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Determination of Seedling Root Rot Fungal Pathogens and Disease Prevalence Rates in Cotton (*Gossypium hirsutum* L.) Fields of the Tigris Basin*

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Abstract: This study was conducted to identify the fungal pathogens responsible for seedling root rot in cotton (*Gossypium hirsutum* L.) cultivated in the Tigris Basin, to determine their prevalence, to characterize these pathogens at morphological and molecular levels, and to assess their pathogenicity in order to contribute to the development of effective disease management strategies. In this study, during the growing season 2021-2022 surveys were conducted on May and June along the Tigris Basin, where cotton is intensively cultivated. Diseased cotton samples were collected from a total 79 separate cotton fields. Fungal agents causing seedling root rot, including *Fusarium* species (*Fusarium* spp., *Fusarium oxysporum*, *F. solani*, *F. moniliforme* (*verticillioides*), *F. chlamydosporum*, *F. proliferatum*, *F. acuminatum*), *R. solani, Macrophomina phaseolina*, *Phytium* spp., *Alternaria* spp., *Ulocladium* sp., *Cladosporium* spp., *Chaetomium* spp., *Thielaviopsis basicola, Phoma* spp., *Phymatotrichopsis omnivora, Sclerotinia* sp., and *Sordaria tomentoalba* were isolated. As a result of pathogenicity tests among the agents causing seedling root rot, *Thielaviopsis basicola, Alternaria* spp., *Fusarium moniliforme, Fusarium oxysporum* and *Fusarium solani* were determined to be highly pathogenic. The disease rate of fields in the study was between %2-16. The prevalence of the disease in the studied fields was %100. In the region, it is essential to implement sustainable approaches for managing fungal diseases, including raising awareness among growers, using resistant cultivars, reducing pesticide use, ensuring proper field drainage, practicing crop rotation, and adopting biological control methods.

Keywords: Cotton, disease incidence, fungal pathogen, pathogenicity, root rot

1. Introduction

The cotton plant (*Gossypium hirsutum* L.), an important species of the *Gossypium* genus from the Malvaceae family, is one of the most significant fiber plants widely cultivated in temperate and tropical regions. Cotton is one of the oldest industrial plants and has various uses with processed cotton fibers posing great economic importance, providing added value and employment to producing countries (Rehman et al., 2019; Majumdar et al., 2019). USA, India, Pakistan, China, and Brazil account for approximately 70% of the world's cotton production as of 2020-2021 (Tokel, 2021). Cotton, which is cultivated over vast areas in Türkiye, also holds a significant share of export income. According to 2020-2021 Turkish Statistical Institute data, 86% of the cotton cultivation areas in Türkiye are located in the provinces of Şanlıurfa, Diyarbakır, Adana, Hatay, Aydın, and İzmir (Anonymous, 2021). Cotton cultivation has been practiced in the Tigris Basin of Diyarbakır province since the 1900s. As cotton is sown annually in this region, the crop is affected by diseases, pests, and weeds. These factors can lead to loss of plant resistance against diseases and even complete drying resulting in total crop losses.

Cotton is susceptible to various fungal diseases. Among these, fungal agents causing seedling root rot are particularly significant. Globally, four major pathogens cause seedling root rot. These are *Rhizoctonia solani* Kühn [telemorph

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Thanatephorus cucumeris (A.B. Frank) Donk], *Fusarium* spp., *Pythium* spp., *Verticillium* spp. and *Thielaviopsis basicola* (Agrios, 1998; Devay, 2001; Rothrock and Buchanan, 2017). These pathogens are soil-borne, and the initial symptoms of the disease appear on young roots. The bark of the root first changes color and softens, and then it rots. The roots and root collar of infected seedlings turn brown, and thin out, and the seedlings wilt and then begin to dry out. The disease can cause patches of empty spaces in the field. In a study conducted by Mamirov and Boyraz (2021) in Söke Plain of Aydın province, to determine the prevalence of seedling root rot pathogens and to investigate some biological and chemical treatments against the disease, they isolated fungi such as *Rhizoctonia solani*, *Fusarium* spp., *Pythium* spp., and *Macrophomina phaseolina* from collected plant samples. In their study, Dinler and Benlioğlu (2013) investigated the vegetative compatibility groups of *Verticillium dahliae* fungi obtained from cotton-growing areas in Aydın province. They isolated 47 *Verticillium* spp. fungi from 48 fields across 12 districts in Aydın. Refai et al. (2022) conducted a study to determine the pathogenicity of *R. solani*, *F. moniliforme*, and *M. phaseolina* isolates obtained from cotton roots. The researchers observed that these isolates were pathogenic, adversely affected seed germination and root length, and caused a significant mortality rate in cotton seedlings.

This study, aimed to identify the pathogens causing seedling root rot and determine their prevalence in the Tigris Basin, where cotton is extensively cultivated. The study aims to provide a detailed examination of the disease agents encountered in cotton cultivation. Due to the sampling and pathogen identification methods used in the study, novel and significant findings are added to the literature. Furthermore, identifying species with high pathogenicity allows for the development of specific control strategies against these pathogens, offering practical solutions for cotton producers. In this regard, the study contributes both to the accumulation of scientific knowledge and provides concrete recommendations for agricultural practices.

2. Materials and Methods

2.1. Material

The study material consists of diseased plant samples collected from cotton fields along the Tigris Basin in the Bismil-Çınar-Sur districts of Diyarbakır. Fungi is isolated from the samples. The study also involved various culture media [Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Water Agar (WA), Alcohol Water Agar (AWA)], laboratory materials, chemicals used for seed viability tests, and chemical materials necessary for molecular studies.

2.2. Disease survey in the field

The survey was conducted using a systematic sampling method during the seedling stage of cotton between May and June months of 2021-2022. Fields were randomly inspected for diseases. In cases where diseases were observed, sampling points were selected based on the size of the fields (Bora and Karaca, 1970).

Sampling was performed as follows: For fields up to 10 decares, samples were collected from 5 points, totaling 100 plants. For fields between 10 and 50 decares, samples were collected from 10 points, totaling 200 plants. For fields larger than 50 decares, samples were collected from 15 points, totaling 300 plants.

Plants showing signs of root rot were counted, and the disease incidence in each field was determined based on the prevalence of infection and field contamination. This approach helped assess the extent of disease spread in the region.

2.3. Isolation of fungal pathogens

Diseased plant samples collected from each field were placed in paper bags- and stored- at +4 °C in the labratory. Firstly the diseased cotton samples were washed with tap water and 0.5-1 cm long tissue samples were taken from the lesions on the roots and root collars. These pieces were then surface-disinfected by soaking in 1% NaOCl (Sodium hypochlorite) solution for 90 seconds. The samples were subsequently rinsed twice in sterile water and dried with drying papers. After the drying process, the tissue pieces were inoculated onto pre-prepared culture media: PDA with 150 mg L^{-1} Streptomycin, MEA, WA, and AWA. The cultures were then incubated at approximately 24 °C. After 5 days of incubation, the fungal colonies that developed were examined under a microscope and classified. Pieces were taken from the hyphal tips at the colony edges and transferred to PDA and 1/10-dilution PDA media to obtain pure cultures. Agar disks taken from these pure cultures were stored in slant agar tubes at +4 °C for pathogenicity tests.

2.4. Morphological characterization

The isolated fungi were grouped for identification based on characteristics such as color, growth rate, and colony appearance on the PDA medium. Detailed features, such as spores and hyphae, were examined under a microscope,

and identifications were made at the genus level. Species-level identifications were based on sources including Booth (1977), Gerlach and Nirenberg (1982), Nelson et al. (1983), Burgess et al. (1988), Allen (1990), Keith (1996), Barnett and Hunter (1998), Agrios (2005), Leslie and Summerell (2006), Domsch et al. (2007), Watanabe (2010) and Sneh et al. (2013). Morphological identifications of the species involved in the study were carried out using these identification keys. Among the identified isolates, fungi selected for pathogenicity test were preserved in slant agar tubes at +4 °C.

2.5. Molecular characterization

DNA isolation for 31 isolates grown on PDA medium at 24 °C for 5-7 days was performed according to the Cetyltrimethylammonium Bromide (CTAB)-Genomic DNA Isolation protocol developed by Doyle and Doyle (1987) and modified by Karaca et al. (2005). Polymerase Chain Reaction (PCR) was conducted using primers specific to the Internal Transcribed Spacer (ITS) gene region. The PCR conditions are detailed (Table 1 and Table 2). The ITS primers used for PCR are presented (Table 3).

Table 1. Quantities of components used for PCR

Component	Quantity (1x)
Templete	3 µl
Primer F	1 µl
Primer R	1 µl
dNTP	1 µl
Taq	0.3 µl
Taq Buffer	3 µl
$MgCl_2$	2.5 µl
BSA	1 µl
dH_2O	12.2 µl
Total	25 µl

Table 2. PCR conditions

Step	Temperature (ºC)	Time (min.)	Number of cycles (cycle)
Initial	95	5	1
Denaturation	94	1	
Denaturation	48	1	35
Annealing	72	1	
Extension			
Final extension	72	5	
Hold	4	∞	

Table 3. The ITS primers used in PCR

Primer F:
5` GAAGTAAAAGTCGTAACAAGG 3`
Primer R:
5` TCCTCCGCTTATGATATGC 3`

The sequences of the PCR products sent for sequencing were compared using the Basic Local Alignment Search Tool (BLAST) function in the National Center for Biotechnology Information (NCBI) database. The sequence data obtained from the ITS gene region for all isolates were analyzed and converted into Mega format using the Mega 11 program. A phylogenetic tree was then constructed using the Neighbor-Joining method.

The PCR products sent to Medsantek were read in one direction using the forward primer.

2.6. Pathogenicity

For the pathogenicity test, a soil mixture was prepared by combining 1/3 peat, 1/3 garden soil, and 1/3 perlite. This mixture was placed in autoclavable bags and sterilized at 121 °C for 20 minutes. The fungal inoculums are prepared by 150 g of wheat bran mixed with 15 ml of water, placed in glass jars, and sterilized in an autoclave at 121 °C for 20 minutes (Turhan, 1992). Fungal colonies that completed their development on petri dishes were divided into 4 equal parts and inoculated into the wheat bran medium in jars. The jars were then incubated at 25 °C for 15 days. The obtained inoculums were mixed at a ratio of 1/20 with the potting soil, while control pots received clean wheat bran without inoculum (Turhan, 1992).

For the study, seed cotton was surface-disinfected by soaking in NaOCl for 5 minutes and then dried between commercial paper towels. Five seeds were sown in each pot containing 2 liters of soil. The experiment was set up in vivo with 3 replicates, and irrigation and maintenance tasks were performed periodically.

2.7. Evaluation

Observations were made throughout the experiment. Seedlings that failed to emerge or that emerged and then dried out were noted for evaluation. 40 days after planting, as the plants were in the seedling stage, an assessment was conducted. Following the pathogenicity study, all plants in the experiment were removed from the pots and inspected.

The degree of damage to the root and root collar was assessed using a 0-4 scale for each replicate, and the disease severity was determined according to the Townsend-Heuberger method. The average severity of the disease and the resulting groupings were obtained (Townsend and Heuberger, 1943).

The root and root collar rot scale is as follows: 0= No symptoms, 1= Up to 1/3 of the root-root collar affected, 2= 1/3 to 2/3 of the root-root collar

affected, 3= More than 2/3 of the root-root collar affected, 4= Seedling completely dried out.

The calculation of disease percentage (DP) is shown in Equation 1 below.

$$DP = \left[Total\ number\ of\ plants\ \mathrm{x}\frac{(n\ x\ V)}{(Z\ x\ N)}\right] \mathrm{x}\ 100 \quad (1)$$

Here, n represents the number of plants in different damage groups, V represents the levels of damage severity groups, N is the total number of plants in the control, and Z is the highest scale value.

Data for the isolates obtained from the study were analyzed using the JMP statistical program according to a randomized complete block design. The resulting groupings were classified based on the Least Significant Difference (LSD) test at a significance level of 95% (0.05).

3. Results

3.1. Disease incidence and prevalence in survey areas

The isolated fungi, along with the disease and prevalence rates, are presented in Table 4. According to Table 4, numerous fungi were isolated from plants showing seedling root rot symptoms in all surveyed areas. Although the disease incidence varied across districts and villages, it ranged from 2% to 16%. Plants showing symptoms of the disease were detected in all fields and throughout the region, and the incidence of the disease was determined to be 100% (Table 4).

3.2. Morphological characterization

The proportions of the isolated fungi are illustrated in Figure 1. Based on the isolation results, a total of 274 fungal isolates were obtained from cotton plants showing disease symptoms collected from different fields. These isolates were first grouped among themselves. It was found that *Fusarium* species were the most prevalent among the isolated fungi. The most frequently isolated species was *F. oxysporum*, with 59 isolates. The other isolates included 50 of *F. solani*, 31 of *A. alternata*, 20 of *Pythium* spp., 17 of *Fusarium* spp., 13 of *F. verticillioides* (syn. *F. moniliforme*), 12 each of *R. solani* and *F. proliferatum*; 10 of *M. phaseolina*, 8 of *Ulocladium* spp. Additionally, 5 isolates were *Phoma* spp., 4 of *F.chlamydosporum*, 3 each of *Penicillium* spp., *T. basicola*, and *Alternaria* spp., 2 each of *F. equiseti*, *F. culmorum*, *Cladosporium* spp., *V. dahliae* and *Gliocladium* spp., and 1 each of *F. avenaceum*, *F. acuminatum*, *F. graminearum*, *G. roseum*, *Trichoderma* spp., *Colletotrichum* spp., *Chaetomium* spp., *Stemphylium* spp., *Mucor* spp., *Curvularia* spp., *P. omnivora, S. tomentoalba*, *Bipolaris* spp. and *Sclerotinia* spp. (Figure 1).

The macroscopic and microscopic images of some species most frequently isolated from diseased cotton plants collected from surveyed cotton fields, and which caused the highest disease severity in this study, are shown in Figures 2-6.

Figure 2 shows the appearance of *Fusarium oxysporum* on the growth medium and under the microscope. This species was the most frequently isolated in this study, with rapid growth on PDA medium, completely covering the Petri dish after approximately one week of incubation (Figure 2a). The macroconidia of *F. oxysporum* are located on branched conidiophores (Figure 2b), are thin-walled, and typically have 3-5 septa. The microconidia are generally abundant, cylindrical, oval-ellipsoid, curved, or straight (Figure 2c).

Figure 3 shows the appearance of *Fusarium solani* on the growth medium and under the microscope. It was the second most frequently obtained species from the surveyed areas. On different growth media, it initially forms cottony and white colonies, which later develop into slightly purplish-pinkish colonies (Figure 3a). Long monophialides develop on the conidiophores of *F. solani*, producing monoconidia (Figure 3b). The microconidia of *F. solani* are slightly wider, more oval, and thicker-walled compared to those of *F. oxysporum* (Figure 3c).

Figure 4 shows the appearance of *Thielaviopsis basicola* on the PDA growth medium, which was observed to be dark brown to black in color (Figure 4a). The pathogen produces two types of spores: endoconidia and chlamydospores. Endoconidia are cylindrical, with rounded ends, colorless, and lack septa. The chlamydospores of the pathogen are thick-walled, dark-colored, disk-shaped, and form chains (Figure 4b), with each chain containing between 5 and 8 chlamydospores (Figure 4c).

Figure 5 shows the appearance of *Rhizoctonia solani* on the growth medium. Initially, the developing colonies are colorless, but they later exhibit a camel-hair brown appearance (Figure 5a). The hyphae of the fungus are septate; initially colorless (Figure 5b) but darken with age. When the hyphae branch, they form a 90º angle (Figure 5c).

Figure 6 shows the appearance of *Macrophomina phaseolina* after approximately one week of incubation. The fungus was observed to completely cover the Petri dish, initially appearing dark gray on the growth medium and

Table 4. Diyarbakır province tigris basin, regions surveyed in 2021-2022, field numbers, cultivation areas, disease rate, isolated fungi and prevalence rates in the region (%)

Field no	Province	District	Village	Area (da)	Disease rate (%)	Isolated fungi	Prevalence rate (%)
1	Diyarbakır	Bismil	Üçtepe	90	16	*Rhizoctonia solani* *Alternaria alternata* *Fusarium* spp. *Fusarium solani* *Fusarium oxysporum* *Pythium* spp.	100
2	Diyarbakır	Bismil	Üçtepe	60	14	*F. solani* *Stemphylium* spp. *Pythium* spp. *F. oxysporum* *Ulocladium* spp.	
3	Diyarbakır	Bismil	Üçtepe	110	5	*A. alternata* *F. oxysporum*	
4	Diyarbakır	Bismil	Üçtepe	150	8	*F. solani* *F. oxysporum* *F. moniliforme* *M. phaseolina* *Ulocladium* spp.	
5	Diyarbakır	Bismil	Üçtepe	100	9	*F. solani* *F. oxysporum* *A. alternata*	
6	Diyarbakır	Bismil	Üçtepe	120	4	*F. oxysporum* *A. alternata* *F. solani* *M. phaseolina* *Trichoderma* spp.	
7	Diyarbakır	Bismil	Üçtepe	70	12	*F. oxysporum* *Ulocladium* spp. *A. alternata* *F. solani* *Pythium* spp.	
8	Diyarbakır	Bismil	Üçtepe	80	12	*R. solani* *F. solani* *F. oxysporum* *Fusarium* spp.	
9	Diyarbakır	Bismil	Üçtepe	75	10	*A. alternata* *F. oxysporum* *Pythium* spp. *F. solani*	
10	Diyarbakır	Bismil	Üçtepe	180	9	*M. phaseolina* *A. alternata* *F. solani* *F. oxysporum* *Pythium* spp.	
11	Diyarbakır	Bismil	Üçtepe	150	8	*F. solani* *Pythium spp.* *F. oxysporum* *M. phaseolina*	
12	Diyarbakır	Bismil	Üçtepe	120	9	*T. basicola* *F. oxysporum* *F. proliferatum* *A. alternata* *F. solani* *V. dahliae*	

Table 4. (Continued)

Field no	Province	District	Village	Area (da)	Disease rate (%)	Isolated fungi	Prevalence rate (%)
13	Diyarbakır	Bismil	Üçtepe	150	8	*F. oxysporum* *F. solani* *Colletotrichum* spp.	100
14	Diyarbakır	Bismil	Üçtepe	100	10	*Pythium* spp. *Fusarium* spp.	
15	Diyarbakır	Çınar	Karalar	210	4	*F. oxysporum* *F. solani* *F. proliferatum*	100
16	Diyarbakır	Çınar	Karalar	250	7	*F. solani* *F. oxysporum*	
17	Diyarbakır	Çınar	Karalar	200	8	*F. oxysporum* *R. solani* *Fusarium* spp.	
18	Diyarbakır	Çınar	Karalar	150	6	*F. oxysporum* *F. solani* *R. solani*	
19	Diyarbakır	Çınar	Karalar	150	6	*F. oxysporum*	
20	Diyarbakır	Çınar	Belenli	250	4	*F. oxysporum* *F. solani* *A. alternata*	100
21	Diyarbakır	Çınar	Belenli	200	12	*Pythium* spp. *Ulocladium* spp. *Chaetomium* spp. *F. solani* *F. oxysporum*	
22	Diyarbakır	Çınar	Belenli	170	8	*M. phaseolina* *F. proliferatum* *F. oxysporum* *Phoma* spp. *A. alternata*	
23	Diyarbakır	Çınar	Belenli	200	5	*Pythium* spp. *F. oxysporum* *Penicillium* spp. *Fusarium* spp. *R. solani*	
24	Diyarbakır	Çınar	Belenli	200	9	*Phoma* spp. *F. oxysporum* *F. solani* *F. acuminatum*	
25	Diyarbakır	Çınar	Belenli	120	8	*F. oxysporum* *F. solani* *A. alternata* *Phoma* spp.	
26	Diyarbakır	Çınar	Belenli	270	5	*F. oxysporum* *A. alternata*	
27	Diyarbakır	Çınar	Kutluk	250	9	*M. phaseolina* *R. solani* *Pythium* spp. *Fusarium* spp. *F. avenaceum*	100
28	Diyarbakır	Çınar	Kutluk	110	7	*F. solani* *F. oxysporum*	
29	Diyarbakır	Çınar	Kutluk	150	10	*A. alternata* *Fusarium* spp. *Pythium* spp. *R. solani*	
30	Diyarbakır	Çınar	Has Köyü	200	6	*A. alternata* *F. proliferatum* *Pythium* spp.	100

Table 4. (Continued)

Field no	Province	District	Village	Area (da)	Disease rate (%)	Isolated fungi	Prevalence rate (%)
31	Diyarbakır	Bismil	Korukçu	230	8	*F. oxysporum* *Phoma* spp. *A. alternata* *F. solani*	100
32	Diyarbakır	Çınar	Has Köyü	50	7	*F. oxysporum* *F. solani* *F. chlamydosporum*	100
33	Diyarbakır	Çınar	Has Köyü	150	8	*A. alternata* *R. solani* *F. oxysporum*	
34	Diyarbakır	Çınar	Aktepe	160	6	*F. oxysporum* *Penicillium* spp. *F. solani* *F. acuminatum* *V. dahliae*	100
35	Diyarbakır	Çınar	Aktepe	150	9	*F. oxysporum* *F. proliferatum* *A. alternata* *Sclerotina* spp.	
36	Diyarbakır	Çınar	Aktepe	150	7	*F. oxysporum* *A. alternata* *Gliocladium* spp.	
37	Diyarbakır	Çınar	Öncüllü	200	5	*F. oxysporum* *F. solani*	100
38	Diyarbakır	Çınar	Aktepe	250	4	*F. oxysporum* *F. proliferatum* *A. alternata*	100
39	Diyarbakır	Çınar	Aktepe	180	8	*F. oxysporum* *F. solani*	
40	Diyarbakır	Çınar	Öncüllü	200	15	*F. moniliforme* *F. proliferatum* *Ulocladium* spp. *Fusarium* spp.	100
41	Diyarbakır	Çınar	Aktepe	150	7	*A. alternata* *Pythium* spp. *F. equiseti*	100
42	Diyarbakır	Çınar	Aktepe	150	9	*Pythium* spp. *Fusarium* spp. *T. basicola*	
43	Diyarbakır	Bismil	Kazancı	250	6	*Ulocladium* spp. *A. alternata* *Fusarium* spp.	100
44	Diyarbakır	Bismil	Kazancı	200	8	*Pythium* spp. *F. solani* *Penicillium* spp. *F. oxysporum* *A. alternata*	
45	Diyarbakır	Bismil	Kazancı	150	11	*Pythium* spp. *A. alternata* *F. solani* *F. moniliforme* *F. oxysporum* *Fusarium* spp.	
46	Diyarbakır	Bismil	Kazancı	150	9	*F. oxysporum* *F. solani* *A. alternata* *F. culmorum*	
47	Diyarbakır	Bismil	Türkmen Hacı	200	4	*F. solani* *Pythium* spp. *F. oxysporum*	100

Table 4. (Continued)

Field no	Province	District	Village	Area (da)	Disease rate (%)	Isolated fungi	Prevalence rate (%)
48	Diyarbakır	Bismil	Türkmen Hacı	300	5	*F. oxysporum* *F. culmorum* *F. solani* *Mucor* spp. *A. alternata*	100
49	Diyarbakır	Bismil	Türkmen Hacı	250	7	*F. oxysporum* *F. solani* *A. alternata*	
50	Diyarbakır	Bismil	Türkmen Hacı	150	6	*F. oxysporum* *A. alternata* *Fusarium* spp.	
51	Diyarbakır	Bismil	Göksu	150	9	*F. oxysporum* *A. alternata* *F. solani*	100
52	Diyarbakır	Bismil	Köseli	200	7	*F. oxysporum* *F. solani* *A. alternata*	100
53	Diyarbakır	Bismil	Of Köyü	100	4	*A. alternata* *F. solani* *F. oxysporum*	100
54	Diyarbakır	Bismil	Of Köyü	150	11	*M. phaseolina* *F. oxysporum* *F. solani* *A. alternata*	
55	Diyarbakır	Bismil	Of Köyü	140	6	*F. oxysporum* *F. solani*	
56	Diyarbakır	Bismil	Çöltepe	100	5	*Ulocladium* spp. *F. oxysporum* *F. solani* *F. acuminatum*	100
57	Diyarbakır	Bismil	Gültepe	200	5	*F. chlamydosporum* *F. oxysporum* *F. proliferatum* *Sordaria tomentoalba*	100
58	Diyarbakır	Çınar	Yuvacık	150	2	*F. equiseti* *F. solani* *Bipolaris* spp.	100
59	Diyarbakır	Çınar	Şükürlü	100	4	*Pythium* spp. *F. oxysporum* *F. solani*	100
60	Diyarbakır	Çınar	Şükürlü	130	6	*Curvularia* spp. *F. oxysporum* *R. solani* *F. chlamydosporum*	
61	Diyarbakır	Çınar	Şükürlü	100	3	*F. chlamydosporum* *Phymatotrichopsis omnivora* *Gliocladium* spp. *F. oxysporum*	
62	Diyarbakır	Çınar	Şükürlü	200	2	*R. solani* *F. solani* *M. phaseolina*	
63	Diyarbakır	Çınar	Şükürlü	150	2	*R. solani* *M. phaseolina* *Pythium* spp. *Fusarium* spp. *Alternaria* spp.	
64	Diyarbakır	Çınar	Başaklı	100	3	*F. oxysporum* *F. solani*	100

Table 4. (Continued)

Field no	Province	District	Village	Area (da)	Disease rate (%)	Isolated fungi	Prevalence rate (%)
65	Diyarbakır	Bismil	Darlı	180	5	*R. solani* *F. oxysporum*	100
66	Diyarbakır	Bismil	Ambar	150	2	*F. moniliforme* *F. oxysporum* *T. basicola*	100
67	Diyarbakır	Bismil	Ambar	120	4	*F. proliferatum* *M. phaseolina* *F. moniliforme* *Fusarium* spp.	
68	Diyarbakır	Bismil	Ambar	150	3	*F. solani* *Fusarium* spp. *F. proliferatum* *F. graminearum*	
69	Diyarbakır	Bismil	Ambar	250	3	*F. oxysporum* *F. moniliforme* *Cladosporium* spp.	100
70	Diyarbakır	Bismil	Ambar	200	2	*F. moniliforme* *F. solani*	
71	Diyarbakır	Bismil	Ulam	150	2	*Pythium* spp. *F. solani*	100
72	Diyarbakır	Bismil	Ulam	170	6	*Cladosporium* spp. *Fusarium* spp. *F. solani* *F. moniliforme* *F. oxysporum*	
73	Diyarbakır	Çınar	Başaklı	150	3	*F. moniliforme* *F. solani*	100
74	Diyarbakır	Sur	Sarılar	100	5	*F. oxysporum* *F. moniliforme* *Phoma* spp. *Ulocladium* spp.	100
75	Diyarbakır	Sur	Sarılar	100	4	*F. solani* *F. proliferatum* *Alternaria* spp.	100
76	Diyarbakır	Sur	Kervanpınar	100	5	*F. moniliforme* *Gliocladium roseum* *F. moniliforme* *F. solani*	100
77	Diyarbakır	Çınar	Şükürlü	200	9	*F. solani*	100
78	Diyarbakır	Çınar	Şükürlü	150	8	*F. moniliforme* *F. proliferatum*	100
79	Diyarbakır	Çınar	Şükürlü	180	11	*F. solani* *F. oxysporum* *Alternaria* spp. *Fusarium* spp. *Sclerotinia* spp.	100

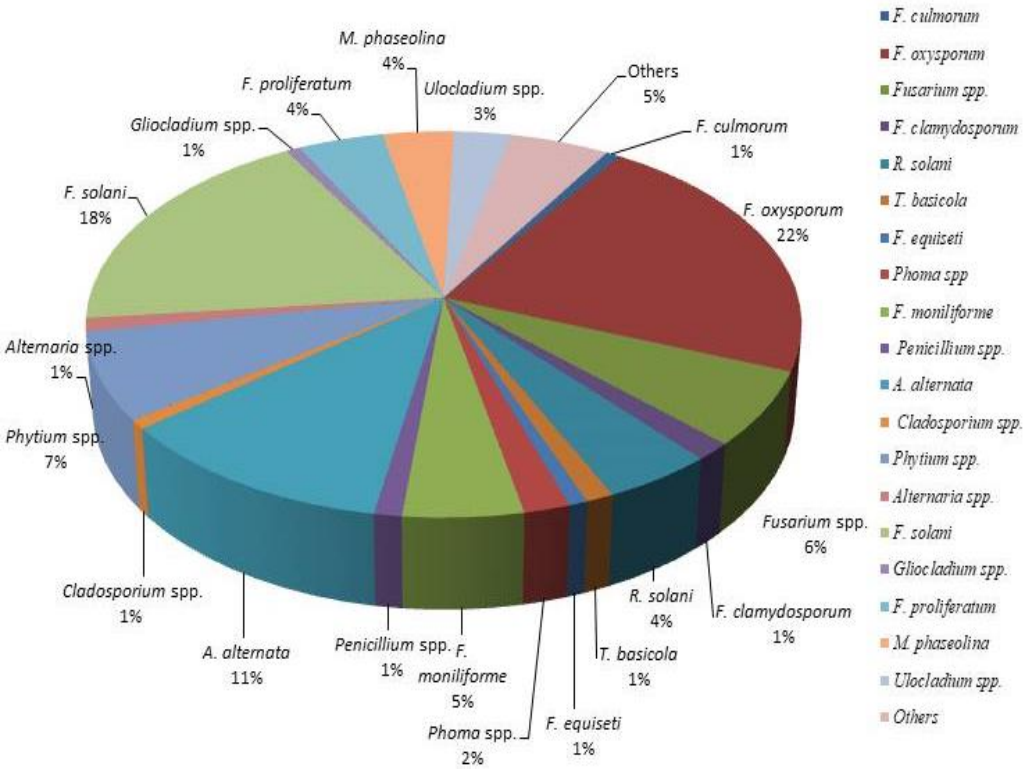

Figure 1. Presence rates of fungi

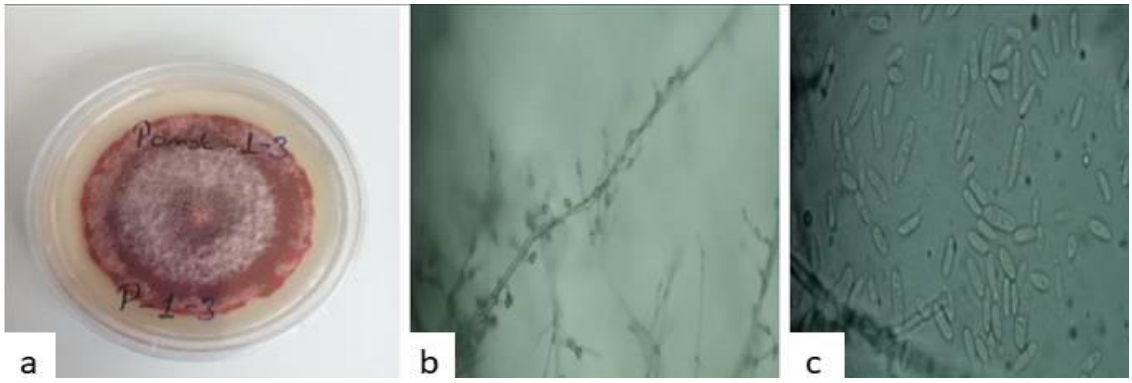

Figure 2. Appearance of *Fusarium oxysporum* on the growth medium (a) and under the microscope (b, c)

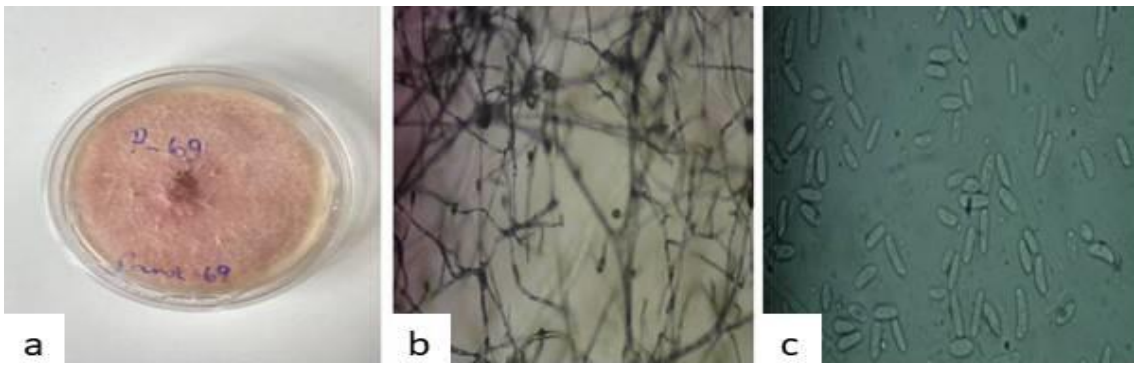

Figure 3. Appearance of *Fusarium solani* on the growth medium (a) and under the microscope (b, c)

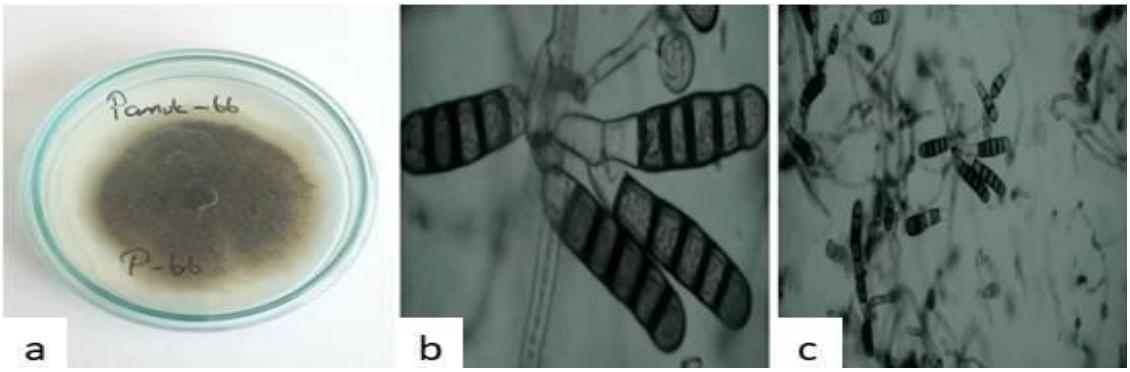

Figure 4. Appearance of *Thielaviopsis basicola* on the growth medium (a) and under the microscope (b, c)

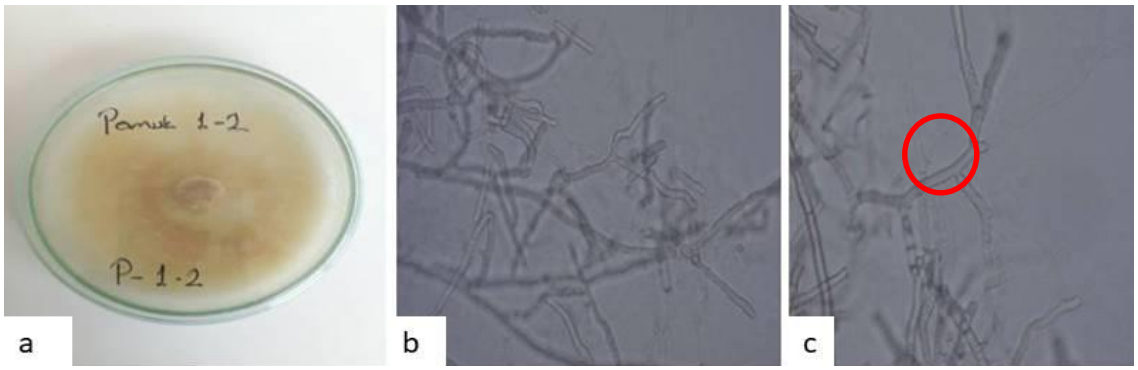

Figure 5. Appearance of *Rhizoctonia solani* on the medium (a) and under the microscope (b, c)

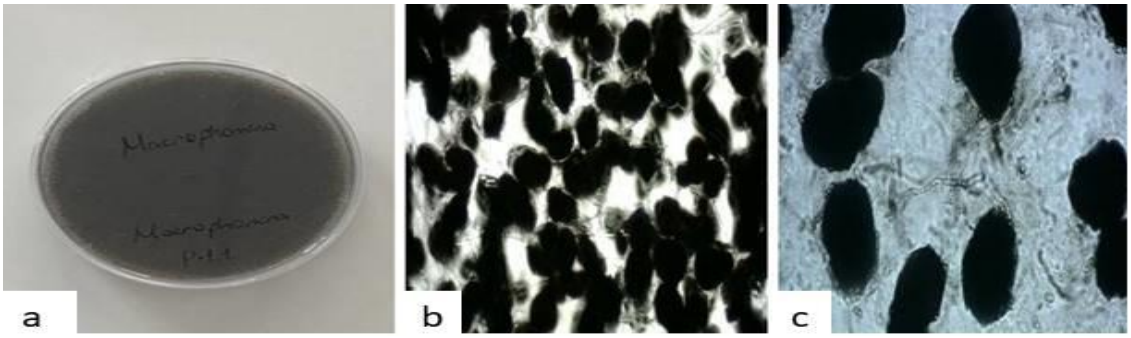

Figure 6. Appearance of *Macrophomina phaseolina* on the growth medium (a) and under the microscope (b, c)

later turning black (Figure 6a). Sclerotia form on the hyphae of the pathogen (Figure 6b), and these sclerotia are oval and irregular in shape (Figure 6c).

3.3. Molecular characterization

For the molecular phylogenetic analysis of the identified isolates, genomic DNA was obtained from the isolated fungal DNA. The gel electrophoresis images of some of the obtained DNA are shown (Figure 7a). Nucleotide sequence studies were conducted using primers (forward and reverse) specific to the ITS gene region. PCR amplification produced DNA products with bands approximately 650 bp in size, corresponding to the ITS gene. The obtained bands are illustrated (Figure 7b).

As a result of the studies, the base sequences obtained from the PCR products sent for sequencing were compared using the BLAST function in the NCBI database. The sequence analysis of all isolates based on the ITS gene region revealed the matched isolates, which are listed.

The sequencing results were processed using the BioEdit program, where errors and repeated sections were cleaned. The sequences were then

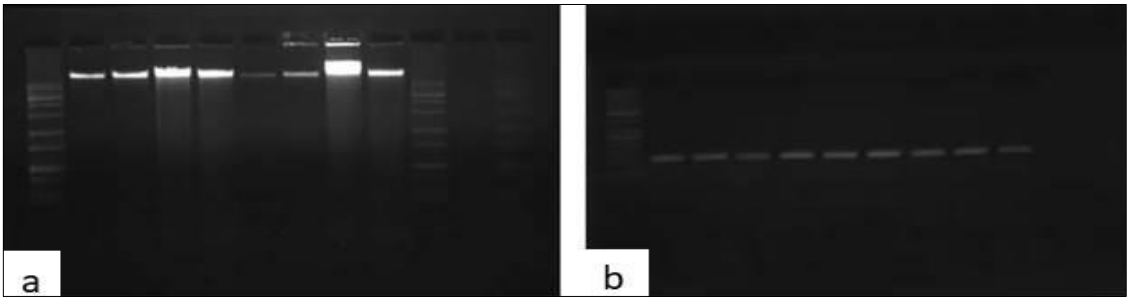

Figure 7. Gel electrophoresis result image of some isolates obtained by the CTAB DNA isolation method (a), Image of some PCR products on a 1% agarose gel (b)

aligned in FASTA format, and the results were recorded. The resulting phylogenetic tree is shown (Figure 8).

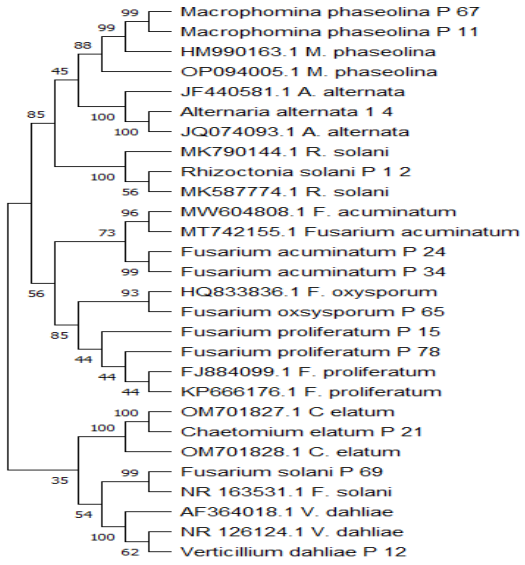

Figure 8. Phylogenetic tree with Mega11 program Neighbor-Joining, bootsrap metod with 500 replicates

From the 13 isolates, 9 different species were selected. To support the identification of these species, ITS region base sequences for these species were retrieved from the NCBI database and used to construct a phylogenetic tree (Figure 8). The selected species, along with the reference sequences obtained, were grouped together, showing distinct clusters among them.

3.4. Pathogenicity study

A pathogenicity and molecular diagnostic studies were conducted with selected isolates from the isolated fungi. The results of the study with these selected isolates are presented (Table 5).

The differences among the pathogenicity treatments were found to be statistically significant at the 5% level. The coefficient of variation (CV) value of the study was 11.49%, indicating that the pathogenicity study was conducted under suitable conditions (Table 5). According to Table 5, the isolate with the highest disease severity was identified as *T. basicola* P.66, while the second highest disease severity was caused by *F. moniliforme* P.4. The third pathogen causing high disease severity was identified as *F. acuminatum* P.24, followed by *Alternaria* spp. P.75 in fourth place. The isolate with the lowest disease severity was found to be *Ulocladium* spp. P.2**.** Other highly pathogenic and frequently isolated fungi included *F. oxysporum* and *F. solani* (Table 5).

Images of the diseased plants from the experiment are shown (Figure 9). In the pathogenicity tests, the fungi used caused color changes in the plant roots and root collars, manifesting as browning or blackening. Additionally, it was observed that the plants did not develop lateral roots and exhibited poor root growth. After evaluation, tissue samples were taken from diseased plants for re-isolation. The re-isolation results yielded the same isolates, thereby fulfilling the requirements of Koch's postulates.

4. Discussion and Conclusion

Cotton, a plant of significant agricultural importance in Türkiye and South Anatolia Region, faces many adverse factors from planting to harvest. Among these, pathogens causing rot in seedling roots are the primary concern. The activity of these pathogens increases in fields with high soil moisture and is more severe in areas with continuous consecutive planting. In the Tigris Basin, cotton has been produced for many years. Since crop rotation is not practiced in this region, pathogens causing seedling root rot continue to remain viable in the soil each year. This situation can lead to severe effects on cotton plants, especially during rainy spring seasons, causing pre- and post-emergence damping-off and resulting in damage that forces farmers to replant. In this study, root rot pathogens were isolated from

Table 5. Average disease severity of fungi contaminated with soil in pot study and the groups formed

Sequence no	Isolate no	Fungi	Disease severity (%)
1	P. 66	*Thielaviopsis basicola*	81.66 A
2	P. 4	*Fusarium moniliforme*	76.66 AB
3	P. 24	*F. acunminatum*	76.66 AB
4	P. 75	*Alternaria* spp.	75.00 ABC
5	P. 6	*Fusarium oxysporum*	71.66 A-D
6	P. 11	*Macrophomina phaseolina*	71.66 A-D
7	P. 73	*Fusarium moniliforme*	68.33 B-E
8	P. 35	*Fusarium oxysporum*	65.00 C-F
9	P.45	*Fusarium solani*	65.00 C-F
10	P. 57	*Sordaria tomentoalba*	65.00 C-F
11	P. 69	*Fusarium solani*	65.00 C-F
12	P.76	*Fusarium solani*	63.33 D-G
13	P. 79	*Sclerotinia* spp.	63.33 D-G
14	P.32	*F. chlamydosporum*	61.66 D-H
15	P. 78	*Fusarium proliferatum*	61.66 D-H
16	P. 1-5	*Pythium* spp.	60.00 E-H
17	P. 1-4	*Alternaria* spp.	58.33 E-H
18	P. 15	*Fusarium proliferatum*	58.33 E-H
19	P. 25	*Phoma* spp.	58.33 E-H
20	P. 61	*Phymatotrichopsis omnivora*	58.33 E-H
21	P. 34	*Fusarium acunminatum*	56.66 F-I
22	P. 1-2	*Rhizoctonia solani*	56.66 F-I
23	P. 21	*Chaetomium* spp.	55.00 F-J
24	P. 30	*Fusarium proliferatum*	53.33 G-J
25	P. 63	*Rhizoctonia solani*	53.33 G-J
26	P. 65	*Fusarium oxysporum*	53.33 G-J
27	P. 68	*Fusarium spp.*	53.33 G-J
28	P. 74	*Fusarium oxysporum*	53.33 G-J
29	P. 72	*Cladosporium* spp.	51.66 HIJ
30	P. 1-3	*Fusarium* spp.	51.66 HIJ
31	P. 67	*Macrophomina phaseolina*	46.66 IJ
32	P. 2	*Ulocladium* spp.	45.00 J
33	P Control	Control	0.00
LSD			3.93
LSD			0.46
CV (%)			11.49

LSD: Least Significant Difference, CV: Coefficient of Variation

Figure 9. Symptoms caused by patogens in the root collar of cotton seedlings

all surveyed and sampled fields, with disease prevalence in the region ranging between 2% and 16%. The high disease incidence could be attributed to the heavy rainfall during the spring months of 2021-2022 cotton production season, which caused damage to plants from root rot pathogens.

Consequently, during this season, some fields experienced empty patches due to the damage caused by these pathogens and had to be replanted. The study isolated numerous *Fusarium* species from the *Fusarium* genus. *Fusarium* wilt has been reported as the most common vascular disease in cotton, frequently encountered in agricultural areas of tropical regions (Chitarra, 2014). The most important of these *Fusarium* species were determined as *F. oxysporum* and *F. solani* besides these species, *F. chlamydosporum*, *F. proliferatum*, and *F. acuminatum* were also identified and found to be pathogenic. Among the *Fusarium* species, *F. acuminatum* and *F. moniliforme* were determined to have high virulence based on the pot trial results. These species have been reported by Karcılıoğlu (1976), to cause seedling root rot and wilt in cotton. In addition, various pathogens such as *Fusarium oxysporum* have been reported to cause symptoms including leaf spots, wilting, defoliation, and necrosis on cotton leaves, flowers, bolls, and stems (Mustafa et al., 2017). Similarly, other *Fusarium* species, such as *Fusarium solani*, are also known to induce symptoms in cotton plants (Zhu et al., 2019).

As a result of the survey, two isolates of *R. solani* (P.1-2, P.63) were selected. These isolates were tested in a pot experiment under laboratory conditions, where they were found to moderately infect cotton plants. Although the plants developed, their roots turned significantly brown, and the seedlings were affected by the disease. Re-isolation confirmed the presence of these isolates. Additionally, Erdoğan (2015) emphasized in their study that *Rhizoctonia solani* is a very significant pathogen causing seedling root rot. Additionally, Sağır et al. (1995), isolated *Rhizoctonia solani*, *Pythium* spp., and *Fusarium* spp., as well as *Verticillium dahliae*, as causal agents of damping-off in cotton based on their study conducted in cotton-growing areas of the Southeastern Anatolia Region.

In the pathogenicity study, isolate P.66 of *Thielaviopsis basicola* was found to cause the highest disease severity in plants, at a rate of 81.66%. In a study conducted, it was stated that the seedling root rot pathogens (*Rhizoctonia solani*, *Pythium* spp., *Fusarium* spp., *Thielaviopsis basicola*, *Alternaria* spp., *Aspergillus* spp.) cause the most destructive diseases worldwide and lead to significant yield losses in cotton (Yılmaz, 2009). In Australia, black root rot (*Thielaviopsis basicola*) spread rapidly during the 1990s and has emerged as a significant threat to the sustainability of the cotton industry (Nehl et al., 2004). Infected plants typically appear stunted and chlorotic. *T. basicola* has been reported to cause characteristic blackening of the roots due to the destruction of the root cortex, which contains the fungal chlamydospores (Hood and Shew, 1997; Mims et al., 2000). Generally, it has been noted that the pathogens responsible for root rot disease include *Rhizoctonia solani*, *Pythium* spp., *Fusarium* spp., and *Thielaviopsis basicola*. The isolate coded as P.75, which is *Alternaria* spp., was observed to cause a disease severity of 75.00%. Indeed, Kaya and Zorba (2021) have mentioned that *Alternaria* spp. species have many host ranges and cause significant yield losses. It was determined that *Macrophomina* spp., coded as P.11, caused a disease severity of 71.66%. Additionally, Kaur et al. (2012), stated that *M. phaseolina*, which has a wide host range, causes yield losses in many crops. To assess virulence, other pathogens used in the study, including *Pythium* spp., *Phoma* spp., *Chaetomium* spp., *Ulocladium* sp., *Phymatotrichopsis omnivora*, *Cladosporium* sp., and *Sclerotinia* sp., caused low to moderate damage, such as browning of the roots, and impeded plant development and emergence in cotton seedlings. The disease severity and variance analysis table of the pathogens is presented (Table 5).

Morphological identifications of the fungi were supported by molecular studies. DNA isolation and PCR analyses were performed on 31 isolates selected for pathogenicity tests. The sequence analysis of these isolates showed a similarity ranging from 95% to 100% with matched isolates in the GenBank database.

The study identified several pathogens responsible for seedling root rot in cotton. These soil-borne fungal pathogens cause damage to cotton plants before emergence, leading to seed rot and hindrance of seedling emergence, and post-emergence, they contribute to root weakening, stunted plant growth, and plant death. This results in sporadic gaps in fields and necessitates re-plowing under conditions favorable for pathogen development. The fungal pathogens responsible for cotton seedling root rot cause significant yield loss and economic damage. For these reasons, instead of planting cotton in the same area every year, crop rotation should be practiced. Efforts should be made to use resistant varieties, and breeding

programs should focus on developing varieties resistant to diseases, host weeds, and disease-carrying pests. Additionally, proper drainage of cotton fields is needed. Comprehensive studies on the fungal pathogens in the region should be conducted, and based on the results, producers in the area should be informed and guided to take the necessary preventive measures.

Ethical Statement

The authors declare that ethical approval is not required for this research.

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Declaration of Author Contributions

Investigation, Data Curation, Formal Analysis, Visualization, Writing-Review & Editing, *B. ARSLAN*; Conceptualization, Material, Methodology, Supervision, Writing-Review & Editing, *M.H. AYDIN*. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

References

Agrios, G.N., 1998. Plant Pathology, 3rd Edition. Academic Pres inc., San Diego.

Agrios, G.N., 2005. Introduction to Plant Pathology (5th Ed.). Elsevier Academic Press Publication, Amsterdam.

Allen, S.J., 1990. *Thielaviopsis basicola*, a new record on cotton in Australia. *Australasian Plant Pathology*, 19(1): 24-25.

Anonymous, 2021. Agricultural Product Markets. Republic of Türkiye Ministry of Agriculture and Forestry, Directorate of Strategy Development, Economic Policy Research Foundation of Türkiye (TEPAV). (https://arastirma.tarimorman.gov.tr/tepge/Menu/27/Tarim-Urunleri-Piyasalari), (Accessed Date: 10.11.2023). (In Turkish).

Barnett, H.L., Hunter, B.B., 1998. Illustrated Genera of Imperfect Fungi. American Phytopathological Society (APS Press), St. Paul, Minnesota.

Booth, C., 1977. *Fusarium*: Laboratory Guide to the Identification of the Major Species. Common Wealth Mycological Institute, Kew, Surrey, England.

Bora, T., Karaca, G., 1970. Measurement of Disease and Damage in Cultivated Plants. Ege University, Faculty of Agriculture, Publication No: 167, İzmir, Türkiye. (In Turkish).

Burgess, L.W., Liddelland, C.M., Summerell, B.A., 1988. Laboratory Manual for *Fusarium* Research (2nd Ed.). Blackwell Publishing, University of Sydney, Sydney.

Chitarra, L.G., 2014. Identification and control of the main diseases of cotton. Embrapa Cotton-Folder/Brochure/Guide (INFOTECA-E), (https://www.infoteca.cnptia.embrapa.br/handle/doc/986846), (Accessed Date: 10.11.2023).

Devay, J.E., 2001. Seedling diseases. In: T.L. Kirkpatrickand C.S. Rothrock (Eds.), *Compendium of Cotton Diseases* (2nd Ed.), APS Press, St. Paul, Minnesota, ABD, pp. 13-14.

Dinler, H., Benlioğlu, S., 2013. Vegetative compatibility groups of *Verticillium dahliae* Kleb. isolates obtained from cotton fields in Aydın Province. *Plant Protection Bulletin*, 53(2): 85-99.

Domsch, K.H., Gams, W., Anderson, T.H., 2007. Compendium of Soil Fungi, (2nd Ed.). Verlag, Eching, Germany.

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure from small quantities of fresh leaf tissues. *Phytochemical Bulletin*, 19(1): 11-15.

Erdoğan, O., 2015. Biological control of soil-borne fungal pathogens in cotton using *Fluorescent Pseudomonads*. *Turkish Journal of Agricultural and Natural Sciences* 2(3): 268-275.

Gerlach, W., Nirenberg, H., 1982. The Genus *Fusarium*-A Pictorial Atlas. Monograph/Atlas, Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Germany.

Hood, M.E., Shew, H.D., 1997. Initial cellular interactions between*Thielaviopsis basicola* and tobacco root hairs. *Phytopathology*, 87(3): 228-235.

Karaca, M., İnce, A.G., Elmasulu, S.Y., Onus, A.N., Turgut, K., 2005. Coisolation of genomic and organelle DNAs from 15 genera and 31 species of plants. *Analytical Biochemistry*, 343(2): 353-355.

Karcılıoğlu, A., 1976**.** Studies on the fungal agents causing damping-off in cotton in the Gediz Basin, their damage severity, and pathogenicity. (Unpublished) Ph.D. Thesis, Ege University Faculty of Agriculture, İzmir, Türkiye. (In Turkish).

Kaur, S., Dhillon, G.S., Brar, S.K., Vallad, G.E., Chand, R., Chauhan, V.B., 2012. Emerging phytopathogen *Macrophomina phaseolina*: Biology, economic importance and current diagnostic trends. *Critical Reviews in Microbiology*, 38(2): 136-151.

Kaya, B., Zorba, N.N., 2021. Effects of *Alternaria* genus members on fruits and vegetables. *The Journal of Fungus*, 12(2): 223-239.

Keith, S., 1996. Fuskey-*Fusarium* interactive key. *Agriculture and AgriFood*, 3(1): 24-34.

Leslie, J.F., Summerell, B.A., 2006. The *Fusarium* Laboratory Manual. Blackwell Publishing, Oxford.

Majumdar, G., Singh, S.B., Shukla, S.K., 2019. Seed production, harvesting, and ginning of cotton. In: K.

Jabran (Ed.), *Cotton Production*, Wiley Online Library, pp. 145-174.

Mamirov, S., Boyraz, N., 2021. Detection of fungi that cause seedling root rot in cotton planting areas of Aydın Province Söke District and determination of the efficacy of some fungicides against seedling root rot disease. *Journal of Bahri Dagdas Crop Research*, 10(2): 169-179.

Mims, C.W., Copes, W.E., Richardson, E.A., 2000. Ultrastructureof the penetration and infection of pansy roots by *Thielaviopsis basicola*. *Phytopathology*, 90(8): 843-850.

Mustafa, R., Hamza, M., Kamal, H., Mansoor, S., Scheffler, J., Amin, I., 2017. Tobacco rattle virüs based gene silencing of enoyl-CoA reductase and its role in resistance against cotton wilt disease. *Molecular Biotechnology,* 59(6): 241-250.

Nehl, D.B., Allen, S.J., Mondal, A.H., Lonergan, P.A., 2004. Black root rot: A pandemic in Australian cotton. *Australas. PlantPathol*, 33(1): 87-95.

Nelson, P.E., Toussoun, T.A., Marasas, W.F.O., 1983. *Fusarium* Species*:* An Illustrated Manual for Identification. Pennsylvania State University Press, University Park, London.

Refai, T., Ali, İ.N.M, Nabi, H.M.M.A., Aly, A.E., Khalil, M.I.I., Abd-Elsalam, K.A., 2022. Pathogenicity assay of some soil-borne fungi isolated from cotton seedlings. *Egyptian Journal of Agricultural Research*, 100(1): 41-48.

Rehman, A., Jingdong, L., Chandio, A.A., Hussain, I., Wagan, S.A., Memon, Q.U.A., 2019. Economic perspectives of cotton crop in Pakistan: A time series analysis (1970-2015). *Journal of the Saudi Society of Agricultural Sciences*, 18(1): 49-54.

Rothrock, C.S., Buchanan, M.S., 2017. The seedling disease complex on cotton. In: K.R. Reddy and D.M. Oosterhuis (Eds.), *Seeds and Seedlings in Cotton*, National Cotton Council of America, pp. 85-100.

Sağır, A., Tatlı, F., Gürkan, B., 1995. Studies on the diseases of cotton in the Southeastern Anatolia Region. Proceedings of the Symposium on Plant Protection Problems and Solution Proposals in the GAP Region, April 27-29, Şanlıurfa, Türkiye, pp. 5-9. (In Turkish).

Sneh, B., Jabaji-Hare, S., Neate, S., Dijst, G., 2013. *Rhizoctonia* species: taxonomy, molecular biology, ecology, pathology and disease control. Springer Science& Business Media, Dordrecht.

Tokel, D., 2021. World Cotton Agriculture and Its Contribution to Economy. *MANAS Journal of Social Studies*, 10(2): 1-16.

Townsend, G.K., Heuberger, J.W., 1943. Methods for estimating losses caused by diseases in fungicide experiments. *Plant Disease Reporter*, 27(17): 340-343.

Turhan, G., 1992. Unterdrückrung des *Rhizoctonia*-Befalls durch einen neuen Mykoparasiten, Stachybotrys elegans. *48. Deutsche Pflanzen schuztagung*, October 5-8, Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Göttingen, Almanya, pp. 223-226.

Watanabe, T., 2010. Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species. CRC Press, Boca Raton, Florida.

Yılmaz, Ö., 2009. The determination of some pesticide's efficieny on some soil pathojens that caused dampng-off in cotton areas. Master's Thesis, Ege University Graduate School of Natural and Applied Sciences, İzmir, Türkiye. (In Turkish).

Zhu, Y., Abdelraheem, A., Sanogo, S., Wedegaertner, T., Nichols, R., Zhang, J.F., 2019. First report of *Fusarium solani* causing *Fusarium* wilt in Pima cotton (*Gossypium barbadense*) in New Mexico. *U.S.A. Plant Disease*, 103(12): 3279.

CITATION: Arslan, B., Aydın, M.H., 2025. Determination of Seedling Root Rot Fungal Pathogens and Disease Prevalence Rates in Cotton (*Gossypium hirsutum* L.) Fields of the Tigris Basin. *Turkish Journal of Agricultural Research,* 12(2): 162-177.