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Fungal and Fungus-like Species Associated with Weeds in Sunflower Fields in the Thrace Region, Türkiye: Incidence of Infected Weeds

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ABSTRACT

Classical biological weed control involves the identification of fungal and fungus-like species that are capable of infecting weeds without causing damage to related crop plant in agricultural areas. This study identified both fungal and fungus-like species on weeds, which were present in sunflower production areas in Tekirdağ and Kırklareli provinces in the Thrace region, Türkiye, and determined the incidence of diseases caused by these species. A total of 1,340 weed samples from various districts in Tekirdağ and Kırklareli provinces were examined. The results showed the presence of *Albugo amaranthi, Peronospora variabilis, Alternaria alternata* and *Dichotomophthora lutea* on the leaves of *Amaranthus retroflexus, Chenopodium album, Lactuca serriola* and *Portulaca oleracea,* respectively. Identification was based on cultural, morphological, and molecular characteristics of the species. The incidence of diseases varied between districts within the provinces. Downy mildew caused by *P. variabilis* had the highest incidence rate, followed by white rust caused by *A. amaranthi.* The presence of *A. amaranthi* on *A. retroflexus* is a new record, and *L. serriola* is a new host for *A. alternata* in Türkiye. Additionally, the occurrence of *D. lutea* on *P. oleracea,* which is present as a weed in sunflower fields, was demonstrated.

Keywords: sunflower, fungus and fungus-like species on weeds, incidence, Thrace region of Türkiye.

INTRODUCTION

Weeds represent one of the most important factors reducing yields in all agricultural areas around the world. By competing with cultivated plants for essential resources, such as water, nutrients and sunlight, they can prevent the growth of these plants, ultimately resulting in a loss of yield. The majority of weed infestation is either managed mechanically or through the application of herbicides (Christensen et al., 2009). However, the use of intensive mechanization techniques has the risks of soil erosion and a loss of soil fertility (Guccione and Schifani, 2001). Herbicide applications, intended to increase crop yields, have resulted in considerable adverse effects on environment, human and animal health. Furthermore, the improper use of herbicides has contributed to the emergence of herbicide-resistant weeds, including those with multiple herbicide resistances, and has caused harm to non-target organisms and changes in weed populations (Scavo and Mauromicale, 2020). Integrated weed management approach (IWM), which is based on the integration of indirect and direct control methods, has been shown to be an effective strategy for reducing the use of herbicides. This approach is dependent on the weed species, climatic conditions, soil characteristics, irrigation method, form of plant farming, socio-economic constraints and farmer's expectations (Gunsolus and Buhler, 1999). It is known that biological control with phytopathogenic fungi make an important contribution to IWM (Cai and Gu, 2016). The common characteristics of weed species and the development of fungus or fungus-like organisms on them differ according to regions (Te Beest et al., 1992; Scavo and Mauromicale, 2020). In the context of the classical strategy of biological weed control, in order to reveal the possibilities of biological control of weeds in a region with fungal species, it is necessary to determine the species that are adapted to

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weeds in important crop production areas in that region and do not cause disease in that crop. This strategy is followed by the development of mycoherbicides. However, the origin of mycoherbicides is typically endemic to a particular region and are therefore used to control indigenous weeds (Hasan and Ayres, 1990).

The sunflower (*Helianthus annuus* L.) is one of the most extensively cultivated oil crops in the world, after soybean and coconut, with Türkiye ranking sixth in the world with production amount of 2.55 million tons (FAO, 2022). The Thrace region has the highest sunflower production (754 501 tons) in Türkiye, where the provinces of Tekirdağ and Kırklareli play an important role in producing sunflower. Weeds have a common distribution in sunflower production areas in Türkiye, as well as to parasitic weeds and fungal diseases. Important weed species in sunflower cultivation areas in Türkiye include *Convolvulus* spp. (Convolvulaceae), *Amaranthus retroflexus*, *Chenopodium album*, *C. vulvaria* (Amaranthaceae), *Cynodon dactylon*, *Echinochloa crus-galli*, *Elymus repens* (Poaceae), *Cyperus rotundus* (Cyperaceae), *Raphanus raphanistrum*, *Sinapsis arvensis* (Brassicaceae), *Heliotropium europaeum* (Boraginaceae) and *Cirsium arvense* (Asteraceae) (Arslan and Kara, 1997; Dindar and Kara, 2016; Karabacak and Uygur, 2017; Özkil et al., 2019). Previous studies in different regions of Türkiye, excluding the Thrace region, have revealed the presence of the following fungal species in these weeds: *Albugo bliti*, *Alternaria amaranthi* and *Rhizoctonia solani* in *A. retroflexus*; *Cercospora chenopodii*, *Peronospora farinosa* and *P. variabilis* in *C. album*; *Puccinia obtegens*, *P. punctiformis* and *Rhizoctonia solani* in *C. arvense*; *Albugo convolvulacearum*, *Erysiphe polygoni*, *E. convolvuli*, *Rhizoctonia solani* and *Septoria convolvuli* in *Convolvulus arvensis*; *Alternaria alternata* in *Convolvulus galaticus*; *Puccinia cynodontis* and *Uromyces cynodontis* in *C. dactylon*; *Claviceps purpurea*, *Puccinia recondita* in *E. repens*; and *Albugo candida* in *R. raphanistrum* (Uygur et al., 1993; Demirci and Zengin, 1995; Demirci et al., 1997; Erper et al., 1997; Karamanlı, 2005; Tunalı et al., 2009; Kitiş and Karaca, 2011; Özaslan, 2011; Kara et al., 2020). In all of the above-cited studies, with the exception of the study conducted by Uygur et al. (1993), the species were only identified; however, the incidence of fungal species was not determined. Considering the importance of sunflower cultivation in the region of Thrace, a survey of the fungi, which were present on weeds in sunflower production areas was carried out in the current study. The results presented here provide detailed information on morphological and molecular characteristics of the fungal and fungus-like species as well as the incidence of the weeds infected by each species.

MATERIAL AND METHODS

Study area

The survey was conducted in the 11 and 7 districts of Tekirdağ and Kırklareli provinces in Thrace region, respectively, during June and August of 2021. A total of 50 fields where sunflower was cultivated were visited, and 1.340 samples of weeds were examined for the presence of fungal and fungus-like agents. Subsequently, the samples were placed in paper bags and transported to the laboratory for further analysis.

Characterization of isolated fungal and fungus-like organisms

Leaves covered with sporulation of biotrophic fungi or fungus-like organisms was directly examined under a stereomicroscope (S6D; Leica Microsystems CMS, Wetzlar, Germany) first, and then their mycelia, spores and fungal fruiting bodies were examined under a microscope (DM 1000 LED; Leica). The leaves with spots were initially superficially disinfected with 70% ethanol (1 min), then they were rinsed in sterile distilled water (2 min) under a laminar flow hood. From each type of lesion, plant fragments of 0.5-1 cm in length, including diseased and healthy tissues, were cut with the sterile scalpel and placed in petri dishes containing Potato Dextrose Agar (PDA, Merck, Darmstadt, Germany) medium. The petri dishes were incubated in a cooled and

controlled incubator (Binder GmbH, Tuttlingen/Germany) at 23±2°C for 7-10 days in the dark. Characteristics of reproductive structures were observed under a microscope (Leica). Single spores were used to create pure cultures of each fungal species. Single spore representatives from all fungus species were inoculated onto three different media, namely Malt Extract Agar (MEA), Potato Carrot Agar (PCA) and Potato Dextrose Agar (PDA), and incubated at 23±2°C for 8-15 days. The colony appearances, including colour, aerial mycelium formation, and other characteristics, and the mycelial growth rates were recorded on these media in order to demonstrate the cultural and morphological characteristics of the species. The dimensions of conidia on PDA media were determined using the Leica Application Suite (LAS) software, 50 conidia were employed from each species to measure. DNA extraction, PCR amplification and sequencing of the isolates were performed by the Central Research Laboratory of Tekirdağ Namık Kemal University (NABİLTEM). The genomic DNA of biotrophic fungi was directly extracted from sporulation on leaves of infected host plants. The isolates were identified by sequencing the Internal Transcribed Spacers (ITS) region of ribosomal DNA with primer pairs ITS4 (5'-TCCTCCGCTTATTGATATGC -3') and ITS5 (5'-GGAAGTAAAAGTC GTAACAAGG-3' (White et al., 1990), mitochondrial COX2 region with primer pairs Cox2/F (5'-GGCAAATGGGTTTTCA AGATCC-3') and Cox2/R (5'-CCATGATTAA TACCACAAATTTCACTAC-3') (Hudspeth et al., 2000), the complete ITS region of rDNA with primer pairs DC6 (5'-GAGGGACTTTTGGGTAATCA-3') and LR0 (5'-GCTTAAGTTCAGCGGGT-3') (Choi et al. 2006), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) region with primer pairs gpd1 (5'-CAACGGCTTCGGTCGCATTG-3' ve gpd2 (5'-GCCAAGCAGTTGGTTGTG C-3') (Berbee et al., 1999). In addition, the rDNA internal transcribed spacer (ITS) region was also amplified using primers was amplified using primers ITS5 (White et al., 1990) and p3 (5'-GCCGCTTCACTCGCCGTTAC-3') (Kusaba and Tsuge, 1995). The DNA fragments that were subjected to sequencing with the primers underwent analysis in the NCBI (National Center for Biotechnology Information) database using BLASTn. Sequences for the isolates of each species were deposited with accession numbers in GenBank based on different gene regions.

Determination of incidence of diseases

Weeds exhibiting diverse disease symptoms, discernible at the macroscopic level, were accepted as contaminated with fungal agents during the surveys. The incidence of diseased weeds was determined by randomly throwing 0.25 m-square wooden frame for five times/da area and examining at least 10 weeds in each frame. The following formula were used for calculation of incidence (%) of weeds infected with fungal or fungus-like species DI) (Odum, 1971): DI (%) = $(\sum x/n) \times 100$, where *x* represents the number of weeds infected with fungal or fungus-like species, and n represents total number of weed in sampling field.

Statistical Analysis

The Fisher's Exact Test or Chi-Square Test was performed to compare the incidence of weed species infected with fungal species across districts in each province. Fisher's Exact Test was used in the case of several expected frequencies being below 5, while the Chi-Square Test was applied where expected frequencies were above 5. Both tests were conducted using JMP Pro 17 for Windows (SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

Characteristics of fungus-like and fungal species determined on weeds

The survey revealed that weeds in sunflower production area of Tekirdağ and Kırklareli provinces were affected by two fungus-like (*Albugo amaranthi* and *P. variabilis*) and two fungal species (*A. alternata* and *Dichotomophthora lutea*). However, the impact of these pathogens

varied depending on the weed species. The fungus-like biotrophic oomycetes *Albugo amaranthi* (Schwein.) Kuntze [synonym: *Wilsoniana amaranthi* (Schwein.)] was determined to be the causal agent of white rust on the leaves of *A. retroflexus* (Figure 1a). It has been observed that the pathogen forms small yellow spots on the adaxial surface of leaves (Figure 1b), and white or pale yellow sporangial sorus that are fluffy and vary in size and shape on the parts below these spots, (Figure 1c). The pathogen formed its sporangia (Figure 1d) as a chain at the end of the cylindrical sporangiophore. Sporangia were formed in two groups, with diameters ranging from 12.46 to 14.85 µm and from 16.01 to 19.63 µm. No oospores were found. GenBank BLASTn search revealed that the cox2 sequences of the isolate AlbA1 was identical (>99%) to many isolates (Accession No: MN533957.1, AY913805.1, MK335465.1, JN849486) of *A. amaranthi*.

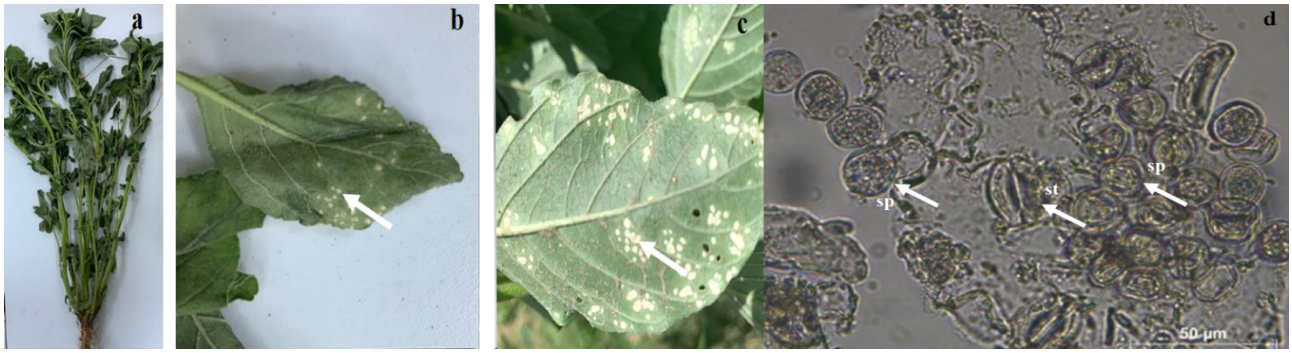

Figure 1. Symptoms and morphological characteristics of white rust caused by *Albugo amaranthi* on *Amaranthus retroflexus*. (a) *A. retroflexus*, (b) Typical yellow spots (arrow) on adaxial surface of leaf, (c) white mass (arrow) consisting of sporangiophores and sporangia on abaxial surface of leaf, and (d) microscopic appearance of A. amaranthi showing sporangia (sp) and sporangia chain near stomata (st)

The other fungus-like biotrophic oomycetes, *Peronospora variabilis* Gum., which were detected on *C. album* (Figure 2a), formed yellow spots on adaxial surface (Figure 2b) and a grayish fungal sporulation on the abaxial surface of the leaves (Figure 2c). The sporangia of this species, germinates directly without the production of zoospores. Consequently, the sporangium is also referred to as conidia. Microscopically, the pathogen was identified as *Peronospora* on the basis of the presence of colorless conidiophores with tree-shaped, straight to slightly curved, dichotomously branched at acute angle (Figure 2d), which conidia were attached at the tip. The conidia (Figure 2e) were pale brown-olivaceous in colour, generally broadly ellipsoidal to ellipsoidal, sometimes obovoid in shape, with a length of 24.73-27.77 µm and a width of 18.77-21.173 µm. The sequence the isolate PerVa1 based on Cox2 gene region showed 100% similarity with the sequences of numerous *P. variabilis* isolates (Accession No: MK408662.1, KJ654199.1, KF269678.1, KF269677.1).

Alternaria alternata (Fr.) Keissl formed small spots with light brown edges on the leaves of *Lactuca serriola* (Figure 3a and 3b). This species developed dark green coloration on PDA medium (Figure 3c), olive-green coloration on MEA and PCA (Figure 3d and 3e, respectively), and there was a decrease in aerial mycelia dense on PCA medium. The growth rates were 4.89 mm/day, 5.07 mm/day and 6.96 mm/day on PDA, MEA and PCA media, respectively. The conidia were observed to form chains on simple conidiophores (Figure 3f and 3g). The mean conidia dimensions were found to range between 10.10 and 26.48 µm in length and 6.18 and 11.19 µm in width. A BLAST analysis of the isolate (AltA7) revealed that it exhibited 100% similarity to numerous *A. alternata* isolates in the GenBank, according to the ITS gene region (Accession No: MN615420.1, MT573466.1, MT573464.1, MN481948.1) and the GPD gene region

(Accession No: MW818018.1, MW818017.1, MW818016.1, MW818015.1).

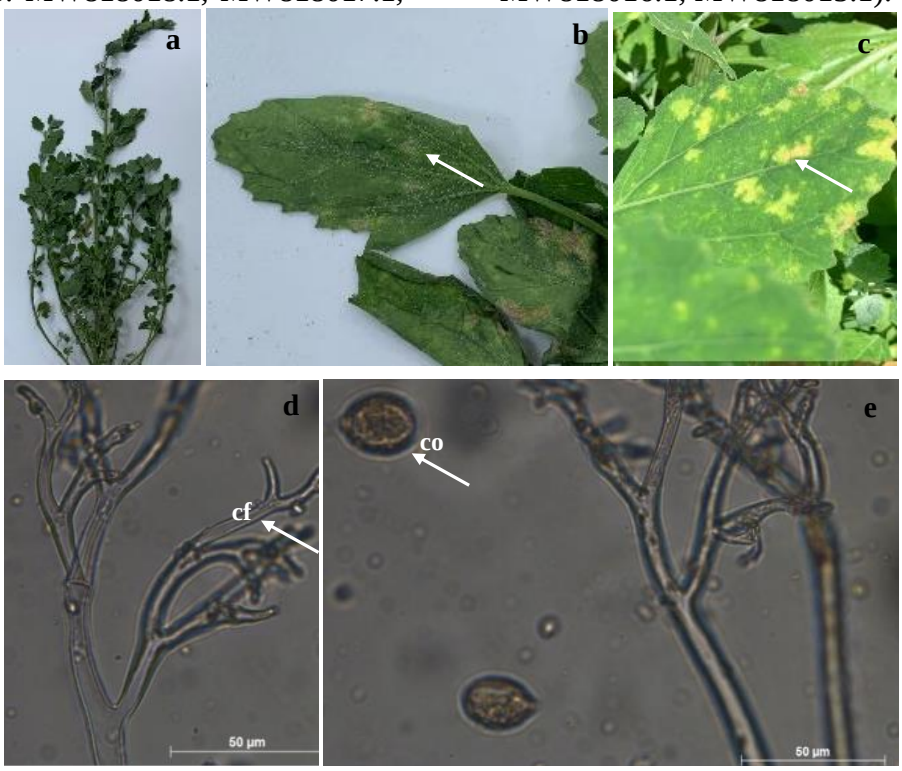

Figure 2. Symptoms and morphological characteristics of downy mildew caused by *Peronospora variabilis* on *Chenepodium album*. (a) *C. album*, (b) Typical yellow spots (arrow) on adaxial surface of the leaf, (c) Heavy sporulation (arrow) consisting of conidiophores and conidia on the abaxial surface of the leaf, (d) conidiophores (cf) and (e) conidia (co)

Dichotomophthora lutea de Hoog & Oorschot (sin: *Dichotomophthora indica* P.N. Rao) was isolated from necrotic oval to irregular and brown spots on the leaves of *Portulaca oleracea* (Figure 4a and 4b). The fungus exhibited a dark brown color and dense cottony aerial mycelia on PDA and MEA medium (Figure 4c and 4d). The color of the colony was reddish brown and the cottony aerial mycelia were less frequent on PCA medium (Figure 4e). The growth rates of the organism on PDA, MEA and PCA media were 8.52 mm/day, 6.39 mm/day and 6.51 mm/day, respectively. Unbranched or irregularly branched conidiophores exhibited a lobe at the apex while conidia were typically formed individually on the lobes or on conidigenous heads (Figure 4f-4h). These conidia were usually straight, rarely very slightly curved, and elliptic to cylindrical in shape, with a slightly narrowed and rounded tip (Figure 4i). The average size of the conidia was from 11.25-52.64 x 6.04-12.68 µm (length x width). It also formed sclerotia (Figure 4j and 4k). A BLAST analysis revealed that the isolate (DichoL1) exhibited 99.82% similarity to numerous *D. lutea* isolates (Accession No: 158420.1, LT990650.1, LT990649.1, LT990648.1) stored in the GenBank, as determined by the ITS gene region.

The cox2 sequence of the isolate AlbA1 is registered in the National Center for Biotechnology Information (NCBI) GenBank database under Accession No. OM371333. The isolate PerVa1 is assigned Accession No. OM371334 for the Cox2 gene region. Additionally, the ITS sequence and GPD sequence of the isolate AltA7 can be accessed via GenBank under Accession Nos. OM368598 and OM371335, respectively. The isolate DichoL1 is listed with Accession No. OM368620 for the ITS gene region. All these sequences are publicly available in the NCBI GenBank database for verification and further research.

Figure 3. Symptoms cultural and morphological characteristics of leaf spot caused by *Alternaria alternate* on *Lactuca serriola*. (a) *Lactuca serriola*, (b) Typical spots (arrow) on the leaf, (c, d, e) Colonies on PDA, MEA and PCA, respectively, (f) Conidia (arrow), (g) Conidia chain (arrow)

Incidence of the diseased weeds

The diseases caused by fungus-like and fungal species on weeds in Tekirdağ province varied according to weed species and the districts where they were found. Among biotrophic fungal species, *A. amaranthi*, the agent of white rust disease, was detected on *A. retroflexus* only in the Ergene district (Table 1). The incidence of *A. retroflexus* infected with *A. amaranthi* in this district (7.31%) differed significantly from that in the districts of Süleymanpaşa, Hayrabolu, Malkara and Saray (Fisher's exact test, P=0.0053, P=0.0005, P=0.0005 and P=0.0081 across districts, respectively). *C. album* attacked by *P. variabilis* showed a significantly higher infection rate (44.4%) in Şarköy compared to other Tekirdağ districts (Fisher's exact test, p<0.0001 across Süleymanpaşa, Hayrabolu, Malkara, Ergene and Saray; P=0.003 and P=0.0012 across Kapaklı and Marmara Ereğlisi, respectively; Chi-square test, P<0.0001, P=0.0002 and P=0.0001 across Çorlu, Çerkezköy and Muratlı, respectively). The infection rate of *C. album* by the same species in Malkara (5.85%), which followed Şarköy, was significantly different from Ergene (Fisher's exact test, P=0.0219). *D. lutea* was present on the leaf spots of *P. oleracea* in Hayrabolu, but its infection rate among the districts was not significantly different.

D. lutea was not found in any district of Kırklareli province (Table 2). *A. retroflexus* in Lüleburgaz and *L. serriola* in Babaeski had the sypmtoms of *A. amaranthi* and *A. alternata* on the leaves, respectively. However, there was no significant difference among districts regarding the incidence of these fungal infections. *P. variabilis* on *C. album* was found in Babaeski, Kofçaz and Lüleburgaz. Significant differences were detected when comparing the incidence of *C. album* infected with *P. variabilis* in Kofçaz to Central, Lüleburgaz, Pehlivanköy, Pınarhisar and Vize (Fisher's exact test, P<0.0001, P=0.0002, P=0.0115, P=0.0398 and P=0.015 across the districts, respectively). Babaeski also showed differences for the presence of infected *C. album* with this species compared to Central (Chi-square test, P=0.0004) and Lüleburgaz (Chi-square test, P=0.0009) and no significant difference was found between Babaeski and Kofçaz.

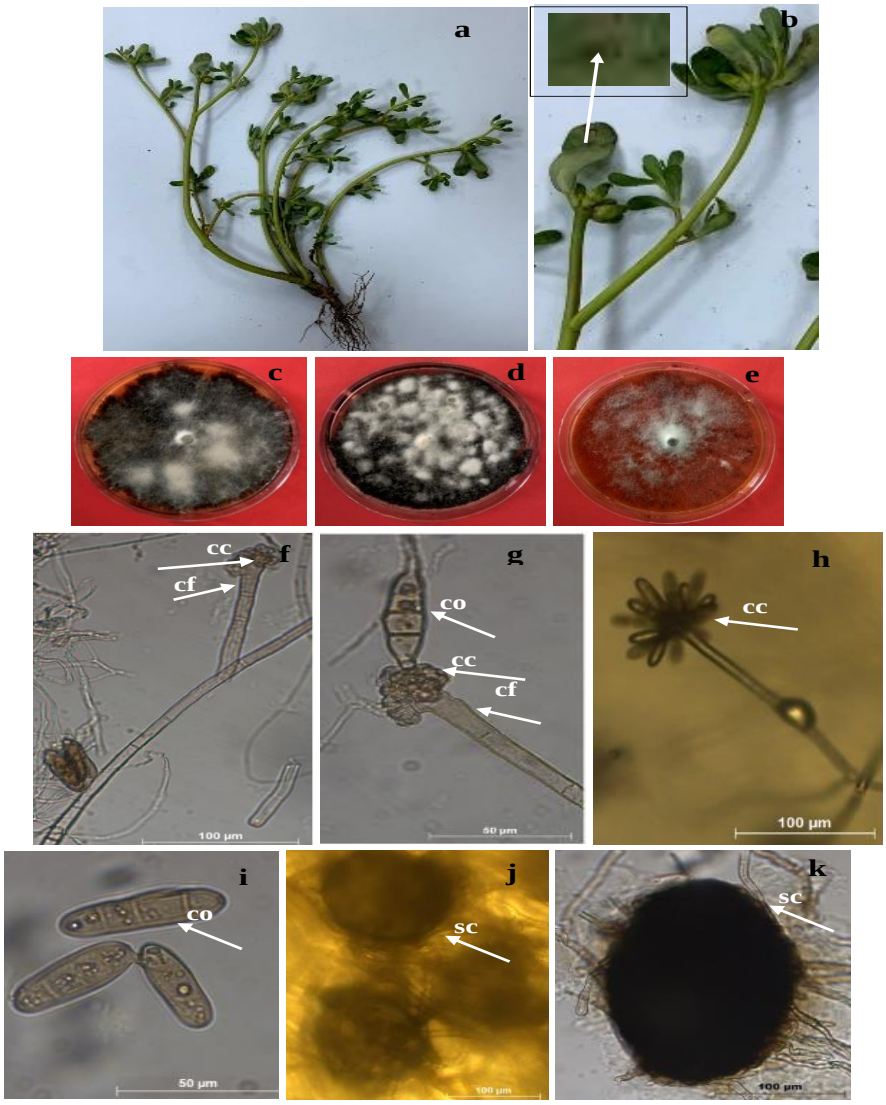

Figure 4. Symptoms, cultural and morphological characteristics of leaf spot caused by *Dichotomophthora lutea* on *Portulaca oleracea*. (a) *P. oleracea*, (b) Typical spots (arrow) on the leaf (arrow), (c, d, e) Colonies on PDA, MEA and PCA, respectively, (f, g, h, i) Conidiophore (cf) lobed at apex (arrow), conidigenous cells (cc) and conidia (co) (arrows), (j and k) sclerotia (sc)

Table 1. Incidence (%) of weed species infected with different fungi species in Tekirdağ province

Districts	No. of weeds sampled	Incidence (%) Weed species[a] - fungi species[b]		
		A. retroflexus - A. amaranthi	*C. album - P. variabilis*	*P. olarecea - D. lutea*
Süleymanpaşa	111	0.00	2.70	0.00
Hayrabolu	202	0.00	2.47	0.49
Malkara	205	0.00	5.85	0.00
Şarköy	27	0.00	44.44	0.00
Çorlu	39	0.00	2.56	0.00
Ergene	82	7.31	0.00	0.00
Saray	98	0.00	4.08	0.00
Kapaklı	15	0.00	0.00	0.00
Çerkezköy	24	0.00	0.00	0.00
Marmara Ereğlisi	17	0.00	0.00	0.00
Muratlı	26	0.00	0.00	0.00
Total	846	0.71	4.37	0.12

[a]*A. retroflexus = Amaranthus retroflexus, C. album = Chenopodium album, P. olarecea = Portulaca olarecea*.
[b]*A. amaranthi = Albugo amaranthi, P. variabilis = Peronospora variabilis, D. lutea = Dichotomophthora lutea*.

Table 2. Incidence (%) of weed species infected with different fungi species in Kırklareli province

Districts	No. of weeds sampled	Incidence (%) Weed species[a] - fungi species[b]		
		A. retroflexus - A. amaranthi	*C. album - P. variabilis*	*P. olarecea - D. lutea*
Babaeski	141	0.00	12.05	0.70
Central	96	0.00	0.00	0.00
Kofçaz	29	0.00	24.13	0.00
Lüleburgaz	145	3.03	2.42	0.00
Pehlivanköy	25	0.00	0.00	0.00
Pınarhisar	16	0.00	0.00	0.00
Vize	22	0.00	0.00	0.00
Total	494	1.01	5.67	0.20

[a]*A. retroflexus = Amaranthus retroflexus, C. album = Chenopodium album, L. serriola = Lactuca serriola*.
[b]*A. amaranthi = Albugo amaranthi, P. variabilis = Peronospora variabilis, A. alternata = Alternaria alternate.*

The present study identified the occurrence of two fungus-like species (*A. amaranthi* and *P. variabilis*) and two fungal species (*A. alternata* and *D. lutea*) on weeds in sunflower production areas in the region of Thrace. The presence of these species exhibited variability depending on the weed host. Of these, *A. amaranthi*, which causes white rust disease, was reported for the first time in this study on the leaves of *A. retroflexus* in Turkey. However, some authors (Erper et al., 1997; Özaslan, 2011) described *A. bliti* as the causal agent of white rust on *A. retroflexus* in Turkey. *A. amaranthi* has been observed on the same weed species in different countries such as Czech Republic (Dietrich and Muller, 2001), Poland (Ruszkiewicz-Michalska and Michalski, 2005), Germany (Jüttersonke, 1996; Spring et al., 2005), United Kingdom (Jones and Baker, 2007) and Iran (Mirzae et al., 2021). In the present study *Peronospora variabilis* was observed on the host weed *Chenopodium album* exhibiting symptoms of downy mildew. Although this pathogen was previously identified as occurring on *C. album* in the provinces of Ankara in the Middle Anatolia region (Göbelez, 1963) and Hatay in the Eastern Mediterranean region (Kara et al., 2020) of Türkiye, to the best of our knowledge, this is the first confirmation of downy mildew caused by *P. variabilis* on *C. album* in the Thrace region of Türkiye. Globally, *P. variabilis* has been reported on *C. album* in countries including Argentina, China, Germany, Italy, Ireland, Latvia, Pakistan, Poland, the Netherlands, Romania, South Korea, Spain, Switzerland and the United States (Riethmuller et al., 2002; Goker et al., 2007; Choi et al., 2008; Choi et al., 2010; ARS, 2019; Nolen et al., 2022; Fondevilla et al., 2023). A previous study identified *A. alternata* in *C. galaticus*, *Sorghum halepense*, *Vicia sativa* and *Xanthium strumarium* in wheat and cotton cultivation areas in Diyarbakır/Türkiye (Özaslan, 2011). Furthermore, the presence of this fungus was reported in *Alchemilla pseudocartalinica*, *Campanula lactiflora*, *Digitalis ferruginea*, *Euphorbia ablongifolia*, *Pteridium aquilinum*, *Rumex acetosella*, *Rumex crispus*, *Sambucus ebulus*, *Silene vulgaris* and *Sedum spurium*, which were important weed species in pasture areas in Trabzon/Türkiye (Asav et al., 2015). However, it has been recorded on *L. serriola* in South Korea (Kim and Choi, 2020). This is the first report of *A. alternata* on *L. serriola* in Türkiye. *Drechslera lutea* was identified on brown leaf spots of *Portulaca oleracea* in sunflower fields in Tekirdağ. This species was identified in *P. oleracea* grown as a crop in the United States (Baudoin, 1986), Türkiye (Erzurum province in Eastern Anatolia region) (Eken, 2003), Iran (Heidari et al., 2018) and the Netherlands (Marin-Felix et al., 2019). The species was identified as the causal agent of leaf spot of *P. oleracea*, which is found as a weed among cultivated plants in the field in India (Rao, 1966) and Cuba (Camino-Vilaro et al., 2019), as in our study. The morphological characteristics of the fungus-like species (*A. amaranthi* and *P. variabilis*) and both cultural and

morphological characteristics of the fungal species (*A. alternata* and *D. lutea*) identified in this study were found to be similar to those previously recorded for these species (Rao, 1966; Baudoin, 1986; Eken, 2003; Choi et al., 2008; Choi et al., 2010; Heidari et al., 2018; Marin-Felix et al., 2019; Kara et al., 2020; Kim and Choi, 2020; Mirzae et al., 2021; Nolen et al., 2022). In the present study, the identification of these species was additionally confirmed through DNA sequencing based on the analysis of different gene regions, as previously described in numerous published articles. (Reithmuller et al., 2002; Spring et al., 2005; Göker et al., 2007; Choi et al., 2008; Choi et al., 2010; Kara et al., 2020; Kim and Choi, 2020; Mirzae et al., 2021; Nolen et al., 2022; Fondevilla et al., 2023). The diseases caused by fungus-like and fungal species on weeds in Tekirdağ and Kırklareli provinces exhibited variation according to the districts in which they were identified. This study is the first investigation into incidence of different weeds infected by different species in sunflower fields, providing initial findings. As a classic biological weed control strategy using fungi, it is very important that the fungal species identified do not cause disease in the crop in the area where the weeds are present. *A. alternata*, identified in this study, causes leaf spot in sunflower (Wang et al., 2014; Kgatle et al., 2018), suggesting that *L. serriola*, its host, might act as a reservoir and inoculum source for sunflowers. However, no other fungal or fungus-like species found in this study have been reported to cause disease in sunflower.

CONCLUSIONS

This study highlighted the potential importance of the biotrophic fungal species *A. amaranthi* and *P. variabilis* on *A. retroflexus* and *C. album*, respectively, in the classical biological control of these problematic weeds in sunflower production. *Peronospora variabilis*, a fungus-like species, on *Chenopodium album* in the districts of Tekirdağ and Kırklareli provinces, and *Albugo amaranthi*, also a fungus-like species, on *Amaranthus retroflexus* in Tekirdağ districts, with high incidence rates of infected plants, can be important for the biological control of these weeds in sunflower production areas in the Thrace region and not only. Future research should focus on the epidemiology and host-pathogen interactions of these species to optimize their use in integrated weed management strategies.

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