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Investigation, Identification and Pathogenicity Assessment of Leaf and Soil-Borne Fungal Diseases Causing Yield Reduction in Vegetables in Antalya

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Abstract: Vegetables significantly contribute to Antalya, Türkiye's economy. This study aimed to investigate and identify fungal pathogens causing leaf and soil-borne diseases in leafy vegetable crops. Surveys were conducted in 2021, focusing on morphological and microscopic diagnosis, as well as the prevalence rate of these diseases in five regions (Aksu, Serik, Muratpaşa, Kepez, and Korkuteli). Isolates were obtained from both the leaves and roots of symptomatic plants, and pathogenicity tests were conducted. Survey results showed that lectuca downy mildew was the most important disease, with a 40% plant infection rate and 16.9% disease severity in most parts of the region. In areas where parsley was cultivated, *Fusarium* sp. was identified as the predominant soil-borne pathogen at 23.82%, and *Sclerotinia sclerotiorum* was prevalent in lettuce at 14.28%. The lowest incidence of *Albugo candida* was observed in cress. In conclusion, this study provides crucial insights into the prevalence of fungal diseases and the specific pathogens responsible for damage to lettuce, parsley, dill, cress, mint, and basil cultivation in Antalya province. The incidence and prevalence of these fungal diseases were assessed through surveys in Antalya. In conclusion, this study provides important information on fungal disease agents and their prevalence in lettuce, parsley, dill, cress, mint and basil cultivated areas in Antalya province.

Keywords: Disease incidence, leafy vegetable, prevalence.

Antalya'da Sebzelerde Verim Azalmasına Neden Olan Yaprak ve Toprak Kökenli Fungal Hastalıklarının Araştırılması, Tanımlanması ve Patojenitesi

Öz: Sebze tarımının Türkiye ve Antalya ekonomisine önemli katkısı vardır. Bu çalışma, yaprağı yenen sebzelerde verim kaybına neden olan yaprak ve toprak kökenli fungal patojenlerin araştırılmasını ve tanımlanmasını amaçlamıştır. Çalışma, 2021 yılında, Antalya ilinde yoğun sebze yetiştiriciliği yapılan Aksu, Serik, Muratpaşa, Kepez ve Korkuteli ilçelerinde yürütülmüştür. Hastalık etmeni fungal patojenlerin simptomolojik, morfolojik ve mikroskobik tanılamaları yapılmış ayrıca hastalık şiddeti ve yaygınlık oranları belirlenmiştir. Hastalık belirtisi gösteren bitkilerin hem yapraklarından hem de köklerinden izolatlar elde edilerek patojenite testleri yapılmıştır. Survey sonuçlarına göre, %40 bitki enfeksiyon oranı ve %16,9 hastalık şiddeti ile Mildiyö en önemli hastalık olarak tespit edilmiştir. Maydanoz yetiştirilen bölgelerde *Fusarium* sp. %23,82 oranı ile toprak kaynaklı baskın patojen olarak tanımlanmış olup bunu marulda %14,28 *Sclerotinia sclerotiorum* izlemiştir. En düşük görülme sıklığı ise terede *Albugo candida* olarak bulunmuştur. Sonuç olarak bu çalışma, Antalya ilinde marul, maydanoz, dereotu, tere, nane ve fesleğen yetiştirilen alanlarda fungal hastalık etmenleri ve yaygınlığı hakkında önemli bilgiler sunmaktadır.

Anahtar kelimeler: Hastalık şiddeti, yaprağı yenen sebzeler, yaygınlık oranı,

1. Introduction

Sustainable agriculture and reliable food are among the most important issues for all countries in the world. Among agricultural products, vegetables have an important place in human nutrition in Turkey as well as in the world. (Gupta &Prakash, 2009). The leafy vegetables consumed in their raw state, which are essential to our tables, are parsley, dill, cress, mint, basil and lettuce. These vegetables hold significant importance in our lives due to their appearance, colors, flavors and nutritional values. According to the 2021 TUIK data, Antalya has 3,556,424 da of agricultural area and 493,193 da of this agricultural area are engaged in vegetable agriculture. Within this total production, the production amount of leafy vegetables (lettuce, parsley, cress, mint and dill) is 60,677 tons (TUIK, 2021).

Failure to adhere to crop rotation in the areas where production takes place leads to vegetables being exposed to soil-borne diseases. The use of unlicensed and uninformed plant protection products by producers poses a threat to human health and the environment. In our country, studies on edible vegetables are generally limited to studies on the presence of the disease. Studies

on fungal diseases in edible vegetables in Turkiye; They can be listed as *Albugo candida* in cress and arugula, *Erysiphe heraclei* in dill, *Septoria petroselini* and *Plasmopara petroselini* in parsley, *Alternaria* spp, *Botrytis cinerea, Sclerotinia sclerotiorum* in lettuce, and *Peronospora belbahri* and *Botrytis cineria* in basil (Kurt 2003; Soylu & Soylu 2003; Ellialtıoğlu et al.,2007; Onaran and Yanar, 2009; Soylu et al. 2010; Ünlü and Boyraz 2010; Özer et al., 2018; Canpolat et al 2019; Uzunoğulları et al. 2022; Günaçtı, 2022;). Among the efforts towards control these diseases, fungicide applications against Septoria leaf spot disease in parsley and grey mold disease caused by *Botrytis cinerea* in lettuce are included (Polat and Coşkuntuna 2014; Tok, 2008).

Table 1. Same Vegetable Production Areas in Antalya, 2021
Çizelge 1*. Antalya ilinde bazı sebze üretim alanları miktarı, 2021*

District	Product					
	Lettuce	**Parsley**	**Dill**	**Cress**	**Mint**	**Basil**
Serik	4.435	95	15			10
Aksu	3.870	475	25	35		8
Muratpaşa	1.670	105	30	100		12
Kepez	1.112	430	55	15	40	6
Korkuteli	1.100	285	20	18		14
Totaly (da)	**12.187**	**1.390**	**145**	**168**	**40**	**50**

Antalya province has the most important place in the production and sales of leafy vegetables and is also the production region with the highest pesticide residue problem in these products. There have not been sufficient studies on fungal diseases of edible vegetables in the region. Antalya province has the most important place in the production and sale of leafy edible vegetables and is also the production region with the highest problem of pesticide residues in these products. There is no research on the incidence and prevalence of disease-causing fungi in leafy vegetables consumed in Antalya. In this study, it was aimed to define fungal diseases morphologically and microscopically and to determine disease occurrence rates and disease incidence rates in some leafy vegetable production areas in the region.

2. Material and Methods

Samples simple random sampling method were taken according to the disease symptoms in the plants and the disease rate was calculated by counting.

2.1. Survey and isolation of fungal pathogens

Isolates were collected in 2021 from 45 total field including lettuce, parsley, dill, cress, mint and basil plants in Antalya (Table 1). Samples simple random sampling method were taken according to the disease symptoms in the plants and the disease rate was calculated by counting. Approximately 14,000 da are grown annually in Antalya for fresh consumption. According to the simple random sampling method, selected fields were examined for disease symptoms, prevalence, and incidences (Bora and Karaca, 1970). Survey studies were programmed to cover at least 1% of the cultivation area. A total of 45 fields were investigated, and plant samples with root and crown rot, wilting and drying symptoms were collected. Plant samples brought to the laboratory were cut into small pieces including healthy and diseased parts and subjected to surface disinfection in 1% sodium hypochlorite (NaOCl) solution for 2-4 minutes. The diseased plant parts were then rinsed with sterile distilled water, dried thoroughly between sterile blotting papers and transferred to Petri dishes containing Potato Dextrose Agar (PDA-Merck) and Synthetic Nutrient Agar (SNA) medium under aseptic conditions. The samples were incubated at 22±2°C for and the developing fungi were purified for identification and then stored in slant agar at +4°C. Samples were brought to the Mycology Laboratory of the Biological Control Research Institute (BMAE). The disease rate in the production area was calculated from the percentage of infected plants. After the disease rates in the production area were determined, the prevalence rate was compared to the current average. (Bora ve Karaca, 1970).

2.2. Microscobic examination and morphological identification

Identification of soilborne pathogens, samples were taken from the root, crown and stem parts of diseased plants, and the identification of cultures developed as a result of isolation was carried out morphologically and microscopically using diagnostic keys in the literature (Agrios, 1998; Barnett & Hunter, 1998; Ellis,

1971;1976; Sneh et al., 1991; Leslie et al., 2006). Identification of obligate pathogens *(Erysiphe* spp., *Peronospora* spp.) based on morphological characteristics (column, color, spore shape and size) has been made. For microscopic analysis, leaf samples showing hairy mycelial development and chlorotic symptoms were determined under the microscope from plant leaves collected from the fields (Bulajic et al.,2009; Nawrocki 2004; Nawrocki and Mazur 2007; Soylu & Soylu 2003). Microscopic studies were investigated using a light microscope, the Olympus BX43 microscope and equipped with a digital camera.

2.3. Pathogenicity

Soil borne fungal pathogens were tested for pathogenicity according to soil inoculation methods described by Kunwar et al. (1989) and Ahmad and Sharma (1990). Wheat culture was prepared for inoculation into the soil. Wheat seeds soaked in water for 10 minutes were boiled. After the prepared inoculum was autoclaved in glass bottles, inoculation was made with discs taken from the colonies for proper growth of the fungi. Bottles were kept in incubator at 24–25 °C for 15 days. Fungal inoculum was first mixed into the potting soil one week after sowing. Then, 10 seeds per pot were planted in parsley seeds; lettuce seedlings were planted in such a way that there would be three seedlings in each pot. In control pots, only wheat was mixed, and disinfected seeds were planted. Trials with three replications have been made. Controls were inoculated with sterile water only. After inoculation, plants were kept at 20°C in a greenhouse. Disease assessments were performed four weeks later. The pathogen was re-isolated from inoculated plants using the method described above. Re-isolation of the pathogen was carried out and compared with the original inoculum to fulfill Koch's postulates.

2.4. Assessment of disease incidence and disease prevalence

During field surveys conducted in 2021, a lot of foliar and soilborne fungal diseases were observed in Antalya province. The surveys were carried out in November and December 2021, when optimum conditions for the diseases were available. Randomly selected 100 plants were taken from each field in all areas where lettuce, parsley, dill, cress, mint and basil are grown in fields in Antalya. The incidence of fungal diseases was determined according to the presence or absence of disease on the leaves and rots examined. The disease incidence and prevalence were calculated using the formulas below (Bora and Karaca 1970).

$$\textit{Disease incidence (\%)} = \textit{Number of diseased plant} \div \textit{total number of plants evaluated} \times 100 \quad (1)$$

$$\textit{Disease Prevalence (\%)} = \textit{Number of disease established fields} \div \textit{total number of surveyed fields} \times 100 \quad (2)$$

3. Result and discussion

A total of 270 plant samples showing disease symptoms were collected from areas where lettuce, parsley, dill, mint, and basil were cultivated during the 2021 growing season in Antalya.Survey studies were carried out in the leafy vegetable areas of Antalya province in 2021, and information about these areas is given in Table 2. As a result of the studies *B. lactucae R. solani, S. sclerotiorum* and *Fusarium* spp. in lettuce; *S. petroselini, Fusarium* spp. and *A. alternata* in parsley; *E. heraclei and Fusarium spp.* in dill; *Fusarium* spp. in mint, *P. belbahrii, B. cineria, Fusarium* spp. in basil and *Albugo candida* in cress were detected .

Isolations revealed that the most commonly identified fungus genus was *Fusarium* sp. It was also determined that *S. sclerotiorum* is common disease, causing damage to plants green parts. Other isolated fungi included *R. solani, A. alternata., E. heraclei, B. lactucae, P. belbahrii, B. cineria S. petroselini* and *Albugo candida.* These fungi showed varying levels of prevalence and the potential to cause damage in fields. As a result of isolations, downy mildew was the most important disease, with a 40% plant infection rate and 16.9% disease severity in most parts of the region. In areas where parsley was cultivated, *Fusarium* sp. was identified as the predominant soil-borne pathogen at 23.82%, and *Sclerotinia sclerotiorum* was prevalent in lettuce at 14.28%. The lowest incidence of *Albugo candida* was observed in cress.

White rot disease, caused by *Sclerotinia sclerotiorum,* has been reported to result in product losses of up to 95% in lettuce production worldwide (Clarkson et al., 2004; Smolinska and Kowalska, 2018; Mullen, 2001; Chitrampalam et al., 2011). Soil-borne pathogens such as *Fusarium oxysporum, Phoma exigua, Rhizoctonia solani, Sclerotinia sclerotiorum, Verticillium dahliae,* and *Pythium* spp. can cause diseases in lettuce such as wilting, root, and root collar rot (Dixon, 1984; Koike et al., 2007). Onaran and Yanar (2009) reported that *Sclerotinia sclerotiorum* is the most damaging disease in lettuce based on their study in the

Aegean Region, particularly in Izmir, Manisa and Aydın provinces.

Table 2. Fungal disease pathogens and prevalence detected in some vegetables
Çizelge 2. *Fungal hastalık etmenlerinin bazı sebzelerde bulunma oranı ve hastalık şiddeti*

Crops	Totaly area (da)	Surveyed area (da)	Disesesd survey area (da)	Disease Prevelance (%)	Disease Incidance (%)									
					Fusarium spp.	*Rhizoctonia solani*	*Alternari alternata*	*Sclerotinia sclerotiorum*	*Septoria petroselini*	*Bremia lactucae*	*Botrytis cineria*	*Peronospora belbahrii*	*Albugo candida*	*Erysiphe heracle*
Lettuce	12.187	20	8	40	14,64	8,83	-	14,28	-	16,9	-	-	-	-
Parsley	1.390	8	2	25	23,82	-	3,84	-	19,26	-	-	-	-	-
Dill	145	5	2	40	3,08	-	-	-	-	-	-	-	-	4,88
Cress	168	5	1	20	-	-	-	-	-	-	-	-	1,22	-
Mint	40	3	1	33,3	6,53	-	-	-	-	-	-	-	-	-
Basil	50	4	3	75	2,38	-	-	3,12	-	-	1,32	12,5	-	-

Parsley is susceptible to several fungal pathogens, with the most significant being Septoria leaf spot disease caused by *Septoria petroselini*, *Pythium* spp., *Fusarium* spp., *Rhizoctonia solani*, *Sclerotinia Sclerotiorum*, *Alternaria radicina*, *Alternaria petroselini*, *Cercospora* spp. and *Plasmopara petroselini* (Raid and Roberts, 2004; Kurt, 2003; Kurt & Tok, 2006, 2019; Soylu et al., 2010; Kurt et al., 2017; Nawrock, 2004; Glawe et al., 2005; Hershman, 1986). Among studies conducted in our country, fungal disease pathogen *Septoria petroselini* was first identified by Kurt in 2003 in Hatay province, where parsley cultivation is most prevalent. In a study conducted to identify fungal pathogens in the Central Anatolia Region, *Alternaria* spp. was detected in parsley and *Bremia lactucae* in lettuce (Ünlü and Boyraz, 2010).

In a study conducted during the 1981-1982 production season in South New Jersey, *F. oxysporum*, *F. solani*, *P. ultimum*, *P. irregulare*, and *R. solani* were isolated from parsley (Hershman, 1986). In a study by Nawrocki (2004), the most commonly detected pathogens in seedlings were *Alternaria radicina* and *Fusarium* spp., followed by *Cylindrocarpon destructans*, *R. solani* and *Stemphylium botryosum* in lower proportions. To effectively control these diseases, it is essential to use certificated seeds, practice crop rotation and ensure the collection and destruction of diseased plants and harvest residues. Chemical control against these diseases should commence as soon as the first symptoms manifest in the environment (Tok & Kurt, 2019; Anonymous, 2020).

In mint plants, fungal pathogens such as *Puccinia menthae*, *V. daliae*, *A. alternata*, and *R.solani* have been reported (Jurronis and Snieskienei, 2004; Zimowska, 2007). In our 2021 survey study, *Fusarium* spp. was identified as the causative agent of mint wilt disease, with a detection rate of 6.53%. The most commonly preferred control methods for managing this disease involve removing infected plants from the field and implementing crop rotation. It is imperative to promptly remove plants exhibiting wilt disease symptoms and prioritize crop rotation (Kalra et al., 2005; Demir, 2007).

In our study, *Erisiphe heraclei* was detected in dill at a rate of 4.88%. Suitable conditions for the pathogen include low light intensity and high humidity. The affected leaves generally wither and dry. Since the pathogen causes quality losses in the leaves, it is economically significant.

For leafy vegetables, the causative agent of powdery mildew is *Albugo candida* in dill. The disease appears on the upper surface of the leaf as raised white pustules or rings. In our study, *A. candida* was identified as the lowest prevalent disease at a rate of 1.22%.

Basil is one of the aromatic plants commonly used in salads and pasta. In this study, *Peronospora belbahrii* was identified at 12.5%, *Sclerotinia sclerotiorum* at 3.12%, *Fusarium* spp. at 2.38%, and *Botrytis cinerea* at 1.32%. In studies related to basil, *B. cinerea* was first detected in Özer et al. (2018), and *P. belbahrii* was first identified in our country by Günaçtı in 2022.

4. Conclusion

Vegetable cultivation is one of the major sources of income in Antalya. This study was to determine soil and leaf-origin fungal diseases causing a decrease in yield in vegetables such as lettuce, parsley, dill, mint, and basil. Survey studies were conducted in the districts of Serik, Muratpaşa, Korkuteli, and Aksu within Antalya province. As a result of the surveys in the edible vegetable areas of Antalya province, it has been observed that fungal disease agents, especially soil-

borne agents, are abundant, and it is thought that these data provide up-to-date information to the literature for the region.

In Turkey, due to the limited number of licensed plant protection products for leafy vegetables, cultural measures take precedence in the control of fungal diseases. Field studies revealed that farmers were not paying sufficient attention to cultural measures. Notable observations included excessive pesticide application, lack of emphasis on pesticide application periods, and inadequate care in the removal of diseased plants, which serve as a source of inoculum. Implementing biological control studies in leafy vegetable cultivation can lead to the production of high-quality products without pesticide residues. This approach minimizes chemical usage, preserves natural enemies, and mitigates the damage caused to the environment and the economy by unnecessary pesticide use.

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