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Detection and Pathogenicity of *Fusarium proliferatum* Causing Wilt in Cotton in Sanliurfa Province of Turkey

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Abstract
Between 2021 and 2023, a comprehensive survey was conducted using a random sampling method in Sanliurfa province and its districts, which are significant cotton production areas in the GAP Region. *Fusarium* isolates were collected from a large portion of the cotton fields, which were examined homogeneously in the north, south, east, and west directions. The molecular identification and diagnosis of the obtained *Fusarium proliferatum* isolates were performed using two different gene regions, ITS and TEF-1α. Pathogenicity tests were conducted on susceptible cotton varieties, Candia and C-92. As a result of this study, *Fusarium proliferatum* and *Verticillium dahliae* were found to be widespread in cotton-growing areas of Şanlıurfa province and its districts. The varieties affected by these pathogens, were five commercial cotton varieties exhibited symptoms of wilting, yellowing, and drying. The findings will help farmers make informed decisions about which cotton varieties to plant in the coming years. Additionally, the results of this study are expected to contribute to the development of resistant or tolerant cotton lines against the *Fusarium proliferatum* pathogen. This, in turn, will support the establishment of resistance management strategies for combating *Fusarium* wilt disease and aid cotton breeders in their resistance breeding efforts.

Research Article

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1. Introduction

Cotton (*Gossypium hirsutum* L.) is a globally significant fiber crop cultivated across approximately 33 million hectares and plays a crucial role in both agricultural and industrial economies (Sami-Ul-Allah et al., 2015; Icac, 2021). As a key raw material for the textile and food industries, its fibers provide natural cellulose for weaving and textiles, while its seeds, containing 17–25% oil, support the oil industry. Additionally, cottonseed meal is used for animal feed, and linters contribute to paper production, making cotton a strategically important crop (Kücük and Issı, 2019). Cottonseed oil is also gaining importance as a biodiesel raw material (Keskinkılıc, 2014).

Turkey is a major cotton producer, ranking 6th globally. In the 2019/20 season, the country produced 815.000 tons of lint cotton on 520.000 hectares, with an average yield of 1.567 kg ha^{-1} (Icac, 2021). Cotton is primarily cultivated in the Southeastern Anatolia, Aegean, and Mediterranean regions, with Şanlıurfa province alone accounting for 208.792 hectares of cotton fields (Anonymous, 2020a). The cotton sector is vital for Turkey's economy, generating employment in both rural agriculture and urban textile industries (Anonymous, 2020b). However, cotton production is continuously threatened by pathogens, leading to economic losses and reduced fiber quality (Arslan and Ozcınar, 2021).

Some fungal diseases cause significant economic losses in cotton, a strategically important crop both globally and in Turkey. These include *Verticillium* wilt (*Verticillium dahliae*), damping-off (*Rhizoctonia solani* Kühn., *Pythium* spp., and *Fusarium* spp.), *Ascochyta* blight (*Ascochyta gossypii*), and several leaf spot diseases (Agrios, 2005). In Turkey, *Verticillium* wilt has been recognized as the primary cotton disease, and extensive research has been conducted on it for many years. However, in recent years, significant losses from *Verticillium* wilt have not been observed, particularly in the GAP Region, due to the adoption of crop rotation systems and the use of *Verticillium dahliae*-tolerant cotton varieties by farmers. On the other hand, *Fusarium* wilt is a major disease widespread in nearly all cotton-growing areas worldwide (Davis et al., 2006).

Early *Fusarium* infection in cotton plants often results in plant death. Some infected plants may regrow from the base, but this new growth does not produce cotton capsules and typically fails to survive later in the growing season (Davis et al., 2006). The disease initially appears as a localized spot in the field and spreads to healthy areas through rainfall, irrigation water, or soil cultivation tools. In fields irrigated using the furrow method, *Fusarium* wilt is commonly observed in water-saturated areas (Cianchetta and Davis, 2015).

The application of solarization and soil fumigation for controlling *Fusarium* wilt in cotton is quite difficult and economically unfeasible for crops grown in large areas like cotton. However, the protective effect of solarization lasts for only one year (Davis et al., 2006). Chemical control with fungicides has a very limited effect in controlling *Fusarium* wilt (Davis et al., 2006). Many studies have noted that controlling soil-borne diseases like *Fusarium* vascular wilt with conventional synthetic fungicides is quite challenging (Singh et al., 2002; Kim and Hwang, 2007). Therefore, the detection of disease pathogens and the development of resistant varieties is of great importance.

It has been reported that the most effective approach to combating *Fusarium* wilt in cotton worldwide is host resistance (Cianchetta and Davis, 2015). Therefore, knowing the pathogen causing *Fusarium* wilt in cotton fields and planting cotton varieties that are tolerant to the prevalent pathotype will be the most effective and economical method of control.

This study was carried out to determine the presence and pathogenicity of *Fusarium* species in cotton production areas in Şanlıurfa province and its districts and to carry out diagnostic studies of the existing species using molecular analysis methods.

2. Materials and Methods

2.1. Plant material

The plant material for this study consists of cotton plants showing wilt symptoms from cotton growing areas in Şanlıurfa province and its districts in the Southeastern Anatolia Region. Additionally, Candia and C-92 cotton seeds were used as plant material in pathogenicity tests.

2.2. Fungal material

The fungal material for this study consists of *Fusarium* isolates obtained from cotton samples showing wilt symptoms in cotton growing areas of Şanlıurfa province and its districts in the Southeastern Anatolia Region. Additionally, the other materials used in the study include media for fungal culture development, laboratory equipment, incubators, various chemicals, primers, peat, soil, and plastic pots.

2.3. Methods

2.3.1. Field survey and sampling

Surveys were conducted between August and September in 2021 and 2022 during different vegetative growth stages of cotton plants, particularly when the plant's vegetative and generative parts were fully developed. The surveys were carried out using a random sampling method across the districts of Sanlıurfa province. As shown in Table 1, cotton production areas and amounts were recorded, while Table 2 presents the number of sampling points based on field area (Anonymous, 2021). To ensure homogeneous representation in the study area, plant samples were randomly selected from various directions (north, south, east, and west) of the study area. Sampling areas are detailed in Table 3 (Bora and Karaca, 1970).

Table 1. Cotton cultivation areas in Sanliurfa province where surveys were conducted (Anonymous, 2020a)

Province	Districts	Planted Area (ha)
Sanliurfa	Akçakale	33887.4
	Birecik	2.3
	Bozova	6590.6
	Ceylanpınar	8836.6
	Eyyubiye	32582.0
	Halfeti	0
	Haliliye	21908.4
	Harran	30392.4
	Hilvan	6067.2
	Karaköprü	2739.2
	Siverek	17118.2
	Suruç	17953.5
	Viranşehir	30714.2
Total (ha)		208792.0

In the surveyed fields, diagonal lines were followed, and at the specified number of points given in Table 2, 10 plant samples were randomly selected from each point (Nehl et al., 2004). The stems of the selected plants were cut longitudinally in half, and any color changes in the vascular bundles were examined. Among the examined samples, 2-3 plants showing color changes in the vascular bundles were taken to the laboratory. GPS (Global Positioning System) coordinates of the fields were recorded using a global positioning device.

Table 2. Number of sampling points based on field area (Anonymous, 2021)

Field size (decars)	Number of sampling points
<50	5
50-100	10
100-1000	15
>1000	20

The random sampling method was used as the sampling technique, and sampling was conducted according to Bora and Karaca (1970) (Table 3 and Figure 1). Stops were made approximately every 20 km along the road, and inspections were carried out in the fields on both the right and left sides of the road.

Table 3. Province, district, area, and sampling dates for the sampling conducted in the study

Sampling Location	District	Village	Sampling Date	Sampled Area (ha)
Sanliurfa	Central-Haliliye	Cekcek	9 August 2021	1.0
Sanliurfa	Central-Karaköprü	Karaköprü	9 August 2021	6.0
Sanliurfa	Central-Eyyubiye	Ugurlu	9 August 2021	10.0
Sanliurfa	Akcakale	Yusuf	9 August 2021	4.7
Sanliurfa	Akcakale	Koruklu Research Station	9 August 2021	5.0
Sanliurfa	Akcakale	Serince Research Station	9 August 2021	3.0
Sanliurfa	Harran	-	9 August 2021	6.5
Sanliurfa	Central	Tektek	10 August 2021	5.0
Sanliurfa	Central	Tektek Research Station	10 August 2021	3.0
Sanliurfa	Viransehir	Central	10 August 2021	8.0
Sanliurfa	Viransehir	Central	10 August 2021	12.0
Sanliurfa	Viransehir	Elgün	12 August 2024	16.0
Sanliurfa	Ceylanpınar	Central	12 August 2024	3.0
Sanliurfa	Ceylanpınar	Central	12 August 2024	7.0
Sanliurfa	Ceylanpınar	Central	12 August 2024	20.0
Sanliurfa	Bozova	Central	17 August 2021	5.0
Sanliurfa	Bozova	Central	17 August 2021	15.0
Sanliurfa	Central	Osmanbey	17 August 2021	6.0
Sanliurfa	Central	Harran University Campus Cotton fields	17 August 2021	2.5
Sanliurfa	Central	Harran University Campus surroundings	17 August 2021	5.5
Sanliurfa	Akcakale	Central	15 September 2021	11.0
Sanliurfa	Akcakale	Kücük Hacı ekber	15 September 2021	5.5
Sanliurfa	Ceylanpınar	Central	15 September 2021	15.0
				Total:175.7 ha

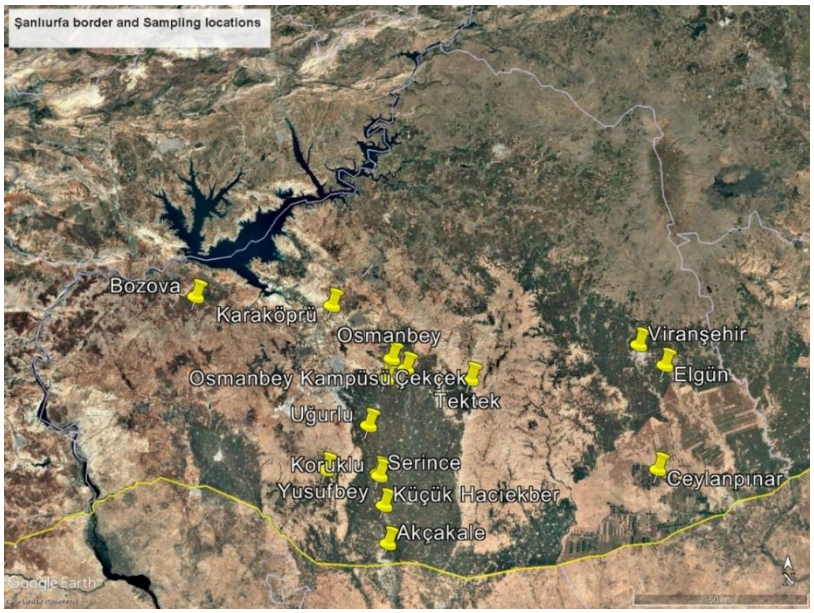

Figure 1. Sampling locations displayed on Google Earth

During sampling of survey, plants showing wilt symptoms were collected from cotton production areas and brought to Plant Health Laboratory of GAP Agricultural Research Institue (GAPTAEM) for isolation. After the incubation period, microscopic identification and diagnostic work were carried out on the fungal isolates, and isolates were obtained. From these isolates, species showing severe wilt symptoms in the field were selected for molecular analysis and pathogenicity testing. In the pathogenicity test, Candia and C-92 cotton seeds were used as sensitive varieties.

2.3.2. Isolation

In the laboratory, each plant showing color changes in the vascular bundles was first washed with tap water, then plant tissues containing both diseased and healthy parts from the vascular tissues were cut into 4-5 mm pieces using a scalpel. These pieces were soaked in a 2% (v/v) sodium hypochlorite solution for 2 minutes and then rinsed twice with sterile distilled water and left to dry between sterile blotting papers. The dried samples were placed in Petri dishes containing Potato Dextrose Agar (PDA) with 100 mg l^{-1} of streptomycin sulfate, with 3-4 tissue pieces per dish. All inoculated Petri dishes were incubated at 25 °C in the dark for 5-7 days (Castano et al., 2014). At the end of the incubation period, colonies resembling *Fusarium* spp. were transferred to fresh PDA medium to obtain pure cultures. The selected isolates, which represented production fields were, transferred to test tubes containing slanted PDA medium, and the stock cultures were stored at +4 °C.

2.3.3. Obtaining single spore cultures and morfological identification

To obtain single spore cultures from the isolates obtained, the following procedure was applied: After the isolates were grown on PDA at 24 °C for 7 days, 3 ml of sterile distilled water containing 1-2 drops of Tween 80 per liter was added to the Petri dishes. The concentration of the resulting spore suspension was adjusted to 10^3 spores ml^{-1} using a Thoma counting chamber. From the spore suspension, 100 µl was transferred onto Petri dishes containing Water Agar (WA, 2%) and spread using a sterile spreader. The Petri dishes were then incubated overnight at 24±2 °C, and the development of the cultures was examined under a compound microscope the following day. Germinated single spores were transferred to PDA using a sterile needle aseptically. The obtained single spore isolates were then transferred to slanted PDA and to drying papers for storage at -20 °C (Vicente et al., 2014). The morphological characteristics and identification of the obtained isolates of *Fusarium* spp. and *F. proliferatum* were made

according to Nelson et al. (1983) and Leslie and Summerell (2006), respectively.

2.3.4. Molecular identification

2.3.4.1. DNA isolation of *Fusarium* sp. isolates

For genomic DNA isolation, *Fusarium* isolates were cultured on PDA (Potato Dextrose Agar) medium for one week. DNA isolation was performed using a DNA isolation-extraction kit (Qiagen DNeasy Plant Kit) with 10-50 mg of fungal spores and mycelial fragments. The quality of the DNA samples was measured using a Nanodrop device, and 5 µl of DNA samples were run on a 1.5% agarose gel at 75V for 45 minutes along with a DNA ladder. As a control, λ DNA with known concentrations (25ng, 50ng, 100ng, 200ng) was used. The gel was stained with Ethidium Bromide and examined under a UV transilluminator, with Polaroid photographs taken. The DNA isolation procedure combined the methods used by O'Donnell et al. (1998), Moricca et al. (1998), Abd-Elsalam et al. (2003), and Mule et al. (2004). Freshly obtained mycelia and fungal spores from PDA were collected using a soft scraping preparation and placed into a 1.5 ml Eppendorf tube, filling the conical portion up to half (50-100 mg). The material in the tube was then crushed until it became powdery.

2.3.4.2. Primers used for *Fusarium* sp.

Primers were selected to genetically differentiate *Fusarium* spp. isolates phenotypically identified and to determine the dispersal maps of the species to different regions. The Internal Transcribed Spacer (ITS) regions of fungal ribosomal DNA (rDNA) are variable sequences that are crucial for distinguishing fungal species through PCR analysis and are commonly used for species identification. For this purpose, initially, Fusarium-specific primers were used to verify whether the isolates were *Fusarium* (Bilgili, 2017). Then, species-specific primers were employed to identify the isolates at the species level (Gonsalves and Ferreira, 2013; Mule et al., 2004). This two-step identification process involved comparing phenotypic and genotypic data using different diagnostic regions (Table 4).

2.3.4.3. PCR analysis

For DNA analysis of the morphologically diagnosed isolates, after the identification at the genus level, the samples were grouped and the isolates were extracted using the "Qiagen DNeasy Plant Kit." PCR analysis was performed using Universal primers (ITS 1 F-ITS 4 R, Tef F-Tef R) (Table 4). A 50 µl reaction mixture was prepared for PCR analysis. The mixture for each reaction consisted of 20 µl master mix, 1 µl genomic DNA, and 29 µl sterile water. After running the PCR products on a 1.5% agarose gel electrophoresis, the results were visualized under UV light. The evaluation for each isolate was based on whether a band was observed for each primer (Arif et al., 2012; Gonsalves and Ferreira, 2013). For this purpose, the optimal concentrations of the components for the amplification were determined according to the optimization studies and conditions outlined by Arif et al. (2012), Mule et al. (2004), Moricca et al. (1998), and Abd-Elsalam et al. (2003). The thermal cycler was programmed, and the study was conducted using *Fusarium* isolates from cotton fields in Sanliurfa.

Table 4. PCR conditions, reaction cycles, temperatures, and durations for ITS and TEF-1α primers

TEF-1α (658 bp)-40 Cycles
TEF-f: ATCGGCCACGTCGACTCT
TEF-r: GGCGTCTGTTGATTGTTAGC
Tef 1-a (40 cycles):
95 °C → 2 min pre-denaturation
94 °C → 1 min denaturation
58 °C → 1 min annealing
72 °C → 2 min elongation
72 °C → 8 min final extension
ITS Region Universal Primers (650 bp)-35 Cycles
ITS1-F: 5'-TCCGTAGGTGAACCTGCGG-3'
ITS4-R: 5'-TCCTCCGCTTATTGATATGC-3'
ITS1-ITS4 (40 cycles):
95 °C → 5 min pre-denaturation
94 °C → 30 sec denaturation
58 °C → 1 min annealing
72 °C → 60 sec elongation
72 °C → 7 min final extension

2.3.4.4. Electrophoresis studies

Agarose Gel Electrophoresis studies were conducted according to the method described by Galitelli and Minafra (1994). PCR products were run on a 1.5% agarose gel. After applying the necessary protocols for DNA isolation and PCR (Qiagen DNeasy plant kit), *Fusarium* sp. isolates were sent for Sanger Sequencing (Molecular Sequence Analysis) to determine the levels of genetic diversity. PCR was performed in a 50 µl volume. For all experiments, isolates that produced clear bands on the gel were sequenced.

In all the studies, sequencing was requested for the isolates showing good bands in the gel. Genomic DNA and PCR analyses were conducted in 2022 at the Plant Protection Department of the Ankara Agricultural Research Institute (AZMAE), Molecular Biology and Biotechnology Unit. Sequencing analyses were performed through external service providers.

2.3.5. Pathogenicity tests

In the pathogenicity tests, the cotton varieties Candia and C-92 were used as plant material. Isolates were obtained from cotton plants in the surroundings of Harran University and Ceylanpınar district. The experiments were conducted in a climate room (24 ± 2 °C with a 12h light $12h^{-1}$ dark cycle) with 5 replications.

A mixture of soil, sand, and peat (1:1:1), sterilized at 121 °C for 30 minutes in an autoclave, was used. The mixture was filled into 250 ml plastic cups, and three cotton seeds were planted in each mixture. When the seedlings reached the cotyledon leaf stage, one plant was left per pot (Figure 2 and 3).

Fusarium isolates obtained from cotton fields in Harran University and Ceylanpınar were grown on Potato Dextrose Agar (PDA; agar 18 g, dextrose 20 g, distilled water 1000 ml) for 14 days. Sterilized distilled water and 1-2 drops of Tween 80 were added to the cultures and shaken gently to obtain spore suspension. The spore suspension was transferred to 16 mm diameter test tubes. After mixing thoroughly with a tube shaker, the spore count was determined using a Thoma chamber (hemocytometer) under a microscope. The inoculum concentration was adjusted to 4×10^6 spores ml^{-1}. Then, 5 ml of the spore suspension was poured into the bottom of each plastic pot, and cotton plants in the 4-6 leaf stage were transplanted into these pots. The plants in the pots were monitored for disease progression for 4 weeks.

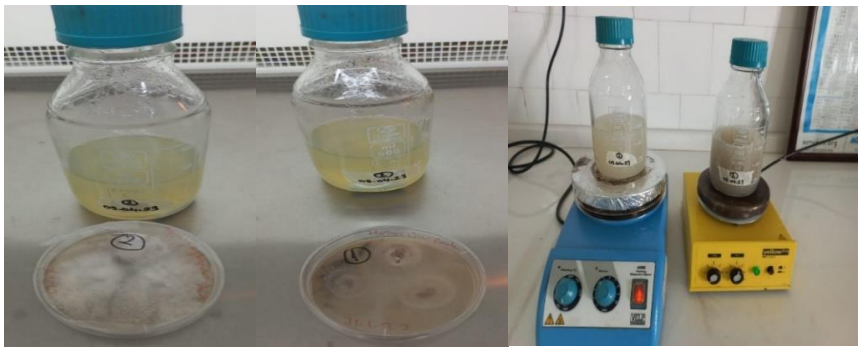

Figure 2. Preparation of inoculums used in pathogenicity tests

Figure 3. Preparation of the pathogenicity tests

2.3.5.1. Disease assessment

Approximately 4 weeks after inoculation, in the assessments, the percentage disease severity for each plant and the lesion areas on all of the plant's true leaves were calculated separately for each leaf according to the modified scale of Ulloa et al. (2006) and Erdogan and Benlioglu (2007) (Table 5), and the average was taken.

Table 5. Green tissue scale used in pathogenicity study

Index	Disease Symptoms
0	No visible chlorosis and epinasty on the leaf
1	Chlorosis on ¼ of the leaf
2	Chlorosis, necrosis, or wilting on ½ of the leaf
3	Chlorosis, necrosis, or wilting on 2/3 of the leaf
4	Leaf is about to shed or has already shed

Disease severity in the assessments was calculated by averaging the lesion areas on all true leaves of each plant, using the scale below to determine the percentage for each leaf. The disease severity values for each leaf were calculated using the index formula provided below (Karman, 1971):

Leaf disease index formula = (0)(a) + (1)(b) + (2)(c) + (3)(d) + (4)(e) / n = (a + b + c + d + e)

Where:

a, b, c, d, e = the number of plants in each scale category

n = total number of plants

3. Results and Discussion

3.1. Morphological identification

Cotton varieties commonly used by farmers in surveyed areas were “Candia, Stoneville-455, Stoneville-458, Progen-Lazer, Stoneville-468, and Stoneville-440. Samples were collected from the fields where these varieties were used, and the symptoms such as wilting and drying were observed. Among the susceptible varieties observed in the field, wilting and drying were noted in the Candia cotton variety, yellowing in the Stoneville-455 variety, and signs of plant drying in the Stoneville-458 and Lazer seed varieties.

Relatively higher levels of drying were observed in the Stoneville-468 and Stoneville-440 cotton varieties. During the survey studies, the Lima and Esperia varieties were observed among the resistant varieties in the field. Al Mahmooli et al. (2013) found the prevalence of *Fusarium proliferatum* in two different *Gladiolus* varieties (Red Majestry and Mascagni) in Oman to be 3% and 12%, respectively. They also reported symptoms of *F. proliferatum* in plants, including wilting, yellowing, and plant mortality, which are similar to the findings of this study. Typical vascular discoloration was observed in the diseased plant samples. *Fusarium* spp. isolates were obtained from all sampled plants showing symptoms of wilting and drying. *Fusarium* isolates were identified from these samples, and 12 different isolates from 12 different fields were selected for further study. Figure 4 shows the mycelia of *Fusarium proliferatum* obtained from some selected cotton survey fields, representing fungal isolates from different fields sampled during the survey.

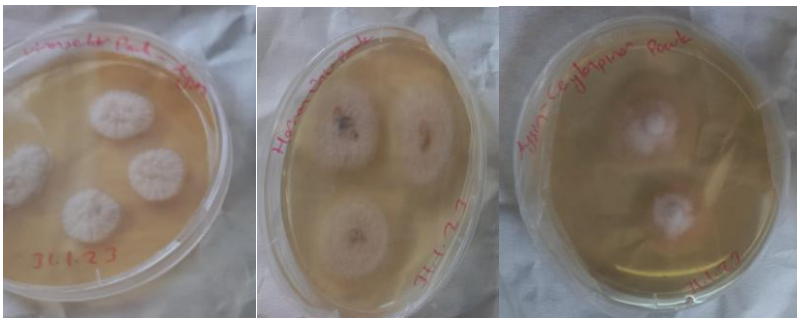

Figure 4. Some fungal isolates of *Fusarium* species obtained from the survey

Similar to the findings of Fattahi et al. (2014), the colony characteristics of *Fusarium proliferatum* grown on PDA medium at 25 °C for 10 days showed a growth of approximatelly 8 cm. The colonies exhibited a color range from cream and purple to dark purple on the surface of the PDA medium. A considerable amount of aerial hyphae was observed, with the colony initially displaying purple and dark purple, which eventually changed to white as it matured. The spore colonies and mycelial growth of the fungus on PDA showed similarities to the symptoms of *Fusarium proliferatum* observed in different plant species, as reported by Gao et al. (2017) and Al Mahmooli et al. (2013).

3.2. Molecular analysis

The DNA bands of 12 fungal isolates, selected from virulent strains obtained through surveys conducted in cotton production areas in Şanliurfa province and its districts, are shown in Figure 5. Table 6 presents the species identification of cotton plant samples analyzed by DNA and PCR, while Table 7 displays the species matched in the NCBI gene database based on the sequencing analysis results of PCR samples. The results indicate that the 12 fungal isolates showed nearly 100% similarity with *Fusarium proliferatum* species in the NCBI database. Samples with good DNA bands were used in Sanger Sequencing and Sequence Analyses.

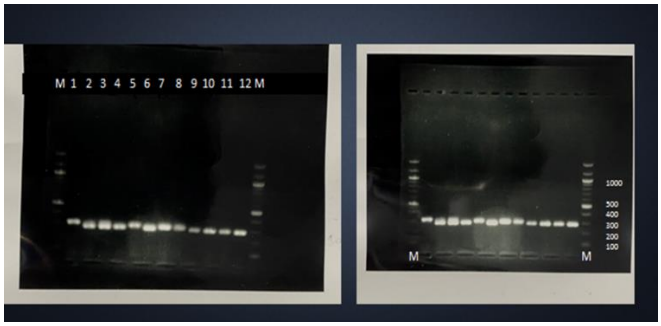

Figure 5. Agarose gel image of TEF amplicons of *Fusarium proliferatum* isolates; M: DNA Marker, 1-12: *F. proliferatum* isolates

Molecular analyses and genomic DNA methods showed parallelism with the result of this study, similar to Zhu et al. (2019) and Zhang and Stewart (2000). Similar results were obtained with the literature of Bilgili (2017) and O'Donnell et al. (1998) regarding the identification of *Fusarium* species by amplification and sequencing of DNA of fungal isolates and the use of ITS-1/ITS-4 and EF-1/EF-2 primers.

Table 6. Molecular analysis results of selected samples from the obtained fungal ısolates

Code of the Fungus Analyzed by PCR Analysis	Species
2	*Fusarium proliferatum*
4	*Fusarium proliferatum*
6	*Fusarium proliferatum*
7	*Fusarium proliferatum*
10	*Fusarium proliferatum*
11	*Fusarium proliferatum*
1	*Fusarium proliferatum*

According to the results of this study, ITS and TEF-1 alpha gene region sequences showed nucleotide similarities ranging from 100% to 98.06% with *Fusarium proliferatum* ITS and EF regions in the NCBI Gene Bank. They were found to be similar to previously published sequences, ITS (100% - KF986684) and TEF-1 alpha (100% - FN252392) (Table 7). Molecular identification of *Fusarium proliferatum* via classical and real-time PCR studies and methods in this study shows paralellism with Amatulli et al. (2012).

Table 7. PCR analysis results of selected fungal isolates from different gene regions (ITS and EF) and their matched species in the NCBI GenBank

Sample No	**Species diognosis**	**Gene region**	**Similarity percent and accession species number**
Cotton -2	*Fusarium proliferatum*	Translation Elongation Factor 1-alpha (EF)	%100- FN252392
Cotton -4	*Fusarium proliferatum*	Translation Elongation Factor 1-alpha (EF)	%99.36 -KC820975
Cotton -7	*Fusarium proliferatum*	Translation Elongation Factor 1-alpha (EF)	%98.09- KF715258
Cotton -11	*Fusarium proliferatum*	Translation Elongation Factor 1-alpha (EF)	%99.38 - KU508352
Cotton -6	*Fusarium proliferatum*	Translation Elongation Factor 1-alpha (EF)	%99.69 - MH628463
Cotton -10	*Fusarium proliferatum*	Translation Elongation Factor 1-alpha (EF)	%99.37- MW845655
Cotton -1	*Fusarium proliferatum*	Translation Elongation Factor 1-alpha (EF)	%99.69-KY178319
Cotton -2	*Fusarium proliferatum*	Internal Transcribed Spacer (ITS)	%99.81- OM956067
Cotton -4	*Fusarium proliferatum*	Internal Transcribed Spacer (ITS)	%99.43 -MT371373
Cotton -7	*Fusarium proliferatum*	Internal Transcribed Spacer (ITS)	%99.81- ON527497
Cotton -1	*Fusarium proliferatum*	Internal Transcribed Spacer (ITS)	%100 - KF986684
Cotton -6	*Fusarium proliferatum*	Internal Transcribed Spacer (ITS)	%100 - ON527491
Cotton -10	*Fusarium proliferatum*	Internal Transcribed Spacer (ITS)	%98.06- KF986684

3.2. Pathogenicity tests

The disease severity index value of two selected aggressive fungal isolates of *Fusarium proliferatum* (Figure 6), in Candia cotton seedlings are shown in Table 8 (Figure 7). As seen in the table, the index value was found to be 1.60 for the Harran University isolate and 1.80 for the Ceylanpınar isolate. According to these results, the Ceylanpınar fungal isolate was found to be more severe in the Candia cotton variety. The disease severity of the Harran University isolate (Isolate-1) was 1.60, while the disease severity of the Ceylanpınar isolate (Isolate-2) was found to be 2.00 in the C-92 cotton variety (Table 9; Figure 7). Based on these results, the Ceylanpınar isolate was determined to be more virulent and aggressive in both cotton varieties.

Table 8. Pathogenicity test results of isolates applied to Candia cotton variety

Isolate No	I. Replication Scale Value	II. Replication Scale Value	III. Replication Scale Value	IV. Replication Scale Value	V. Replication Scale Value	Disease Index Value
Isolate-1 (Harran University)	2	2	2	1	1	1.60
Isolate-2 (Ceylanpınar)	2	2	1	2	2	1.80

Table 9. Pathogenicity test results of isolates applied to C-92 cotton variety

Isolate No	I. Replication Scale Value	II. Replication Scale Value	III. Replication Scale Value	IV. Replication Scale Value	V. Replication Scale Value	Disease Index Value
Isolate-1 (Harran University)	2	1	2	1	2	1.60
Isolate-2 (Ceylanpınar)	2	2	2	2	2	2.00

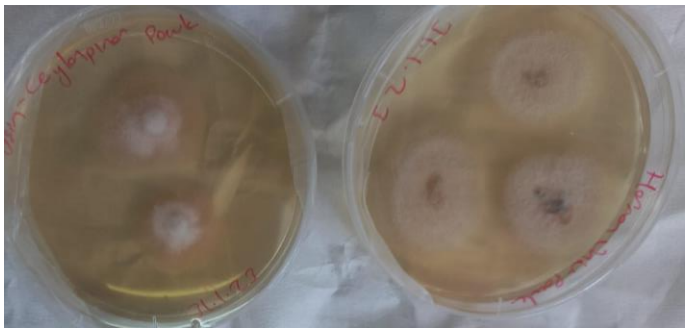

Figure 6. Some fungal isolates used in pathogenicity tests and molecular analyses

Figure 7. Growth stages of the plant in the pathogenicity test

Studies on *Fusarium* wilt in cotton in Turkey have been limited. In one of these, Canıhoş et al. (2000) reported in their TÜBİTAK project (Project No: TOGTAG-1375) that by treating cotton seeds with some herbicides they significantly increased the cotton plant's resistance to *Fusarium oxysporum* f.sp. *vasinfectum* (Fov)-caused wilt disease in the Cukurova Region cotton planting areas. In another study, Şahbaz and Akgün (2016) identified fungal wilt pathogens in cotton planting areas in the Reyhanlı district of Hatay and investigated the effects of various treatments that promote plant resistance on the susceptibility of commonly grown cotton varieties to these pathogens and their role in disease formation. In that study, *Fusarium* spp. was isolated from 46 of the 54 sampled fields (85.2%), *V. dahliae* from 5 fields (9.3%), and both *Fusarium* spp. and *V. dahliae* from 4 fields (7.4%). Pathogenicity tests showed that other *Fusarium* species, except Fov isolates, did not cause disease in cotton, while the disease severity caused by Fov isolates was found to be significantly high.

As mentioned earlier, studies on *Fusarium* wilt in cotton have been quite limited in our country. A portion of the cotton seeds used in cotton production in Turkey are imported. Since *Fusarium* species are soil-borne pathogens and can also be transmitted through seeds, there is a risk that imported cotton seeds may carry different races of *Fusarium* species, potentially contaminating local soils. To date, no studies have been conducted in our country regarding this issue. In this study, the presence of *Fusarium proliferatum* in cotton cultivation areas of Sanliurfa province, along with the resistance/tolerance status and pathogenicity of cotton varieties grown in these fields, have been determined, making significant contributions to both national and global literature.

4. Conclusion

In this study, a comprehensive survey was conducted in cotton cultivation areas of Sanliurfa province and its districts in the GAP region, with the aim of collecting *Fusarium* spp. isolates. These isolates were then both morphologicaly and molecularly identified using specific primers as *Fusarium* spp. pathogens. These fungal isolates were subjected to pathogenicity tests on two susceptible cotton varieties. Their virulence was characterized based on the disease severity on cotton genotypes.

This study, conducted to determine the prevalence of FOV in cotton production areas of Sanliurfa province, revealed that, in addition to the commonly observed *Verticillium* species in cotton fields, *Fusarium* species, specifically *F. proliferatum*, has started to appear in these fields as well. Plant samples collected from cotton plants showing wilting and drying symptoms in clusters, taken from the roots and root collars, were analyzed through morphological and molecular studies. Contrary to expectations, the presence of *F. proliferatum* instead of FOV indicates that climate change is affecting the soil-borne pathogens in cotton production areas, leading to a shift in the species present. Future studies should focus on the presence and prevalence of *F. proliferatum* in the GAP region and other cotton production areas in Turkey. Accordingly, new research should be conducted on breeding cotton varieties resistant to this pathogen. It is believed that this study will contribute to cotton breeding efforts aimed at developing new varieties.

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