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Occurrence of fungal strawberry diseases in Central Anatolia Region of Turkey and reactions of some varieties grown widely against the important pathogens

Orta Anadolu Bölgesinde çilek ekiliş alanlarında görülen fungal hastalıkların tespiti, yaygın olarak yetiştirilen çeşitlerin önemli patojenlere karşı çeşit reaksiyonlarının belirlenmesi

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ABSTRACT

Strawberry diseases, occurring in Central Anatolia region of Turkey was studied in the years 2013-2016 in the strawberry growing areas of Bartın, Kayseri, Konya, and Zonguldak provinces. Totally 515.5 da strawberry fields in the above mentioned provinces were visited and plants showing disease symptoms were counted and their percentages were determined. Foliage and root diseases were determined by using 383 samples collected from various fields. Seventeen fungal pathogens: namely *Alternaria alternata* (Fr.) Keissler, *Boeremia exigua* (Desmazières) Aveskamp, Gruyter & Verkley, *Mycosphaerella fragariae* (Tul.) Lindau., *Colletotrichum acutatum* J.H. Simmonds, *Colletotrichum gleosporoides* (Penz.) Penz., *Botrytis cinerea* (de Bary) Whetzel, *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., *Diaporthe actinidae* N.F. Sommer & Beraha., *Diaporthe eres Nitschke*, *Didymella americana* Saccardo ex Saccardo, *Didymella pomorum* (Thümen) Aveskamp, Gruyter & Verkley, *Diplodia seriata* de Notaris, *Macrophomina phaseolina* (Tassi) Goidanich, *Peyronellae prosopidis*, *Phytophthora cactorum* (Lebert & Cohn) J.Schröter, *Phytophthora plurivora* Jung & Burgess., *Phytophthora kelmania* were identified. *A. alternata*, was the most frequently isolated fungus and was obtained from 41.94% of the plant samples while root rot diseases caused by various fungi occurred on 38.13% of the fields. Reactions of twelve strawberry cultivars; (Festival, Kabarla, Sweetann, Alison, Osmanlı, Kara çilek, Tüylü, Sabrina, Rubigem, San Andreas, Albion, Festival, Monterey) were tested against the three most widespread pathogen; *A. alternata, M. phaseolina* and *L. theobromae*. With the 86% disease severity, Kabarla was found the most susceptible cv. against *A. alternata* while Tüylü was the most susceptible cv. against *M. phaseolina* and *L. theobromae*. Only one variety, Osmanlı, showed low disease intensity against three of the pathogens.

INTRODUCTION

Strawberry (*Fragaria* × *ananassa*) is widely cultivated worldwide for its fruit which is consumed in large quantities, either fresh or in various prepared foods. With 486.705 tons of production, Turkey is the fourth largest producer in the world after China, USA, and Mexico (Faostat 2021). Strawberry production in Turkey is mainly focused in

Mediterranean region and it is followed by Aegean, Thrace and Central Anatolia regions respectively. A lot of fungal, bacterial, and viral diseases occur on strawberries and cause considerable damage not only on the field grown strawberries but also on nursery grown plants (Averre et al. 1992).

Fungal diseases cause economically important crop losses on strawberries grown in the field and under cover in many parts of the world as well as Turkey. The main fungal foliage diseases of strawberries are gray mold (*Botrytis cinerea*), powdery mildew (*Sphaerotheca macularis f.sp. fragariae*), leaf spots (*Mycosphaerella fragariae, Alternaria tenuissima, Alternaria alternata*), anthracnose (*Colletotrichum acutatum, C. dematium*), Cercospora leaf spot (*Cercospora fragariae Lobik*) and downy mildew (*Peronospora potentillae* de Bary). Among the pathogens causing root rots are *Phytophthora cactorum, Pythium* spp., *Rosellinia necatrix* Prill., *Rhizoctonia fragariae* Hussain & W.E. McKeen, *M. phaseolina* (Tassi) Goidanich, *Armillaria mellea* (Vahl:Fr.) P. Kumm. (Maas 1998).

The main crop loss attributed to fungal diseases is root and crown rots caused by *P. cactorum* which is reported in Norway (Eikomo et al. 2000), in Poland (Irzykowska et al. 2005), and in Spain (Porras et al. 2007). Along with *P. cactorum*, another *Phytophthora* sp., *Phytophthora fragaria* was also reported to cause root rot on strawberries, but this species were not found widespread as *P. cactorum* (Eikemo et al. 2003). Both of the species mentioned above are known to survive in soil for long periods because of their resting spores (oospores). Eikemo et al. (2000) pointed out the rapid spread of *P. cactorum* in and between countries by mainly infected seedlings and also mentioned that the disease also spread locally by contaminated irrigation and drainage water and soil cultivation machinery. *P. cactorum* also causes leathery fruit rot on strawberries (Irzykowska et al. 2005).

Maas (1998) and De los Santos et al. (2003) also pointed out the importance of soilborne fungal diseases of strawberries and listed *Verticillium dahliae, P. cactorum, P. fragariae, Pythium* spp., *Rhizoctonia solani*, and *Fusarium* spp. as the most important ones.

Some of the soil borne pathogens such as *Rhizoctonia* spp., *Pythium* spp., *Cylindrocarpon* spp., and *Fusarium* spp. inciting black root rot are also considered important diseases causing significant losses in the strawberry growing countries the former two being the most important (Manici et al. 2005, Martin 2000, Martin and Bull 2002).

Fang et al. (2008) found many pathogens affecting strawberry, including *Fusarium oxysporum, R. solani* (AG-A, AG-C, AG-I, AG-K), *Cylindrocarpon destructans, Phoma exigua, Gnomonia fructicola, P. cactorum, Pythium ultimum, Macrophomina phaseolina, F. oxysporum,* and *R. solani* being the most widespread. *Fusarium oxysporum* f. sp. *fragariae* Winks and Williams was reported to cause about 30% crop loss in South Korea (Nam et al. 2005). Other than these pathogens, *V. dahliae* was also reported to cause about 80% crop loss (Kurze et al. 2001). Due to wide host range and long survival, effective control of this disease is not practicable (Ellis 2008, Gordon et al. 2006).

Strawberry is grown in various regions of Turkey and early production especially comes from the warmer parts, from the low tunnels, mainly from the Mediterranean and Aegean regions. Occurrence of diseases and their control on the production regions were studied by various researchers and more of them were done in the Aegean region especially in Aydın province which is an important strawberry producing area of Turkey. With the study done by Benlioglu et al. (2004) in this region, many of soil-borne pathogens affecting strawberry, such as *R. solani, Fusarium* spp., *Macrophomina* spp., *Phoma* spp., *P. cactorum, Pythium* spp., *V. dahliae* were also found as important pathogens in the order of frequency respectively.

Occurrence of *Lasiodiplodia theobromae* was first reported in 2014 in this region and found highly aggressive (Yıldız et al. 2014). Later on in the same region, Dinler et al. (2015) investigated fungal diseases of strawberry seedlings (plantlets) and found *Fusarium* spp. on 291 seedlings, *Rhizoctonia* spp. on 53, *Cylindrocarpon* sp. on 13, *Macrophomina* sp. on 4 seedling samples out of 2248 strawberry seedlings of Camarosa, Sweetcharlie, Rubygem and Festival cvs. The first study on fungal diseases in this region, on the other hand, was carried out in 1978 as a MSc thesis (Kapkın 1978).

The first study to reveal root rot pathogens of strawberry in the Mediterranean region was done in 1986 (Yürüten et al. 1986). Following this study a PhD study was completed on the pathogens causing root rot in this region and *R. solani* was found as a primary pathogen (Pala 1987).

Phytopathological problems of strawberry was also investigated in Erzurum province of Turkey and gray mold (*B. cinerea*), leaf spot (*M. fragariae*), and soil borne diseases (*Rhizoctonia* spp., *Fusarium* spp., *Pythium* spp., and *Verticillium* sp.) were the main problems (Eken 2008).

There has been an increase on the acreage of strawberry production in Central Anatolia recently and fungal diseases have not been investigated in the region with the only exception of the study of Gürer and Coşkun (1993), carried out in Bartın and Zonguldak provinces.

With another study, carried out in Düzce province of Turkey, in 2012; *Alternaria* spp., *B. cinerea*, *Hainesia lythri*, *M. fragariae, Phoma* sp., *Phomopsis* sp., *R. solani* were isolated from the leaves and petioles; *B. cinerae* from the fruits and *Alternaria* spp., *F. oxysporum, M. phaseolina*, *Phytophthora*

spp. and *R. solani* were isolated from the roots. Pathogenicity of these fungi was proven on detached leaf assay (Sarıgül Ertek et al. 2018).

The aim of this work is to identify fungal diseases in Central Anatolia strawberry growing fields and to search for the reactions of some extensively grown varieties against the most important pathogens.

MATERIALS AND METHODS

Collection of disease samples

About 5% (51.5 ha) of the strawberry growing fields of Kayseri, Bartın, Konya, and Zonguldak were visited and 383 disease samples showing leaf spots, leaf scorch, powdery and downy mildews and wilt symptoms were collected and brought to the laboratory in a cool box. Total number of the samples collected from these provinces were 157, 80, 135, and 30 from Konya, Bartın, Kayseri, and Zonguldak, respectively.

Isolation of the fungi

Samples having root and crown rot first were washed under tap water thoroughly, blot dried and subsamples were taken from the adjacent parts of the intact and necrotized tissues and disinfected by immersing 0.1% NaOCl for 1 or 3 min depending of the tenderness of the tissues. After disinfection, samples were blot dried and small parts about 2-3 mm dissected and placed on the appropriate media. Leaf spots and scorches, fruit rots were treated the same way except duplicate subsamples of some leaf spots were incubated on a humid chamber in addition to plating isolation media. For isolation of *Phytophthora* spp. samples were directly plated on the semi selective medium (CMA-PARP) of Jeffers and Martin (1986). For the isolation of other pathogens Potato Dextrose Agar (PDA) and 2% Water Agar (WA) were used. The Petri plates containing PDA and WA were incubated at 24±1 °C for a week then subcultures were prepared by removing mycelial tips from the edge of the growing colonies under a stereomicroscope. The semi selective Phytophthora medium was incubated at 18-20 °C in darkness and the growing colonies were examined under a microscope for 2-3 days. Subcultures were prepared by removing mycelial tips from the right angle branching mycelia and plating them on Carrot Agar (CA) (40 g thinly grated carrot, 20 g agar and 1000 ml water) as described above. The isolates obtained were transferred to PDA or for Phytophthora spp. Corn Meal Agar (CMA) slants in tubes.

Pathogenicity of the Phytophthora spp. isolates obtained from the roots

Pathogenicity of the *Phytophthora* spp. isolates was tested by soil inoculation method. The isolates were grown on wheat seeds sterilized twice daily at 121 °C by inoculating with culture discs and incubated at 24 °C in darkness for two weeks. This inoculum was added to torf + perlite mixture at 5% (Latorre et. al. 2001). The inoculum was placed in 4 l pots and 5 healthy strawberry seedlings were planted in this inoculum. Five control seedlings were also planted in uninoculated soils. To maintain the sufficient humidity, the pots were regularly watered. The strawberry plants were uprooted after seven weeks and rated for the root rot index. Re-isolations were also done.

Pathogenicity of the Phytophthora spp. isolates obtained from the fruits

Healthy strawberry fruits were used for this test. The fruits were sterilized by immersing them in 10% NaOCl for 15 min and they were rinsed three times by sterile water. After that, the fruits were transferred to sterile Petri plates. Fruit inoculation was done in a sterile cabin by placing mycelial discs of 2 mm diameter cultures on slightly punctured fruit surfaces. Only plane agar discs were used as controls. Inoculated fruits, ten for each isolate were incubated at 24±1 °C and re-isolations were done from the fruits showing diseases symptoms.

Pathogenicity of the other fungi

Pathogenicity of the other soil-borne pathogens was performed by using Toothpicks method described by Benlioglu et al. (2014). For this aim, ten healthy looking runners (stolons) for each isolate about 8-8.5 cm long, not contacted to the soil were dipped into 70% alcohol for 5 min and rinsed by sterile water and each fungus was inoculated as described. Inoculated stolons were kept at 28±2 °C in a humid chamber in Petri dishes. Five to seven days after inoculation, lesion sizes were compared by the controls.

For the pathogenicity of leaf spot and blight pathogens, detached leaf inoculation method was used (Dolar et al. 1994, Sarıgül Ertek et al. 2018). Healthy looking trifoliate strawberry leaves were removed from the plants and disinfected in 1% NaOCl for three min, blot dried and placed into Petri dishes having 2 fold humid blotter papers. Fungal discs of 0.5 cm in diameter, taken from the peripheries of young cultures were placed on the trifoliate leaflets wounded by piercing from 3 to 4 points by a needle. Stipes of the leaves were wrapped by cotton wool and wetted by sterile water in order to keep the turgor of the leaves. Lesion formation and fungal growth was observed until the symptoms onset. Each pathogen was inoculated on ten leaves and disease severity was calculated by the following formula:

$$\text{Percent disease severity} = \frac{\Sigma \text{ (value of each scale x number of samples at each scale)}}{\text{the highest scale value x total no of the samples}} \times 100$$

Scale for root pathogens: 0, no lesion; 1, 1-25% of the stolon have lesion; 2, 26-50% of the stolon have lesions; 3, 51-75% have lesions, 4, 76-100% have lesions.

Scale for foliage pathogens: 0, no disease on the trifoliate leaf; 1, 1/5 of the trifoliate leaf have lesion; 2, 2/5 of the trifoliate leaf infected; 3, 3/5 of the trifoliate leaf infected; 4, 4/5 of the leaf infected; 5, whole leaf infected (Dolar et al. 1994).

Determination the reactions of the cultivars

Reactions of 12 widely grown strawberry cultivars; Festival, Sweetann, Kabarla, Alison, Osmanlı, Kara çilek, Tüylü, Sabrina, Rubigem, San Andreas, Albion, Monterey were tested against the most virulent isolates of three most widespread pathogens; *A. alternata*, *M. phaseoli* and *Lasiodiplodia theobromae*. Detached leaf assay and toothpick assay as described above were used for the reactions of the cultivars for *A. alternata* and *M. phaseoli* and *L. theobromae,* respectively.

Identification of the fungal pathogens of strawberry

The obtained fungi, first were identified at genus level by using published textbooks (Barnett and Hunter 1998, Ellis 1976, Gerlach and Nirenberg 1982, Samson et al. 1996, Tousson 1995). Identification at species level was done by comparing DNA sequences of ITS-4 and ITS-5 regions of the isolates with the sequences deposited in GenBank. For this study; the isolates were grown on PDA and a small amount of mycelia was macerated in liquid nitrogen and DNA isolation was performed by using DNeasy Blood & Tissue Kit (Qiagen). PCR reaction was performed as described by Cobos and Martin (2008) by using 50 µl PCR mixture of the following amounts; 5 µl DNA (about 10 ng), 5 µl 10x buffer (75 mM Tris HCl, pH 9.0, 50 mM KCl, 20 mM (NH4)2SO4) (Biotools), 2 µM each of primers (MWG-Biotech), 5 mM MgCl2, 2 mM dNTPs, 2 U Taq polimerase (Biotools), 5 µl bovine serum albumin (BSA) (10 mg/ml; Sigma Aldrich). The primers used were; ITS-4 (5'-TCC TCC GCT TAT TGA TAT GC-3') and ITS-5 (5'-GGA AGT AAA AGT CGT AAC AAG G-3'). The PCR mixture was pre-denatured at 96 °C for 5 min; followed by 35 cycles of denaturing at 94 °C for 30 s, annealing at 52 °C for 30 s and 72 °C for 90 s, followed by final hold at 72 °C for 7 min. PCR products were run 1.5% agarose gel in 1X TBE (40 mM Tris-borate, 1 mM EDTA, pH: 8.0). PCR products were sent to BM Laboratories (Ankara, Turkey) for sequencing facility.

RESULTS

Identification of the fungi

The following genera and species were identified from 402 samples collected from 47 fields of Kayseri, Bartın, Konya, and Zonguldak provinces about 515.5 ha; *A. alternata, Boeremia exigua, M. fragariae, C. acutatum, Colletotrichum gleosporoides, B. cinerea, L. theobromae, Diaporthe actinidae, Diaporthe eres, Didymella americana, Didymella pomorum, Diplodia seriata, F. oxysporum, M. phaseolina, Peyronellae prosopidis, P. cactorum, Phytophthora plurivora, Phytophthora kelmania.*

Occurrence of the diseases

Three pathogens; *A. alternata, M. phaseoli* and *L. theobromae* were the most widespread diseases in strawberry fields of four provinces of Central Anatolia and the former was obtained from 41.94%, and the latter two root pathogens were obtained 38.13% of the fields, evaluated the at two growth stages Table 1.

Table 1. Percentages of infested areas by three pathogens

Provinces	Pathogens		
	Alternaria alternata	*Macrophomina phaseolina*	*Lasiodiplodia theobromae*
Zonguldak, Çaycuma	25	25	25
Zonguldak, Ereğli	38.7	12.9	0.0
Bartın, Merkez	31.8	37.8	9.1
Kayseri, Tomarza	19.6	35.3	29.4
Kayseri, Merkez	33.3	19.6	29.4
Konya, Akşehir	19.3	0.0	0.0

Alternaria leaf spot (*A. alternata*) occurred in about half of the fields, producing intensive yellowing and reddening on leaves (Figure 1a). First symptoms leaf spots were irregular in shape, 3 to 6 mm in diameter, and blackish brown. Later on, yellowing occurred around the leaf spots (Figure 1b). *A. alternata* shows characteristic concentric growth in PDA media (Figure 1c). Conidial chain can be seen easily under 10x microscope magnificent (Figure. 1d).

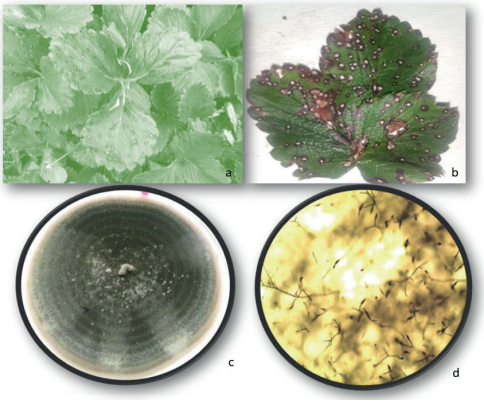

Figure 1. Various aspects of *Alternaria alternata* a) a general view from an infected field showing yellowing and reddening b) leaf spots with purple margins and whitish centres, c) consantric colonial growth d) conidia of *Alternaria alternata* under 10x microscope magnicifient

Two pathogens; *M. phaseoli* and *L. theobromae* were isolated the most frequently from the plants showing decline and

necrotic roots. The plants infected by *M. phaseoli* lost their vigour and dried (Figure 2a). This pathogen caused reddish brown root discoloration (Figure 2b) and produced grayish black growth with microsclerotia of average 105 × 74 µm (Figure 2c,d) on PDA medium.

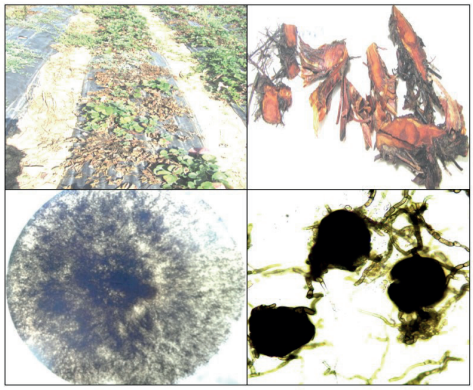

Figure 2. a) a field showing severe root rot caused by *Macrophomina phaseoli,* b) sections of infected roots having reddish discoloration, c) dark colour growth on PDA d) microsclerotia of *Macrophomina phaseoli*

L. theobromae was also isolated from the wilted and dead plants having necrosis on roots. This pathogen first produced a whitish growth on PDA, later on the growth turned to olive colour to dark brown (Figure 3a). The pathogen produced pycnidia having one celled, hyaline or two celled, dark coloured conidia of 25.42 ± 2.12 × 12.87 ±1.08 µm when incubated about 20-30 days (Figure 3b).

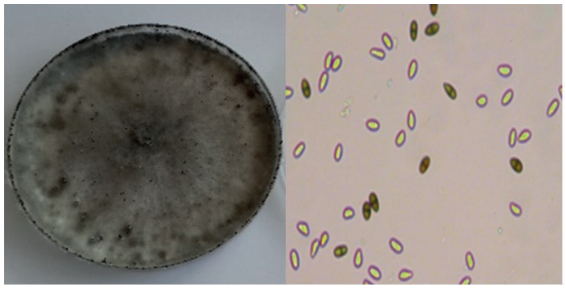

Figure 3. a) olive coloured, 5 days old growth of *Lasiodiplodia theobromae* on PDA, b) hyaline and dark coloured conidia

Reactions of common strawberries against three of the most widespread diseases

Disease intensities produced by the three widespread pathogens on eleven strawberry varieties are given in Table 2. Strawberry varieties showed different reactions against the three pathogens. The varieties Festival, Alison, Tüylü, Osmanlı, and Sabrina had the lowest percentages of diseases against *A. alternata*, while the varieties Karaçilek, Osmanlı, Rubigem, Sabrina, Monterey, and Albion against *M. phaseoli*; Karaçilek, Alison, Osmanlı, were against *L. theobromae.* Only one variety, Osmanlı, showed low disease intensity against three of the pathogen Table 2.

Table 2. Disease intensities produced by three most widespread strawberry pathogens on eleven strawberry varieties grown widely in Central Anatolia region

Variety/ pathogens	*Alternaria alternata*	*Macrophomina phaseolina*	*Lasiodiplodia theobromae*
Festival	31.33± 12.05de	48.75± 8.82 bcd B[2]	67.50± 6.23 cd
Kara çilek	84.67± 10.79ab	17.50± 6.23 ef A	25.00± 5.89 e
Alison	24.00± 8.32e	75.00± 5.33 f A	22.50± 9.82 e
Tüylü	41.33± 8.24de	100± 0 a A	100± 0 a
Osmanlı	26.67± 8.94e	16.25± 8.34 ef B	35± 5.20 e
Rubigem	51.33±12.57cde	28.75±7.22 def B	60± 1.247 cd
Sabrina	40.67± 13.38de	20.0± 3.33 ef B	70.0± 8.16 bcd
San Andreas	54.67± 12.87bcde	63.75± 6.83 b B	80± 8.16 abc
Monterey	76.67± 82.70abc	32.5± 1.16 de B	100± 0 a
Albion	50.67± 13.84cde	37.5± 6.71 cde B	90± 6.66 ab
Kabarla	96.67± 2.27cde	57.5± 6.77 bc A	56.25± 6.52 d
Sweetann	64.00± 4.23 bcd	61.25± 3.46 b A	59.72± 3.47 cd

Molecular identification of the strawberry pathogens

The internal transcribed spacer (ITS) regions of ITS-4 and ITS-5, of the whole pathogenic isolates were amplified and the obtained gene sequences were compared with the ones deposited in GenBank.

Identification of the pathogenic fungi obtained from strawberry plants were done by comparing DNA sequences of ITS-4 and ITS-5 gene regions of all the fungi with the sequences deposited in GenBank. Many pathogens were identified by species level (Table 3).

DISCUSSION AND CONCLUSION

In order to determine diseases on strawberry surveys were done in fields of Kayseri, Bartın, Konya, and Zonguldak provinces in Central Anatolia in 2013-2016. The most widespread disease was Alternaria leaf spot, determined at 41.94% of the fields, and the second widespread disease was root rots caused by *M. phaseoli* and *L. theobromae* which occurred in 38.13% of the fields.

Alternaria leaf spot is frequently occurring disease of strawberries worldwide (Cho et al. 1980, Fu et al. 2019, Mehmood et al. 2018, Wassenear and Scheer 1989) and it is also known in Turkey (Sarıgül Ertek et al. 2018).

Wilting and root rot symptoms, especially reddish necrotic areas, were also observed in many fields and various pathogenic fungi were isolated from the necrotic root tissues, *M. phaseoli* and *L. theobromae* being the most frequent. Along with these two pathogens; *Fusarium* spp,

Table 3. Molecular identification of pathogenic fungi obtained from strawberry production areas of Central Anatolia region

Species identified	Percent similarity to GenBank records	Accession number of most similar GenBank records	GenBank accession number of isolates from this study
Alternaria alternata	100	MT126620.1 MH384939.1 MK578900.1	MK571621
Boeremia exigua	100	MN077426.1 MK907733.1 MK541618.1	MK571625
Botrytis cinerea	100	MT573470.1 MN589849.1 MN589847.1	MK554490
Colletotrichum acutatum	99.82	MH532959.1	MK571604
Colletotrichum gleosporoides	100	MK474924.1	MK571633.1
Diaporthe actinidae	100	MK541622.1 KT163360.1	MK541622
Diaporthe eres	100	MT561403.1 MK431122.1 MK571633.1	MK571606
Didymella americana	99.80	KY070283.1 KY070282.1 KY070281.1	MK571615
Didymella pomorum	100	MH861278.1 AY904062.1	MK541633
Diplodia seriata	100	KJ921854.1 KJ921853.1 KJ921852.1	MK571617
Fusarium oxysporum	100	MT530269.1 MT530243.1 MT530242.1	MK554485
Lasiodiplodia theobromae	100	MT123030.1 MN909160.1 MH793584.1	MK571624
Macrophomina phaseolina	100	MT735239.1 MT183520.1 MT127393.1	MK571619
Neofusicoccum parvum	100 99 99	MT012295.1 MH057199.1 MH623075.1	MK554486
Peyronellae prosopidis	100	MH866094.1 KF777181.1	MK571630
Phytophthora cactorum	99	EU045748.1 MT558729.1 MT280033.1	KJ603452
Phytophthora plurivora	100	KT306852.1 KF682435.1 JQ730714.1	KJ603449 KJ603450
Phytophthora kelmania	100	KU053244.1 KU053242.1 KU053234.1	KJ603451

Rhizoctonia spp., *Phytophthora* spp. were also obtained from root rots. *M. phaseolina* was also reported from the main strawberry production areas of the world (Freeman and Zveibil 2009, Mertely et al. 2005). Martin (2000) and Manici et al. (2005) on the other hand, reported that soil borne *Rhizoctonia* spp., *Pythium* spp., *Cylindrocarpon* sp. and *Fusarium* spp. are also important soil borne pathogens affecting strawberry production in the world. Occurrence of Fusarium and Rhizoctonia root rots was also reported by other researchers (Freeman and Zveibil 2009, Mertely et al. 2005). Root rots on strawberry have also been determined in different strawberry fields. Yürüten et al. (1986) found out that root rots caused by soil borne pathogens caused serious problems and produced about 40-60% yield loss in the Mediterranean region of Turkey. The same situation is seen in our study area and this situation is attributed to use of infected strawberry plantlets (seedlings) extensively.

Another root rot pathogen of strawberry, *L. theobromae*, has only been encountered at strawberry growing areas in Aydın province in western Turkey so far (Benlioglu et al. 2014) and in our study area, which was found so aggressive on strawberry killing the whole plants in seven days. Our findings suggest that more time should be spent on the disease.

To find a solution for the difficulty of finding disease free seedlings, different plant structures were used for testing pathogenicity. Detached leaf assay as used for other host plants and for strawberry by various authors (Argun et al. 2008, Sarıgül Ertek et al. 2018, Sezer and Dolar 2012) was also found suitable for strawberry since the detached leaves remained intact about 3-4 weeks. Toothpicks method, tested by Benlioglu et al., (2014) for root rot pathogens on the stolons was also applied successfully in our study. In our study, for the pathogenicity of *Phytophthora* spp, especially for *P. cactorum*, strawberry fruits and soil infestation methods were also used successfully.

Testing the susceptibility of twelve strawberry cultivars against three of the most common fungal diseases formed another aspect of our study, which will help growers to choose suitable varieties for their regions. Most of the varieties, except Festival, Alison and Osmanlı, had more than 40% infection when inoculated by *A. alternata*, in other words they were found susceptible (having infections between 40-60%) and highly susceptible (having disease ratios over 60%). The cultivars "Karaçilek" and "Osmanlı" had lower disease intensities against the two of the root rot pathogens while some of the remaining varieties like "Alison", "Rubigem", "Sabrina", and "Monterey" showed resistance to only one of the root rot pathogens. Some varieties; such as "Festival", "Tüylü", "San Andreas", "Kabarla" and "Sweetann" were susceptible against both of the root rot pathogens (Table 2).

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ÖZET

2013-2016 yılları arasında Bartın, Kayseri, Konya ve Zonguldak illeri çilek üretim alanlarında sorun olan çilek fungal hastalıklarının tespiti ve yaygın olan fungal patojene karşı mücadele olanaklarının araştırılması hedeflenmiştir. Bu amaçlar doğrultusunda Bartın, Kayseri, Konya ve Zonguldak illerine ait 515.5 da çilek alanından alınan hastalıklı bitki örnekleri toplanarak laboratuvara getirilmiş ve bu örnekler uygun besi ortamlarına ekilerek etmenler izole edilmiştir. Çeşitli arazilerden toplanan 383 adet yaprak ve kök örneklerinden fungal etmenler izole edilmiştir. 17 fungal patojen: *Alternaria alternata* (Fr.) Keissler, Boeremia exigua (Desmazières) Aveskamp, Gruyter & Verkley, *Mycosphaerella fragariae* (Tul.) Lindau., *Colletotrichum acutatum* J.H. Simmonds, *Colletotrichum gleosporoides* (Penz.) Penz., *Botrytis cinerea* (de Bary) Whetzel, *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., *Diaporthe actinidae* N.F. Sommer & Beraha., *Diaporthe eres* Nitschke, *Didymella americana* Saccardo ex Saccardo, *Didymella pomorum* (Thümen) Aveskamp, Gruyter & Verkley, *Diplodia seriata* de Notaris, *Macrophomina phaseolina* (Tassi) Goidanich, *Peyronellae prosopidis, Phytophthora cactorum* (Lebert & Cohn) J.Schröter, *Phytophthora plurivora* Jung & Burgess., *Phytophthora* kelmania olarak tespit edilmiştir. Yaprak ve köklerden izole edilen bu etmenler uygun metotlarla çilek bitkilerine inokule edilerek patojenisiteleri gerçekleştirilmiştir. Patojenisitesi yapılan etmenlerden en yaygın olarak tespit edilen etmen yaprak lekesi hastalığı (*A. alternata*), %41.94 oranında; çeşitli fungusların sebep olduğu kök hastalıkları ise %38.13 oranında tespit edilmiştir. En yaygın bulunan 3 patojenle (*A. alternata, M. phaseolina* ve *L. theobromae*) çeşit-reaksiyon çalışmaları 12 çilek çeşidi (Festival, Kabarla, Sweetann, Alison, Osmanlı, Kara çilek, Tüylü, Sabrina, Rubigem, San Andreas, Albion, Festival,

Monterey) üzerinde gerçekleştirilmiştir. Buna göre *A. alternata* yaprak lekesine en hassas çeşit %86 hastalık şiddetiyle Kabarla, *M. phaseolina* ve *L. theobromae*'ye karşı hassas olan çeşit ise Tüylü çilek çeşididir. Osmanlı çilek çeşidi her 3 hastalık etmenine karşı diğer çeşitlere göre daha dayanıklı bulunmuştur.

Anahtar kelimeler: çilek fungal hastalıkları, *Alternaria alternata*, *Macrophomina phaseolina, Lasiodiplodia theobromae*

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