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Fungal diseases of strawberry grown in Düzce province of Turkey

Düzce ili çilek üretim alanlarında görülen fungal hastalıklar

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ABSTRACT

Surveys in Düzce province indicated that strawberry cultivation was made in small open fields changing between 0.5 and 5.0 da with mulching. Four strawberry cultivars; Camarosa, Kabarla, Sweet Ann and Osmanlı have been commonly grown in the province. Fungal diseases of strawberry which has gained importance recently in Düzce province of Turkey were studied with the surveys conducted in three periods (15.03.2012; 20.05.2012; 15.09.2012). In the first period for leaf diseases; in the second period for leaf, petiole, fruit, and root diseases and in the third period mainly for root diseases were investigated. *Alternaria* spp., *Botrytis cinerea*, *Hainesia lythri*, *Mycosphaerella fragariae*, *Phoma* sp., *Phomopsis* sp., *Rhizoctonia solani* were isolated from the leaves and petioles; *B. cinerea* from the fruits and *Alternaria* spp., *Fusarium oxysporum*, *Macrophomina phaseolina*, *Phytophthora* spp., and *R. solani* were isolated from the roots. All the fungi obtained caused necrosis on detached leaves at the pathogenicity tests. Detached leaf tests were clearly reflected the pathogenicity of the fungi. *Botrytis cinerea* and *Mycosphaerella fragariae* were the most widespread diseases found in all the fields surveyed. The other fungi showed various rates of disease occurrence.

INTRODUCTION

Strawberry which is a natural hybrid of *Fragaria x ananassa* belonging to Rosaceae family is grown for its edible fruits and its cultivation has been supported by the EU and Turkish government. According to 2015 database, 5.4 million tons of strawberry was produced in the world (Anonymous 2016). Strawberry production was 400 167 tons in 153 918 da in Turkey according to TUIK database in 2017 (Anonymous 2017).

Uselis et al. (2006), reported that the most spread disease

was common leaf spot *Mycosphaerella fragariae* Tul. (Lindau) in Lithuania and prevalence of this disease was found 43%. The most resistant cultivars were Dange, Elkat and Pegasus in cultivar reaction studies. Ellis (2008), indicated that leaf diseases of strawberry can occur from early spring to late autumn in dormant season. However, these diseases cause decrease in the number of leaves, thus this situation makes the plants weaker against root diseases and winter damage. Fang et al. (2008), found that *Fusarium*

oxysporum Schldtl., *Rhizoctonia* spp. (AG-A, AG-C, AG-I, AG-K), *Cylindrocarpon destructans* Zins., *Phoma exigua* Sacc., *Gnomonia fructicola* (Arnaud) Sogonov, comb. nov., *Phytophthora cactorum* Lebert & Cohn, *Pythium ultimum* Trow, *Macrophomina phaseolina* Tass. and *F. oxysporum* pathogens were isolated from strawberries in Western Australia. The most important stem disease was found *F. oxysporum* and the root disease was *Rhizoctonia* spp.

Swift (2014), reported that *Mycosphaerella* leaf spot was very significant pathogen in Colorado. The most resistant cultivars were found respectively Atlas, Cavendish, Earliglow, Jewel, Kent, Lateglow and Tenesse Beauty among 25 cultivar that were grown in Colorado.

There are limited reports dealing with strawberry diseases in Turkey. The most common disease reported is Gray mold caused by *Botrytis cinerea* (de Bary) Whetzel and it was reported from many strawberry cultivation areas; Yıldırım and Turhan (2003) from İzmir, Benlioğlu et al. (1998) from Aydın, Eken (2009) from Erzurum. Benlioğlu et al. (1998) investigated the resistance of *Botrytis cinerea* to commonly used fungicides in Aydın province and they found that about thirty percent of the isolates were resistant against benomyl and procymidone. Root rot and wilt diseases caused by *Fusarium* spp., *Phytophthora* spp., *Pythium* spp., *Rhizoctonia* spp. and *Verticillium* sp. were also reported in several places (Benlioğlu et al. 2002, Eken 2009, Güner and Coşkun 1993, Yıldırım and Turhan 2003). Benlioğlu and Yıldız (2014), found that *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., root pathogen was first report in Aydın in Turkey where strawberry production is very important. Due to being very destructive in pathogenicity test such a short time at 7 days, it really deserves to be investigated deeply. Dinler et al. (2015), used different pathogenicity tests using stolons. Total number of 1014 fungal isolates obtained from root and crown from 2248 strawberry seedlings (Camarosa, Sweetcharlie, Rubygem and Festival cultivars) in 2009-2010 and 2010-2011 growing season in Sultanhisar and Köşk province in Aydın, Turkey. At the end of the pathogenicity tests using stolons, 291 *Fusarium* spp., 153 *Rhizoctonia* spp., 13 *Cylindrocarpon* sp. isolates were found as a pathogen.

A project to improve strawberry cultivation in Düzce was started in 2009 and diseases, which are the main factors on yield loss, were studied. Certified daughter plants of four varieties; Camarosa, Kabarla, Sweet Ann and Osmanlı, were brought from Adana and distributed to the farmers. The aims of this study were to identify and assess pathogenicity of the fungal agents causing important diseases of strawberry in Düzce.

MATERIALS AND METHODS

Study site and sampling

In order to determine strawberry diseases, three surveys were made in three periods (15.03.2012; beginning of flowering, 20.05.2012; start of harvest, 15.09.2012; end of harvest). In the first period leaf diseases; in the second period leaf, leaf stipe, fruit and root diseases and in the third period mainly root diseases were investigated. The survey was done with randomly selected methodology. A total 45.5 da were surveyed from different counties of Düzce (Table 1).

Isolation and identification

The samples were disinfected by dipping in 1% NaOCl for two minutes for leaf and fruit, 3 min for root samples. Isolations were made on water agar (WA) and potato dextrose agar (PDA, Merck) with and without streptomycin sulphate (200 mg/l). The cultures were incubated at 24–28±2 °C with 12 hours alternating Near Ultra Violet (NUV) light and darkness. Root samples were also transferred to the selective media for *Phytophthoras* [PARPNH medium as mg/ml; PCNB 50, pimaricin 10, ampicillin 250, rifampicin 10, nystatin 50, hymexazol 50 in the base medium of grated carrot 40 g/l and agar 20 g/l (GCA)] for isolation. For morphological characteristics of *Phytophthoras*, GCA amended with β -sitosterol 30 mg, tryptophan 20 mg, thiamin 1 mg per liter was used. Golden Delicious apple variety was used for baiting *Phytophthora* spp. from soil. The fungi obtained in this study were identified both microscopic and macroscopic criteria using morphological characteristics described in literatures (Booth 1977, Burnett and Hunter 1998, Ellis 1971, 1976, Erwin and Ribeiro 1996, Gallegly and Hong 2008, Leslie and Summerell 2006).

Pathogenicity test

Pathogenicity of the fungi was made on detached, healthy looking Kabarla cultivar leaves by placing 10 day old culture discs of 0.5 cm in diameter on leaf surfaces disinfected by dipping in 1% NaOCl for 3 min and rinsed in sterile distilled water. In the pathogenicity tests; 5 *Botrytis* sp., 4 *Rhizoctonia* spp., 4 *Fusarium* spp., 3 *Macrophomina* sp., 3 *Alternaria* spp., 1 *Hainesia* sp., 1 *Phoma* sp., 1 *Phomopsis* sp., 1 *Phytophthora* spp. fungal genuses were used. Inoculated leaves were incubated at 22±1 °C in a humid chamber in plastic petri dishes. The measurements were done in seventh and fourteenth days after inoculation. Pathogenicity tests were only done using detached leaves, due to not able to obtain healthy plants. All the pathogens were reisolated from infested leaves in the pathogenicity tests.

Table 1. The areas surveyed and numbers of collected sample in Düzce strawberry cultivation areas

Date	Location	Area studied (da)	Number of samples collected				
			Leaf	Stem	Fruit	Root	Soil
15.3.2012	Beyköy	0.5	1	-	-	-	-
	Çakırlar	1	4	-	1	-	-
	Çamköy	0.5	1	-	-	-	-
	Darıcı	2.5	7	1	1	-	-
	Durak köyü	0.5	1	-	-	-	-
	Konuralp	3	10	1	2	-	-
	Otluoğlu	1	4	-	-	-	-
	Yeni Karaköy	0.5	1	-	-	-	-
	Total	9.5	29	2	4	-	-
20.5.2012	Üçköprü köyü	1	1	-	1	-	-
	Beyköy	0.5	1	-	-	-	-
	Çakırlar	1	1	-	1	-	-
	Darıcı	2.5	4	1	3	3	-
	Kaymakçı	0.5	1	-	-	-	-
	Konuralp	3	5	2	3	3	-
	Otluoğlu	5	7	3	4	3	-
	Yörük	0.5	-	-	-	-	-
	Total	14	20	6	12	9	-
15.9.2012	Tepeköy	2	-	-	-	3	2
	Altunçay	4	4	-	-	5	5
	Kiraztarla	1	-	-	-	1	1
	Mahırağa	1	-	-	-	-	1
	Merkez	1.5	2	-	-	2	1
	Sarımeşe	4	4	-	-	6	5
	Söğütlü	2	2	-	-	1	2
	Topçular	1	-	-	-	1	-
	Yenivakıf	1	1	-	-	1	-
	Değirmenbaş	1.5	1	-	-	2	2
	Düzköy	1	-	-	-	1	-
	Osmanca köyü	2	2	-	-	2	2
Total	22	16	-	-	25	21	
Total number	45.5	65	8	16	34	21	

Table 2. The prevalence of the fungi obtained from strawberry cultivation areas of Düzce province, studied in three periods

Survey period	Disease agents	Area surveyed (da)	Infested area (da)	Prevalence (%)
First	<i>Botrytis cinerea</i>	9.5	9.5	100.0
	<i>Mycosphaerella fragaria</i>	9.5	9.5	100.0
	<i>Alternaria</i> spp.	9.5	4.0	42.1
	<i>Fusarium oxysporum</i>	9.5	0.5	5.6
	<i>Phoma</i> sp.	9.5	0.5	5.6
Second	<i>Botrytis cinerea</i>	14.0	14.0	100
	<i>Mycosphaerella fragaria</i>	14.0	14.0	100
	<i>Alternaria</i> spp.	14.0	6.0	42.9
	<i>Fusarium oxysporum</i>	14.0	5.5	39.3
	<i>Rhizoctonia</i> sp.	14.0	4.0	28.6
Third	<i>Phoma</i> sp.	14.0	0.5	3.6
	<i>Botrytis cinerea</i>	22.0	22.0	100.0
	<i>Mycosphaerella fragaria</i>	22.0	22.0	100.0
	<i>Fusarium oxysporum</i>	22.0	12.0	54.5
	<i>Rhizoctonia</i> sp.	22.0	8.0	36.4
	<i>Macrophomina phaseoli</i>	22.0	7.0	31.8
	<i>Alternaria</i> spp.	22.0	6.0	27.3
	<i>Hainesia lythri</i>	22.0	2.0	9.1
	<i>Phomopsis</i> sp.	22.0	1.0	4.5
<i>Phytophthora</i> sp.	22.0	1.0	4.5	

RESULTS

Ten fungal pathogens were obtained in Düzce province of Turkey. The most widespread fungi were *Botrytis cinerea* and *Mycosphaerella fragariae* in all periods. The other fungi isolated in this study and their prevalence is tabulated in Table 2.

Botrytis cinerea caused brown leaf spots expanding from leaf margins to the whole leaf as a sectoral pattern (Figure

1a). The pathogen produced very profuse gray mold having sparse sporulation on the fruits (Figure 1b). It was also found on petioles. The fungus produced similar spots on the detached leaves in seven and fourteen days (Figure 1c, d).

Mycosphaerella fragariae (anamorph *Ramularia tulasnei*) caused leaf spots with 3–6 mm in diameter having reddish brown edge with whitish center (Figure 2a). When incubated in a moist chamber they produced cylindrical

and 20-30 µm long conidia with 0-4 septa. The conidia sometimes occurred in short chains (Figure 2b).

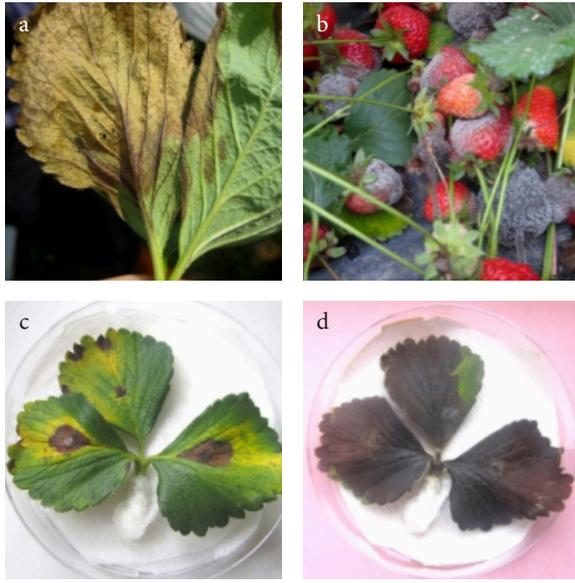


Figure 1. Various aspects of *Botrytis cinerea* a) Leaf spots b) Fruit rot c) Leaf spots on detached leaves after 7 days from inoculation d) Leaf spots after 14 days from inoculation

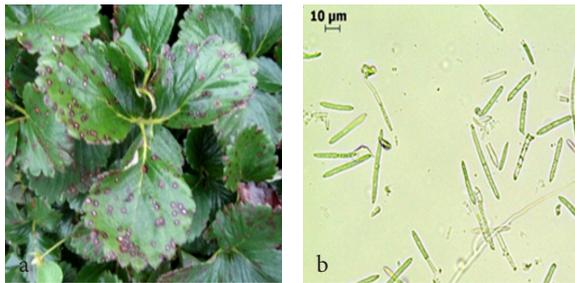


Figure 2. a) Leaf spots caused by *Mycosphaerella fragaria* b) Conidia of *Mycosphaerella fragariae*

Fusarium wilt (*Fusarium oxysporum*) caused various degrees of wilting resulted in plant death. Root sections of wilted plants showed a reddish discoloration in root cortex. The pathogen produced dark red spots on the detached leaves. *Alternaria* leaf spots were observed during first and second period of surveys and caused irregular, small, dark brown almost black, concentric spots. Leaf tissues around the spots turned yellow. Similar spots were produced on detached leaves. *Macrophomina* root rot caused by *Macrophomina phaseoli* appeared as a dry, red brown rot on the roots. The isolate of *Phytophthora* sp. caused leaf necrosis of irregular shape on detached leaves. *Hainesia lythri* causing Hainesia leaf spot found in two fields was isolated from the leaf spots having dark margin with

whitish center and produced small lesions on the detached leaves.

Phomopsis leaf blight was also found in a field from the leaves having sectoral necrosis similar to *Botrytis* infections. *Phoma* sp. was isolated from a field from leaf stipe necrosis.

DISCUSSION

This study showed that the most widespread diseases of strawberries were gray mold caused by *Botrytis cinerea* and common leaf spot caused by *Mycosphaerella fragariae* in Düzce province of Turkey. They were detected in all the surveyed fields. Widespread distribution of these two diseases of strawberry was also reported by various researchers in the world (Duncan 1980, Laugale et al. 2009, Swift 2014, Timudo-Torrevilla et al. 2005, Uselis et al. 2006). The same diseases were also found widespread in Turkey (Eken 2009).

Although very common, another disease, *Mycosphaerella fragaria* is not as destructive as gray mold. Along with the above-mentioned diseases; root rots caused by *Fusarium* spp., *Rhizoctonia solani* Kühn. and *Macrophomina phaseoli* were also found in high percentages in the area. These diseases were also recorded in different countries (Mertely et al. 2005). Presence of the root rot diseases at high rates in the first year of planting in the area raises the question of the distribution of them by the planting material.

Testing the pathogenicity of the fungi, especially for soil borne pathogens, was rather difficult because of not finding disease free seedlings. In this study; detached leaf assay was tested for all the fungi and comparable results were obtained since some of the fungi did not produced symptoms on detached leaves. The method was also used by various researchers and found useful (Argun et al. 2008, Sezer and Dolar 2012, Türkkan and Dolar 2008). Detached leaf assay is also used for pathogenicity of *Phytophthora* spp. (Hansen et al. 2005, Vleeshouwers et al. 1999).

ÖZET

Düzce ilindeki survey çalışmaları çilek yetiştiriciliğinin genellikle 0.5-5.0 da arasındaki küçük alanlarda, açıkta ve malçlama yöntemi ile yapıldığını göstermiştir. İlde 4 çilek çeşidinin (Camarosa, Kabarlar, Sweet Ann ve Osmanlı) yetiştirildiği görülmüştür. Yetiştiriciliğin gittikçe artış gösterdiği Düzce ilindeki çileklerde fungal hastalık etmenleri 3 dönemde yapılan incelemelerle belirlenmiştir. Birinci dönem (15.03.2012) surveyinde yaprak; ikinci dönemde (20.05.2012) meyve, yaprak, sap,

kök; üçüncü dönem (15.09.2012) sürveyinde ise daha çok kök hastalıkları araştırılmıştır. Düzce ilinde çileklerde yaprak ve yaprak saplarından *Alternaria* spp., *Botrytis cinerea*, *Hainesia lythri*, *Mycosphaerella fragariae*, *Phoma* sp., *Phomopsis* sp., *Rhizoctonia solani*; meyvelerden *B. cinerea*; köklerden *Alternaria* spp., *Fusarium oxysporum*, *Macrophomina phaseolina*, *Phytophthora* spp., *R. solani* izole edilmiştir. Koparılmış yapraklar üzerinde yapılan patojenisite çalışmaları sonucunda elde edilen tüm funguslar, yapraklarda nekrozlar oluşturmuştur. Koparılmış yaprak testleri fungusların patojenisitelerini net olarak göstermiştir. *Botrytis cinerea* ile *Mycosphaerella fragariae* en yaygın funguslar olup, incelenen tüm tarlalarda bulunmuştur. Diğer funguslar değişik oranlarda yaygınlık göstermişlerdir.

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