, and light). Some researchers state that the economic loss threshold for the adult density overwintered by sunn pest is 0.8 individuals/m2 (Lodos 1961, Şimşek and Sezer 1985, Şimşek 1998). It was reported that the overwintered adult Sunn pest density was 2.93 individuals/m² in 1987 when there was an epidemic, and 0.69 individuals/m² in 1991 when there was no epidemic in a study conducted in Tekirdağ between 1987 and 1991 (Kıvan 1991). In another similar study, when 2-7 individuals/m² adults were detected in the chemical control of overwintered adults, and chemical control against nymphs, it is stated as EDT between 3-20 individuals/m² nymphs, although it is usually 10 individuals/m² nymphs.

In addition, pest insect species, and predator insect species were detected in the wheat fields of Siirt province and are listed in Table 2. Among the predator species given in Table 2 are *Chrysoperla carnea* (Stephens) (7 adult/100 sweep nets), *Coccinella septempunctata* (L.) (12 adult/100 sweep nets) and Nabis sp. (4 adult/100 sweep nets) drawn attention as the most common species. The species belonging to the Chrysopidae family, which are common in both agricultural and non-agricultural areas in our country, as in many parts of the world, are the predators of many harmful insect groups. These species feed on harmful species such as aphids, mites, thrips, pre-adults of whiteflies, and leafhoppers (Kasap et al. 2003). *Chrysoperla carnea* is primarily a predator of aphids, although it feeds on psyllids, mealybugs, spider mites, and other soft-bodied insects (Atlıhan and Özgökçe 2003, Rashid et al. 2012, Souliotis et al. 2002).

Many species belonging to the Coccinellidae family play a leading role in biological control. Undoubtedly, William (2002) stated that species belonging to the Coccinellidae family are the most common of all beneficial insect species. Among these species, *C. septempunctata*, known as the seven-spotted ladybug, has been reported as an important predator of aphids, psyllids, potato beetles, and leafhoppers (Bolu et al. 2007, Erler 2004, Honek and Hodek 1996, Kedici et al. 1998, Mehrnejad et al. 2010). This species has been reported to be common throughout our country (Uygun 1981). Yiğit et al. (2007) reported *C. septempunctata* as a predator of aphids in wheat fields in Antakya (Serinyol) and Reyhanlı (Hatay). Among the coccinellidae species, the most common *C. septempunctata* species was detected in agricultural and non-agricultural areas (Buğday et al. 2015).

Nabis species (Hemiptera: Nabidae) were detected at all sampling locations. Kıvan and Dirik (2016) reported that *Nabis pseudoferus* is a predator of aphids in wheat production areas in Edirne province. Moreover, Nabis species have been shown as predator species in various ecosystems in many studies (Alaoğlu and Özbek 1987, Atlıhan and Özgökçe 2003, Kıvan and Dirik 2016).

Samplings made using a sweep net were made in fields with weeds and cultivated fields. Most of the collected species were insects infesting weeds, especially abundant flowers and pollen-bearing, found in these areas. Simultaneously, insect density and diversity were found to be higher in wheat fields close to meadows, pasture, and wooded areas. The general situation of the region was found to be high in terms of insect diversity in wheat fields. We have observed that particularly Merkez, Kurtalan, and Pervari districts are at a very good level in this respect. Pervari, stands out with its very rich flora, natural flora, and fauna, as it has a microclimate feature due to its special geography.

From the sampling locations, are very common *E. integriceps*. *A. acuminata*, *Dolycoris baccarum* (Linnaeus, 1758), *Carpocoris fuscispinus* (Boheman, 1850), *Carpocoris pudicus* (Poda, 1761), *Aphrodes diminutus* (Ribaut, 1952), and *Cercopis vulnerata* (Rossi 1807) have been identified to the Hemipter species. Furthermore, among Coleopter species, *Tropinota hirta* (Poda, 1761), *Clanoptilus geniculatus* (Germar, 1823), *Haplomalachius flabellatus* (Frivaldzsky, 1835), *Cantharis lateralis* (Linnaeus, 1758), *Cantharis annularis* Ménétriés, 1836, *Cryptocephalus parvulus* Müller, 1776, *Oulema melanopus* (Linnaeus, 1758), *Bruchus* sp. and *Ceutorhynchus* sp. species have been identified. In addition to these, *Melanogryllus* sp., (Orthoptera: Gryllidae) (7-48 adult/pitfall trap) and *Scarites* sp. (Coleoptera: Carabidae) (1-27 adult/pitfall trap) were recorded as common species.

Apart from harmful species, there is a fauna that cannot be underestimated in terms of predator species diversity in the region. Although the wheat fields are sprayed in every period, the number and density of natural enemy species are at a level that cannot be underestimated. For this reason, it is extremely important to develop methods that can be an alternative to pesticide control within integrated pest management programs. Besides, it was concluded that it would be a predator to monitor the population developments of predators and harmful species during the wheat planting period and to conduct studies to determine the activities of important natural enemy species.

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ÖZET

Buğday, günümüzde dünyanın en önemli besin kaynağıdır. Buğday verimini ve kalitesini olumsuz yönde etkileyen birçok zararlı böcek türü tespit edilmiştir. Bu çalışma 2020-2021 yıllarında Siirt ili ve ilçeleri (Merkez, Kurtalan, Eruh, Tillo, Şirvan, Pervari ve Baykan)'nde buğday ekiliş alanlarında bulunan zararlı ve yararlı böcek (predatör) türlerinin belirlenmesi ve zararlı *Eurygaster integriceps* Puton, 1881 (Hemiptera: Scutelleridae)'in yayılışının belirlenmesi amacıyla yürütülmüştür. Örneklemeler nisan-temmuz aylarında 1-2 haftalık aralıklarla yapılmıştır. Çalışmada atrap, çerçeve, çukur tuzaklar ve gözle kontrol yöntemleri kullanılmıştır. Çalışma sonunda 6 takım ve 25 familyaya bağlı 42 tür saptanmıştır. Türlerin dağılımları Coleoptera takımında 12 familyaya bağlı 27 tür, Hemiptera takımında 7 familyaya bağlı 10 tür, Neuroptera takımında 2 familyaya bağlı 2 tür, Hymenoptera, Orthoptera ve Diptera takımlarında 1'er familyaya bağlı 1'er tür olarak kaydedilmiştir. Bu türler içerisinde en yaygın ve yoğun türler: ana zararlı *E. integriceps*, ikincil zararlılar *Aelia acuminata* (Linnaeus, 1758), *Dolycoris baccarum* (Linnaeus, 1758) (Hemiptera: Pentatomidae), *Cephus pygmaeus* (Linnaeus, 1767) (Hymenoptera: Cephidae) ve *Melanogryllus sp.* (Orthoptera: Gryllidae) belirlenmiştir. Çalışmada Siirt ilinin lokal faunası için ilk kayıt niteliğinde olan toplam 16 tür tespit edilmiştir. Ayrıca *E. integriceps*'ın en yüksek ortalama yoğunluğu (2 ergin + nimf / m^2) Kurtalan ilçesi Garzan ve Gözpınarı lokasyonlarında bulunmuştur. Bunlara ilaveten faydalı türler arasında genel predatör *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) ve *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) en yaygın türler olarak dikkat çekmektedir.

Anahtar kelimeler: buğday, *Eurygaster integriceps*, predatör, Siirt, zararlı

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Original article

Microfungi species observed on various weed species in the Yüksekova Basin, Türkiye

Yüksekova Havzasında yabancı otlar üzerinde tespit edilen mikrofungus türleri

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ABSTRACT

Studies on biological control for the sustainable management of weeds that exert serious ecological, economic, and human health problems are attracting increasing attention. Detection of potential biological control agents (pests, pathogens, etc.) on target weed species is the first step in the biological control program. This study aimed to determine the microfungi species found on noxious weed species in the Yüksekova basin situated in Hakkari province, Türkiye. Continued traditional agricultural practices, minimum or no use of pesticides and fertilizers, and better protection of natural flora/fauna compared to other parts of Türkiye were reasons for the selection of the basin in the current study. Field surveys were carried out in different periods during 2020 and 2021. A total of 101 microfungi species were recorded on 79 weed species belonging to 29 families in the basin. The most common fungi species in the basin were in genera Puccinia (29 species), *Alternaria* (18 species), *Uromyces* (14 species), and *Curvularia* (4 species). Weed hosts of the above-mentioned fungi species mostly belonged to Asteraceae (20 species), Fabaceae (7 species), Poaceae (7 species), and Lamiaceae (6 species) families. While 84 microfungi species were recorded on a single host, and the remaining 17 were found on more than one weed species. It has been observed that *Puccinia cyani* (Schleich.) Pass., *Puccinia chondrillina* Bub & Syd., and *Uromyces polygoni-avicullariae* (Pers.) P. Karsten significantly inhibited the growth and development of their host weed species (*Centaurea* spp., *Chondrilla juncea* L., and *Polygonum aviculare* L.) and were able to suppress the populations of the weeds in the fields. The results revealed that it would be beneficial to review the recorded pathogens in terms of biological activity and to carry out detailed field studies in the region.

INTRODUCTION

Weeds and invasive alien plant species can pose serious ecological, economic, and human health risks in agricultural ecosystems and natural areas. Therefore, weed management is indispensable for the sustainability of these areas (Önen 2015). Weeds cause significant yield and quality losses in crops, retard the development of crop plants with

allelopathic effects, serve as hosts for diseases and pests, make soil cultivation and harvesting difficult, and waste resources and time in agroecosystems (Önen 2006, Önen 2013, Önen 2021a, Özaslan et al. 2015, Özer et al. 2001, Zimdahl 2018). Therefore, weeds are one of the most important factors limiting agricultural production. Besides, weeds endanger human and animal health with their poisonous effects (Önen 2021b, Zimdahl 2018). Hence, it is anticipated that the management of weeds will become increasingly important in the future (Önen 2010a, Önen and Özcan 2010, Özaslan et al. 2016).

Yield losses caused by weed infestation can reach up to 10-90% (Önen 1995, Pätzold et al. 2020), which makes weed management mandatory (Önen and Özer 2001, Önen 2020). Herbicides have become an important weed management option, especially in conventional farming systems (Önen and Kara 2008). Herbicides are easier to use, give results in a short time, and are cheaper than other weed control methods, all of which increased their usage (Önen 2021c). The frequent use of herbicides has been posing significant risks to human health and the environment (Önen 2010b). Besides, an increase in herbicide resistance weed species has also been reported from all over the world. Recent years have seen a rise in environmental consciousness due to growing concerns about pesticide residues and a decline in ecosystem services (Önen 2014). Emerging public pressure has increased the tendency towards environmentally friendly alternative approaches such as biological control (Atay et al. 2015, Önen 2014).

Biological control is a weed management strategy that can be defined as preventing the growth/development of weeds and keeping their population below the economic threshold level by using different agents such as insects, fungi, bacteria, and viruses (Atay et al. 2015, Önen and Kara 2008). Although different organisms are used in biological control, fungi have a privileged place due to their high number of species, ability to reproduce easily in artificial environments, specialization to a single host, and suitability for commercial production (Eken and Demirci 1997, Uygur and Uygur 2010). It has been revealed that several microfungi can be used as biocontrol agents against different weed species (Amsellem et al. 2002, Atay et al. 2015, Bailey 2014, Berner et al. 2015, Eken and Demirci 2002, Harding and Raizada 2015, Kiss 2003, Özaslan 2011, Rector et al. 2006, Tepe and Özrenk 1999). Bio-herbicides containing different fungal pathogens are commercially available worldwide for weed management, especially in America, Canada, China, and South Africa (Triolet et al. 2020). For instance, the biocontrol product Lubao No: 1S22Ò has been used in the management of dodder (*Cuscuta* spp.) species since 1987 in soybean fields in China (Winston et al. 2014). Similarly, the bioherbicide "Woad Warrior" with the active ingredient Puccinia thlaspeos has been approved to control Isatis spp. in the USA (Cordeau et al. 2016, Lovic et al. 1988). It is also stated that populations of some weed species can be kept under control in agricultural areas by promoting (natural biological control) fungal pathogens affecting weeds (Atay et al. 2015, Özer et al. 2001, Sırrı and Özaslan 2022). These studies reveal that at least some of the pathogens affecting weeds in natural or agricultural ecosystems have the potential to be used as biocontrol agents. Therefore, the determination of fungal agents on weeds and their effectiveness in agriculture and non-agricultural areas can contribute significantly to the creation of integrated weed control strategies.

Ecological and biological diversity in Türkiye naturally affects microfungi biota and causes significant regional differences in fungal species on weeds and their effectiveness. Therefore, fungal pathogens affecting weed populations show significant spatial and temporal differences in the country (Bahcecioglu and Gjaerum 2003, Demirci et al. 1997, Doğan 2013, Ekici et al. 2012, Erciş 1989, Erdogdu et al. 2010, Erdogdu and Hüseyin 2013, Erper et al. 1997, Kabaktepe 2010, Kirbağ 2004, Özaslan 2011, Özaslan et al. 2013, Özaslan et al. 2015, Sert and Sümbül 2003, Sert 2009, Tunalı et al. 2009, Ulukapı 2016).

Yüksekova Basin in Hakkari province has been considered an important location for potential biological control agents due to its rich and undisturbed flora and fauna, a continuation of traditional agricultural practices, and limited use of pesticides and chemical fertilizers. Therefore, this study aimed to determine the weeds and microfungi on the weeds in the Yüksekova Basin over areas that are used in different ways (agricultural and non-agricultural).

MATERIALS AND METHODS

The study area is located in the Yüksekova district of Hakkari province. The basin is a depression plain surrounded by mountains. The altitude of the plain is between 1950 and 2000 m, its width is 15 km, and its length is 40 km. The region has a generally harsh continental climate due to its altitude and location.

Field studies (surveys) were carried out in 2020 – 2021 to determine the weed species and fungal agents on the weeds. The basin was divided into a 1 × 1 km grid, and 232 points were selected to represent the study area. Surveys were carried out in 4 different periods from the emergence of weeds in the spring to the end of the vegetation period (i.e., May through September). Weeds showing visible signs of disease were detected by surveying an area of 50 m × 50

Table 1. Host weed species and observed microfungi

Genus	Microfungi Species	Host Weed Species
Alternaria	*Alternaria alternariae* (Cooke) Woudenb. & Crous	*Rumex crispus* L.
	Alternaria alternata (Fr.) Keissl.	*Polygonum amphibium* L., *Rumex conglomeratus* Murray
	Alternaria aspera Woudenb. & Crous	*Plantago lanceolata* L.
	Alternaria atra (Preuss) Woudenb. & Crous	*Cerinthe minor* L. subsp. *auriculata* (Ten.) Domac, *Convolvulus arvensis* L., *Salvia verticillata* L. subsp. *verticillata, Ranunculus diversifolius* Boiss. & Kotschy
	Alternaria botrytis (Preuss) Woudenb. & Crous	*Chenopodium album* L. subsp. *album var. album, Senecio doriiformis* DC. subsp. *doriiformis, Medicago sativa* L. subsp. *sativa*
	Alternaria chartarum Preuss	*Equisetum arvense* L., *Calamagrostis epigeios* (L.) Roth
	Alternaria consortialis (Thüem.) W. Groves & S. Hughes.	*Tanacetum balsamitoides* Sch. Bip., *Dactylis glomerata* L. subsp. *glomerata, Ranunculus flammula* L.
	Alternaria dianthicola Weerg.	*Silene vulgaris* (Moench) Garcke var. *vulgaris, Plantago major* L. subsp. *intermedia* (Gilib.) Lange
	Alternaria herbiphorbicula E.G. Simmons	*Cirsium haussknechtii* Boiss., *Dipsacus laciniatu* L., *Silene vulgaris* (Moench) Garcke var. *vulgaris, Nepeta nuda* subsp. *albiflora* (Boiss.) Gams
	Alternaria hispidula Ellis	*Artemisia absinthium* L., *Euphorbia cheiradenia* Boiss. & Hohen., *Medicago sativa* L. subsp. *sativa, Hypericum perforatum* L. subsp. *veronense* (Schrank) H.Linb., *Rumex crispus* L.
	Alternaria lanuginosa (Harz.) Sacc.	*Eryngium campestre* L. var. *virens* Link, *Cichorium intybus* L., *Scorzonera veratrifolia* Fenzl, *Plantago lanceolata* L.
	Alternaria loliicola Meng Zhong	*Lolium perenne* L.
	Alternaria microspora (Moub. & Abbel-Hafez) Gannibal & D.O. Lawr.	*Inula britannica* L.
	Alternaria multiformis (E.G. Simmons) Woudenb. & Crous.	*Inula britannica* L.
	Alternaria obovoidea (E.G. Simmons) Woudenb. & Crous	*Rumex conglomeratus* Murray
	Alternaria oudemansii (E.G. Simmons) Woudenb. & Crous	*Carex distans* L. subsp. *distans*
	Alternaria tenuissima (Kunze) Wiltshire	*Stachys annua* L. subsp. *annua*
	Alternaria septospora (Preuss.) Woudenb. & Crous	*Chenopodium album* L. subsp. *album* var. *album, Anchusa azurea* Mill. var. *azurea, Nepeta nuda* subsp. *albiflora* (Boiss.) Gams. *Alcea striata* (DC.) Alef. subsp. *striata*
Curvularia	*Curvularia lunata (Wakker) Boedijn*	*Cirsium haussknechtii* Boiss.
	Curvularia sorghina R.G. Shivas & Sivan.	*Sorghum halepense* (L.) Pers. var. *halepense*
	Curvularia protuberata Nelson & Hodges	*Stachys annua* L. subsp. *annua*
	Curvularia trifolii (Kauffm.) Boedijn	*Sanguisorba officinalis* L.
Pirenofora	*Brachysporium gracile* (Wallr.) Sacc.	*Convolvulus arvensis* L.
Macrosporium	*Macrosporium malvae* Thüem.	*Alcea striata* (DC.) Alef. subsp. *striata*
Stemphylium	*Stemphylium piriforme* Bon.	*Echinops spinosissimus* Turra subsp. *bithynicus* (Boiss.) Greuter., *Inula britannica* L., *Epilobium hirsutum* L.
	Stemphylium vesicorium (Wallk) E.G. Simmons	*Urtica dioica* L. subsp. *dioica*
Periconia	*Periconia funerea* (Ces.) Mason & M.B. Ellis	*Hordeum bulbosum* L.
Dendryphion	*Dendriphion camosum* Wallr.	*Stachys annua* L., *Polygonum amphibium* L.
Stagonospora	*Dictyoartrinium sacchari* (J.A. Stev.) Damon	*Anchusa azurea* Mill. var. *azurea*

Aecidium	*Aecidium eremostachydis* Petr.	*Phlomoides laciniata* (L.) Kamelin & Makhm.
Uromyces	*Aecidium polygoni-cuspidati* Diet.	*Polygonum aviculare* L.
	Uromyces chenopodii (Duby) Schroet.	*Chenopodium album* L.
	Uromyces epilobii (DC.) Lév.	*Epilobium hirsutum* L.
	Uromyces fischeri-eduardi Magn.	*Vicia tetrasperma* (L.) Schreb.,*Vicia cracca* L. subsp. *cracca*
	Uromyces glycyrrhizae (Rab.) Magn.	*Glycyrrhiza glabra* L. var. *glabra*
	Uromyces gypsophilae Cooke	*Vaccaria hispanica* (Mill.) Rauschert
	Uromyces ononidis Pass.	*Ononis spinosa L. subsp. leiosperma* (Boiss.) Sirj.
	Uromyces pisi (Pers.) de By	*Lathyrus tuberosus* L.
	Uromyces polygoni-avicularae (Pers.) P. Karsten	*Polygonum aviculare* L.
	Uromyces rumicis (Schum.) Wint.	*Rumex crispus* L.
	Uromyces scillarum (Grev.) Wint.	*Bellevalia paradoxa* (Fisch. & C.A. Mey.) Boiss.
	Uromyces sp.	*Centaurea nemecii* Nábělek
	Uromyces striatus J. Schroet.	*Medicago sativa* L. subsp. *sativa*
	Uromyces tuberculatus Fuckel	*Euphorbia cheiradenia* Boiss. & Hohen.
	Uromyces turcomanicum Katajev	*Hordeum bulbosum* L.
Melampsora	*Melampsora euphorbiae* (Ficinus & Schubert) Castagne, 1843	Euphorbia heteradena Jaub. & Spach
Phragmidium	*Phragmidium sanguisorbae* (DC.) J. Schröt., 1889.	*Sanguisorba minor* L. subsp. *minor*
Puccinia	*Puccinia acarnae* Syd.	*Picnomon acarna* (L.) Cass.
	Puccinia centaurea DC.	*Centaurea iberica* Trev. ex Spreng.
	Puccinia chaerophylli Purton	*Chaerophyllum crinitum* Boiss.
	Puccinia chondrillina Bub. & Syd.	*Chondrilla juncea* L.
	Puccinia cichorii (DC.) Belynck	*Cichorium intybus* L.
	Puccinia cnici Mart.	*Cirsium haussknechtii* Boiss.
	Puccinia cyani (Schleich.) Pass.	*Centaurea gigantea* Sch.Bip. ex Boiss.,*Centaurea nemecii* Nábělek
	Puccinia echinopis DC.	*Echinops spinosissimus* Turra subsp. *bithynicus* (Boiss.) Greuter
	Puccinia falcariae (Pers.) Fuckel	*Falcaria vulgaris* Bernh.
	Puccinia ganeschinii Tranz. & Erem.	*Rhaponticum repens* (L.) Hidalgo
	Puccinia graminis Pers.	*Triticum aestivum* L.
	Puccinia isiacae (Thüem.) Wint.	*Isatis tinctoria* L., *Hyoscyamus niger* L.
	Puccinia jaceae Otth.	*Centaurea behen* L.
	P*uccinia lojkaiana* Thüem.	*Bellevalia paradoxa* (Fisch. & C.A.Mey.) Boiss.
	Puccinia mabvacearum Mont.	*Alcea striata* (DC.) Alef. subsp. *striata*
	Puccinia magnusiana Koern.	*Phragmites australis* (Cav.) Trin. ex Steud.
	Puccinia nigrescens Kirch.	*Salvia verticillata* L. subsp. *verticillata*
	Puccinia opopanacis Ces.	*Opopanax hispidus* (Friv.) Griseb.
	Puccinia polygoni Alb. & Schw.	*Polygonum amphibium* L.
	Puccinia polygoni-amphibii Pers	*Polygonum amphibium* L.
	Puccinia pozzii Semadeni	*Chaerophyllum crinitum* Boiss.
	Puccinia praegracilis Arthur	*Dactylorhiza umbrosa* (Karelin & Kirilow) Nevski var. *umbrosa*
	Puccinia punctata Link	*Galium verum* L. subsp. *verum*
	Puccinia ranunculi Blytt	*Ranunculus arvensis* L.
	Puccinia schirajewskii Tranz.	*Klasea radiata* (Waldst. & Kit.) A. Löve & D. Löve subsp. *biebersteiniana* (Grossh.) Greuter
	Puccinia stipina Tranz.	*Salvia verticillata* L. subsp. *verticillata*
	Puccinia taraxsaci (Reb.) Plowr.	*Tanacetum balsamitoides* Sch. Bip.
	Puccinia tiflisensis Petr.	*Cirsium arvense* (L.) Scop.
	Puccinia vagans (DC.) Arth.	*Epilobium hirsutum* L.
Cintractia	*Cintractia caricis* (Pers.) Magn.	*Carex distans* L. subsp. *distans*
Coniothecium	*Coniothecium seriale* Durieau & Mont.	*Falcaria vulgari* Bernh., *Equisetum arvense* L.
Annellophorella	*Annellophorella foureae* (Henn.) M.B. Ellis	*Inula britannica* L.
Dicoccum	*Dicoccum asperum* (Corda) Sacc.	*Lactuca scarioloides* Boiss.

	Ramularia rubella (Bon.) Nannf.	*Falcaria vulgaris* Bernh.
	Ramularia armoraciae Fuckel	*Raphanus raphanistrum* subsp. *raphanistrum* L.
	Ovularia ovata (Fuckel) Sacc.	*Tanacetum balsamitoides* Sch. Bip.
Ramularia	*Ramularia menthicola* Sacc.	*Mentha longifolia* (L.) L. subsp. *typhoides* (Briq.) Harley
Cercospora	*Cercospora megidicaginis* Ell. & Ev.	*Medicago sativa* L. subsp. *sativa*
Cladosporium	*Cladosporium fasciculare* (Pers.) Fr.	*Sanguisorba officinalis* L.
Taeniolella	*Taeniolella plantaginis* (Corda) Hughes	*Bellevalia paradoxa* (Fisch. & C.A. Mey.) Boiss., *Anchusa azurea* Mill. var. azurea
Sporidesmium	*Sporidesmium cladosporii* Corda	*Acanthus dioscoridis* L. var. *dioscoridis, Cirsium arvense* (L.) Scop., *Xanthium strumarium* L. subsp. *strumarium*
Sporidesmium	*Sporidesmium microscopicum*	*Lysimachia vulgaris* L.
Brachysporium	*Brachysporium flexuosum* (Corda) Sacc.	*Pulicaria dysenterica* (L.) Bernh. subsp. *dysenterica*
Coniosporium	*Coniosporium rhizophilum* (Preuss) Sacc.	*Cerinthe minor* L. subsp. *auriculata* (Ten.) Domac
	Coniosporium triticinum L. Gaja	*Lysimachia vulgaris* L.
Scolicotrichum	*Scolicotrichum bonordenii* Sacc.	*Lepidium draba* L.
Trichothecium	*Trichothecium roseum* (Pers.) Link.	*Trifolium repens* L. var. *repens*
Geotrichum	*Oospora lactis* (Fres.) Sacc.	*Nepeta betonicifolia* C.A. Mey. subsp. *betonicifolia*
Bostrichonema	*Bostrichonema alpestre* Ces.	*Phlomoides laciniata* (L.) Kamelin & Makhm.
Diplocarpon	*Diplocarpon alpestre* Ces.	*Polygonum amphibium* L.
Entyloma	*Entyloma crastophilum* Sacc.	*Lolium perenne* L.
Hadrotrichum	*Hadrotrichum sorghi* (Pass.) Ferraris & Massa.	*Sorghum halepense* (L.) Pers. var. *halepense*
Botryotrichum	*Botryotrichum atrogriseum* J.F.H. Beymo	*Rumex conglomeratus* Murray
Physoderma	*Physoderma menthae* J. Schrot.	*Mentha longifolia* (L.) subsp. *typhoides* (Briq.) Harley

m at each sampling point. These plants were accepted as microfungus hosts and their samples with disease symptoms were taken to the herbarium.

In the laboratory, samples were taken from the diseased parts of the host weeds (herbarium specimens), and isolation procedures were performed (Özaslan 2011).

The preparations obtained from pure fungal cultures and the preparations made by scraping (obligate fungi) were examined under the microscope for the identification of microfungi at the genus level. The lesions in plant tissue and features such as conidial structures, branching of the conidiophore, conidia structure, conidia shape, and size were taken into consideration in genus-level diagnoses (Ellis and Ellis 1985, Gannibal and Lawrence 2018a, Gannibal and Lawrence 2018b, Shvartsman et al. 1973). The species-level identification of the fungi samples/properties was made by Retired Professor Elşad Hüseyin (Ahi Evran University, Faculty of Arts and Sciences, Department of Biology). The host weed species were identified using plant specimens in the herbarium of the Plant Flora Laboratory (Siirt University Faculty of Arts and Sciences) and different books related to Turkish flora (Flora of Turkey Davis 1965–1985, Önen 2015, Özer et al. 1996, Özer et al. 1998, Özer et al. 1999, Serin 2008). Identified weed samples approved by Ass. Prof. Dr. Mehmet Fidan (Siirt University Faculty of Arts and Sciences, Biology Department).

RESULTS

A total of 220 plant samples belonging to 79 weed species were collected from the study area. Weed species observed in the study area belonged to 29 different families. Similarly, 101 microfungi (leaf pathogen and obligate) species infecting weeds were observed in the region. Weed and microfungi species recorded from the study area are given in Table 1.

The weed families hosting the highest number of fungi were *Asteraceae* (20 species), *Fabaceae* (7 species), *Poaceae* (7 species), and *Lamiaceae* (6 species). The microfungi detected on weeds belonged to *Puccinia* (29 species), *Alternaria* (18 species), *Uromyces* (14 species), and *Curvularia* (4 species). A total of 84 microfungi species (e.g. *Puccinia chondrillina*, *P. cnici*, *P. magnusiana*, *P. cyani*, *P. falcariae*, *Stemphylium vesicorium*, and *Uromyces polygoni-avicullariae*) determined just on a single weed in the region. The remaining 17 species (e.g. *Alternaria atra*, *A. alternata*, *A. botrytis*, *A. chartarum*, *A. consortialis*, *A. dianthicola*, *A. herbiphorbicula*, *A. hispidula*, *A. lanuginosa*, *A. septospora*, *Coniothecim seriale*, *Dendryphion comosum*, *Puccinia cyani*, *P. isiacae*, *Taeniolella plantaginis*, *Sporidesmium cladosporii*, and *Stemphylium piriforme*) were found on more than one weed species

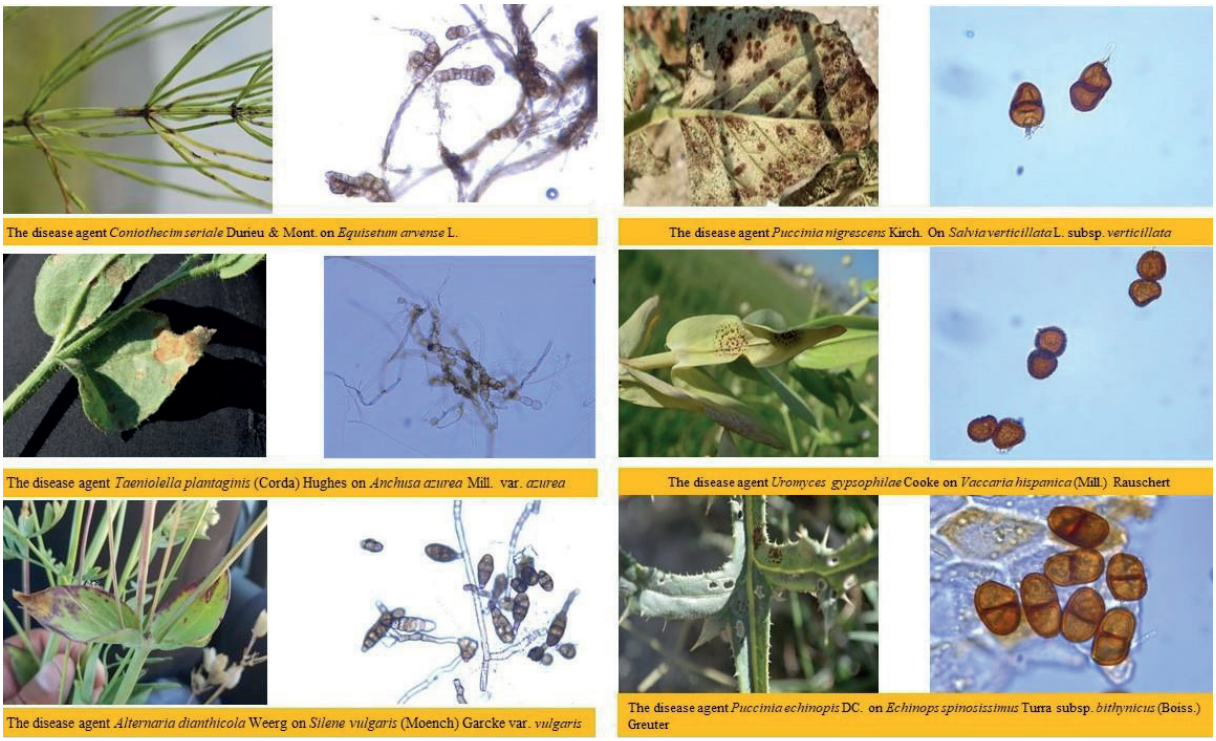

Figure 1. Examples of host weed species and observed microfungi

DISCUSSION AND CONCLUSION

A total of 101 microfungi species were identified from 79 weed species in the Yüksekova basin. It was determined that most of the recorded microfungi species belonged to Alternaria, Puccinia, and Uromyces genera. These are the most common genera in the microfungi biota found on weeds in different regions of Türkiye (Asav et al. 2015, Doğan 2013, Ekici 2011, Erciş and İren 1988b, Erciş and İren 1993, Ertuğrul et al. 2019, Kabaktepe 2010, Özaslan 2011, Özaslan et al. 2013, Özaslan et al. 2015, Özaslan 2016, Sırrı and Özaslan 2022, Ulukapı 2016, Uygun et al. 1994, Uygur et al. 1993). Therefore, the results are in agreement with previous studies. However, it has been determined from field observations that microfungi highly limit the vegetative growth of many weed species in the basin. It has been observed that some microfungi species cause high deformations in the flower, leaf, and stem of host weed species, limit plant growth, and even cause death. For instance, it was observed that Cercospora convolvulicola and Curvularia inaequalis species significantly inhibited the growth and development of Polygonum amphibium under field conditions and suppressed the population density of the weed. Similarly, the development and population density of Anchusa azurea, Salvia verticillata and Galium verum were significantly affected by Dendryphion comosum, Taeniolella plantaginis, and Puccinia punctata, respectively. It was concluded that the effectiveness of microfungi was at the highest level due to the limited use of pesticides and the continuation of traditional agricultural practices in the basin. It has also been stated in previous studies that the activities of microfungi can be increased, especially by reducing the use of pesticides and supporting beneficial organisms in the field, and can contribute to integrated weed control in organic farming (Önen and Kara 2008, Önen 2014).

The activity of some fungal species such as monophage Uromyces polygoni-avicullariae, Puccinia chondrillina, and P. cnici reached significant levels in the study area. These species have been used in the biological control of weeds such as Chondrilla juncea, Cirsium sp., and Polygonum bellardii (Espiau et al. 1997, Michaux 1984). P. chondrillina used in the management of C. juncea in Australia is among the most successful examples of fungi used as a biocontrol agent (Cullen 1976). Besides, different species in these genera can also be used successfully in the biological control of weeds. P. xantii (Julien et al. 1979, Uygun et al. 1994), Phytophthora palmivora (Kenney 1986), Phragmidium violaceum (Adams 1988), Colletotrichum gloeosporioides f. sp. aeschynomene (Kumar 1992) and Ulocladium atrum (Linke et al. 1992) fungi species can be used successfully for the biological control of Xanthium strumarium, Merrenia odorata, Rubus spp., Aeschynomene virginica and Orobanche spp. weed species, respectively. Considering these successful examples, it was concluded that the monophagous fungal species detected in the study area should be evaluated in terms of

biological activity. It is reported that the host-specific P. cyani detected on Centaurea gigantea and endemic C. nemeci in the study area has the potential to be used in the biological control of Centaurea species (Ulukapı 2016). Similarly, it has been reported that P. chordrillina can be extremely effective in the control of C. juncea (Erciş and İren 1988a, Erciş and İren 1993, Nemli 1991).

In conclusion, the results revealed the presence of some promising pathogens. However, to determine the potential of the identified biocontrol agents as myco-herbists; detailed laboratory, greenhouse, and field trials as well as host tests and efficacy studies are required. Moreover, detailed survey studies should be conducted in the future. Observations in the basin have shown that natural biological control can be extremely effective in the region and that some important weed species can be suppressed by microfungi. Although biological control alone is not sufficient for weed management in agricultural production areas throughout the basin, microfungi can play an important role within the scope of integrated control. Therefore, it would be appropriate to adopt and expand organic/good agricultural practices, which aim to protect and support the beneficial organisms in agricultural production, in the region. Thus, both the sustainable use of chemical fertilizers and pesticides and the protection of human/environmental health in the region will be possible, and crop pests, diseases, and weeds will be successfully managed.

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ÖZET

Önemli ekolojik, ekonomik ve sağlık sorunlarına neden olan yabancı otların sürdürülebilir bir yönetimi için biyolojik mücadele çalışmaları giderek daha fazla ilgi görmektedir. Hedef yabancı ot türleri üzerindeki potansiyel biyolojik mücadele ajanlarının (zararlılar, patojenler vb.) tespiti biyolojik mücadelenin ilk adımıdır ve türün biyolojik mücadelesi için temel bilgiler sağlar. Bu çalışmada Hakkari/Yüksekova havzasında zararlı yabancı otlar üzerinde bulunan mikrofungus türlerin belirlenmesi amaçlanmıştır. Bölgede geleneksel tarımsal uygulamaların devam etmesi, ilaç ve kimyasal gübrelerin çok az kullanılması veya hiç kullanılmaması, doğal flora/faunanın Türkiye'nin diğer bölgelerine göre nispeten daha iyi korunması gibi nedenler çalışma alanının seçiminde rol oynamıştır. Çalışma alanında 2020 ve 2021 yıllarında farklı dönemlerde yürütülen sürvey çalışmaları sonucunda; 29 familyaya ait toplam 79 yabancı ot türü üzerinde 101 mikrofungus türü tespit edilmiştir. Havzada en sık rastlanan fungus türlerinin *Puccinia* (29 tür), *Alternaria* (18 tür), *Uromyces* (14 tür) ve *Curvularia* (4 tür) cinslerine dahil oldukları saptanmıştır. Söz konusu fungus türlerine konukçuluk yapan yabancı ot türlerinin ise en fazla Asteraceae (20 tür), Fabaceae (7 tür), Poaceae (7 tür) ve Lamiaceae (6 tür) familyalarına ait oldukları belirlenmiştir. Çalışmada, sadece tek konukçu (yabancı ot) üzerinde 84 mikrofungus türü tespit edilirken, geri kalan 17 tür ise birden fazla yabancı ot türü üzerinde tespit edilmiştir. Çalışmada *Puccinia cyani* (Schleich.) Pass., *Puccinia chondrillina* Bub. & Syd., *Uromyces polygoni-avicularіae* (Pers.) P. Karsten gibi bazı fungus türlerinin konukçu yabancı ot türlerinin (sırasıyla *Centaurea* spp., *Chondrilla juncea* L., *Polygonum aviculare* L.) gelişimini önemli ölçüde engelledikleri ve popülasyonlarını baskılayabildikleri gözlemlenmiştir. Sonuçlar, tespit edilen patojenlerin biyolojik aktivite açısından gözden geçirilmesi ve bölgede detaylı saha çalışmalarının yapılmasının faydalı olacağını ortaya koymuştur.

Anahtar kelimeler: biyolojik mücadele, yabancı ot, mikrofungus, Yüksekova havzası, Hakkâri, Türkiye

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