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Research Article

Identification of *Fusarium* Species in Vegetable Greenhouses in Kumluca District

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ABSTRACT
Kumluca is an important district where many kinds of vegetables are produced and exported to other countries in Turkey. The main purpose of the current investigation was to diagnose the *Fusarium* species in several crop plants such as tomato, pepper, eggplant, cucumber and melon in Kumluca district, Antalya. A total of 272 samples were collected from diseased plants in greenhouses. In this study, eight *Fusarium* species were identified based on morphological characteristics. Considering the pathogenicity test under growth chamber conditions, the disease severity of these species ranged from 48 to 92%. Among the identified species, *Fusarium verticillioides* (92%) exhibited the highest virulence in pepper plants.
Keywords: Soil-borne fungi, greenhouse, morphological characteristic, pathogenicity

Kumluca İlçesi Sebze Seralarındaki *Fusarium* Türlerinin Tanımlanması

ÖZ
Kumluca, Türkiye'de birçok sebze türünün üretildiği ve diğer ülkelere ihraç edildiği önemli bir ilçedir. Bu araştırmanın temel amacı, Antalya'nın Kumluca ilçesinde domates, biber, patlıcan, salatalık ve kavun gibi farklı kültür bitkilerinde *Fusarium* türlerini teşhis etmektir. Seralardaki hastalıklı bitkilerden toplam 272 örnek toplanmıştır. Morfolojik özelliklere dayanarak sekiz *Fusarium* türü tanımlanmıştır. İklim odası koşullarında yapılan patojenite testine göre, bu türlerin hastalık şiddeti %48 ile %92 arasında değişmiştir. Bu türler arasında *F. verticillioides* (%92) biber bitkilerinde en virülent tür olmuştur.
Anahtar kelimeler: Toprak kökenli fungus, sera, morfolojik karakter, patojenisite

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Introduction

Vegetables contain many crucial components including vitamins and antioxidants which have beneficial effects on human health. Owing to its rich vitamin and mineral content, it is indispensable for a healthy life all over the world. During the sedentary production period, most vegetables are often exposed to phytopathogenic fungi, bacteria and viruses, thereby causing plant diseases, leading to crop and yield losses (Rizvi et al., 2017).

Plant diseases caused by fungal pathogens pose an important problem in vegetable production as in other crops. However, it's possible to increase the productivity of agricultural crops by protecting plant health. Disease agents, especially soil-borne fungi cause root rot, drying, wilting, yellowing and white rot. The *Fusarium* genus is widely distributed in soils in many regions of the world, and causes vigorous plant diseases in different host plants (Summerell, 2019).

Fusarium species are considered to be one of the most devastating fungal disease agents in vegetable greenhouses worldwide. It causes significant crop losses not only in Turkey but also in other countries (Jarwis, 1992; Akhtar and Iftikhar, 2017). *Fusarium oxysporum* f. sp. *radicis-cucumerinum*, the causal agent of cucumber root and stem rot is a devastating disease in greenhouse and it has been distributed around the world (Pavlou et al., 2002; Netzer et al., 1977). Watermelon *Fusarium* wilt caused by *F. oxysporum* f. sp. *niveum* constrains watermelon production (Egel and Martyn, 2013). The tomato *Fusarium* wilt (*Lycopersicon esculentum* Mill.), caused by *F. oxysporum* f. sp. *lycopersici* is one of the most prevalent diseases in this crop (Reis et al., 2005; Sudhamoy et al., 2009). This genus is also an economically important wilting pathogen in various crops in Iran (Saremi et al., 2008). The control of *Fusarium* diseases is very difficult and expensive. The use of a resistant cultivar is very important and the most effective disease control measure for *Fusarium* wilt (Beckman, 1987).

The objectives of this study were to (I) diagnose the *Fusarium* spp. that cause economic losses in vegetable greenhouses in the Kumluca district, and (II) determine the pathogenicity of each identified *Fusarium* spp. in their hosts. Thus, the present study is the first detailed research on *Fusarium* spp. in plants cultivated in greenhouses in the Kumluca district of Antalya province.

Materials and Methods

Sampling of symptomatic plants and pathogen isolation

Random samples of symptomatic plants were collected from vegetable greenhouses during the cultivation period from September 2013 to June 2014. A total of 272 samples showing disease symptoms, including yellowing, wilting and vascular browning were collected from tomato, pepper, eggplant, cucumber and melon greenhouses in the Kumluca district, Turkey. The collected samples were stored in plastic bags and taken to the laboratory, and then subjected to isolation procedures. For the isolation process, the roots of the plants were washed thoroughly with tap water and excess moisture was removed on filter paper. Infected tissues were cut into small pieces (approximately 4-5 mm in length) from the plant parts including diseased and healthy tissues. All pieces were surface-sterilized in 2% sodium hypochlorite (NaOCl) solution for 3 minutes and washed twice in sterile distilled water and excess moisture was removed on sterile filter paper. After drying, sterilized tissues were plated on potato dextrose agar (PDA; Difco Laboratories, USA) medium. After incubation of the Petri dishes for 7 days at 25°C in the dark, fungi started to grow from the tissue and were transferred to another Petri dish. In addition to PDA, two different mediums, namely carnation leaf agar (Fisher et al., 1982) and synthetic nutrition agar (Nirenberg, 1976) were used to identify *Fusarium* spp.

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Identification of *Fusarium* species

For the diagnosis of *Fusarium* species, all isolates were grown in different media such as PDA, Synthetic Nutrient Agar and Carnation Leaf Agar (CLA). The lam culture technique was used for identification and incubated for 5-15 days at 25°C in dark conditions (Booth, 1971). After ten days of incubation, the diagnosis was performed using morphological characteristics, according to The *Fusarium* Laboratory Manual (Leslie and Summerell, 2006). The morphological characteristics such as hyphae and reproductive structures were observed under a light microscope (Nicon/Eclipse E 100) and photographed using the Progress Mac Pro Capture program. To determine the colony growth rate of each isolate, radial mycelial growth was measured on 4th day after the incubation.

Pathogenicity tests

Pathogenicity tests were conducted using pot trials under controlled conditions. In pathogenicity tests, *Capsicum annuum* L. cv. D-36, *Lycopersicon esculentum* L. cv. Bestona, *Cucumis sativus* L. cv. Aspendos, *Solanum melongena* cv. Sicilia, *Cucumis melo* L. cv. Balkan were used as plant materials. The growth medium was sterilized twice by autoclaving at 121° C, and 1.1kPa for 60 minutes. Seedlings of the above-mentioned cultivars were transferred to 10 cm diameters pots including autoclaved soil: sand: peat (v:v:v;1:1:1). All the plants were placed in a growth chamber at 25±2°C, 16:8 photoperiod and 65% humidity. The identified isolates were cultured on autoclaved wheat grains in 9 cm diameters Petri dishes. After 10 days of incubation at 25°C in the dark, the plants were inoculated with 4 grams of wheat culture pieces by placing them around the roots. The control plants were inoculated similarly without pathogens in the experiments. Three replicates were made for each isolate. Re-isolation of *Fusarium* species was performed by direct isolation and the species was morphologically identified.

The disease severity was determined after 20 days of inoculation. The wilting symptoms were scored according to the 0-5 scale (Prados-Ligero et al., 2007). The disease severity was calculated by applying the Townsend-Heuberger formula to the obtained scale values (Townsend and Heuberger, 1943).

Statistical analysis

The data were analyzed by using Analysis of Variance (ANOVA). Significant differences among the averages were determined by Tukey's multiple comparison tests ($p<0.05$) using MINITAB statistical software version 16.

Results and discussion

Identified *Fusarium* species

A total of 272 plant samples were collected from all the vegetable greenhouses in the Kumluca district. In this study, according to their morphological and cultural characteristics, the eight *Fusarium* species were identified from vegetable greenhouses as *F. oxysporum, F. poae*, *F. proliferatum, F. sambucinum, F. semitectum, F. solani, F. sporotrichioides* and *F. verticillioides* (Figure 1). Among them, *F. sporotrichioides* and *F. verticillioides* were isolated from cucumber, while *F. proliferatum* was isolated for the first time in pepper from Turkey. The morphological and cultural characteristics of all species mentioned above were parallel with the species descriptions in the Fusarium Laboratory Manual (Leslie and Summerell, 2006) and Introduction to Food-Borne Fungi (Samson et al., 1995). These distinguishing characteristics were shown in Table 1.

The soil-borne fungi spread and devastate a significant proportion of agroecosystems and these fungi are the most destructive class of plant pathogens (Summerell et al., 2003). The morphological and cultural characteristics of *Fusarium* species have been utilized from past to present as the preferred methods for species identification (Fisher et al., 2012). Among the identified *Fusarium* species, *F. oxysporum* is one of the well-known plant pathogenic soil-borne fungi causing diseases on a wide range of host plants including tomato, pepper, cucumber, eggplant, melon etc. and completely distributed all over the world.

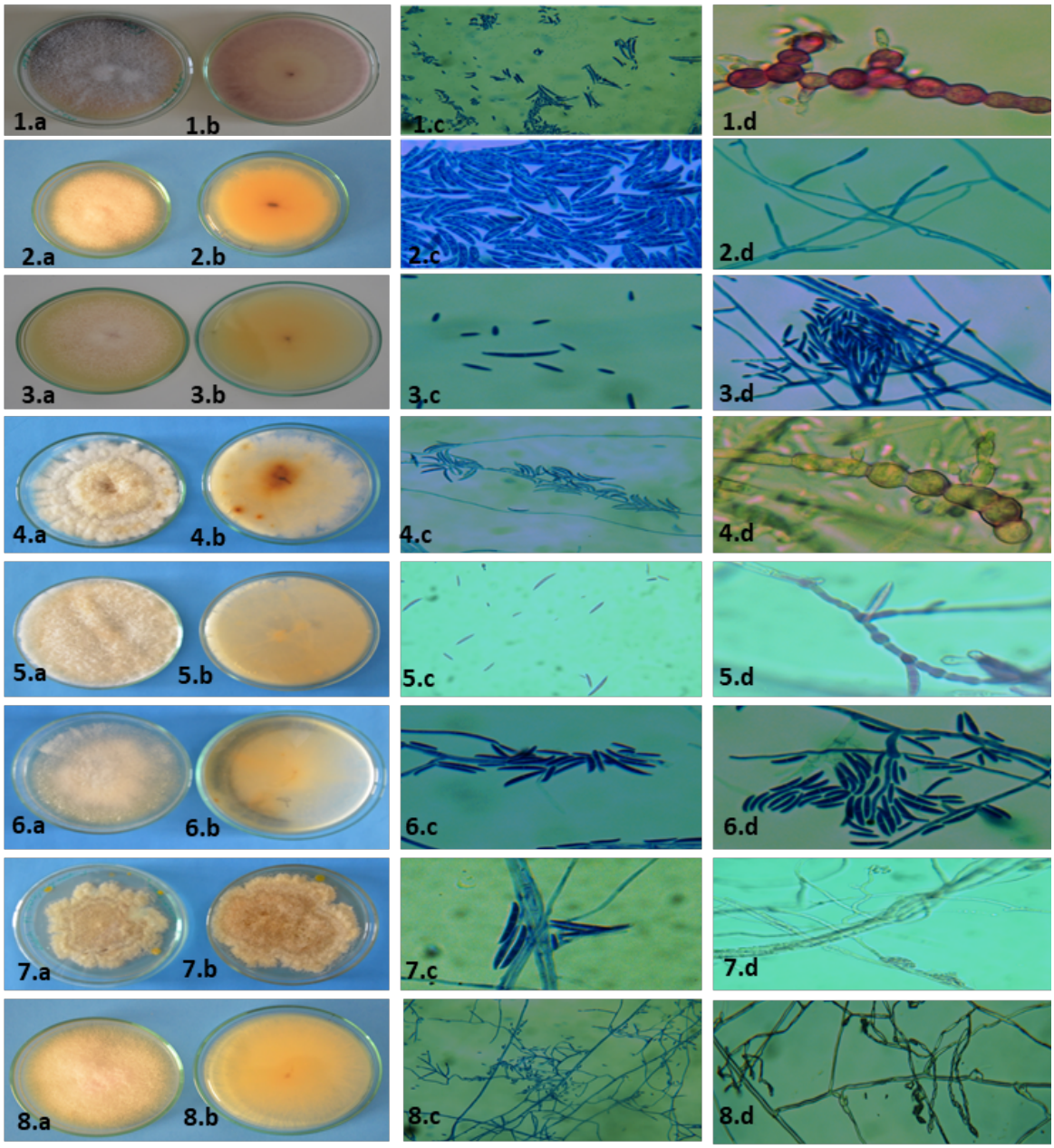

Figure. 1. Macroscopic and microscopic characteristics of *Fusarium* species. ***Fusarium oxysporum***: The colony's upper surface (1.a); The colony's lower surface (1.b); Macroconidia (1.c); Chlamydospore (1.d); ***Fusarium poae***: The colony's upper surface (2a); The colony's lower surface (2.b); Macroconidia (2.c); Monophialide (2.d); ***Fusarium proliferatum***: The colony's upper surface (3.a); The colony's lower surface (3.b); Macro and microconidia (3.c); Polyphialide (3.d); ***Fusarium sambucinum***: The colony's upper surface (4.a); The colony's lower surface (4.b); Macroconidia (4.c); Chlamydospor (4.d); ***Fusarium semitectum:*** The colony's upper surface (5.a); The colony's lower surface (5.b); Macro and microconida (5.c); Chlamydospor (5.d); ***Fusarium solani***: The colony's upper surface (6.a); The colony's lower surface (6.b); Macro and microconidia (6.c); Microconidia (6.d); ***Fusarium sporotrichioides***: The colony's upper surface (7.a); The colony's lower surface (7.b); Macro and microconida (7.c); Phialide (7.d); ***Fusarium verticillioides:*** The colony's upper surface (8.a); The colony's lower surface (8.b); Microconidia and phialide (8.c); Microconidia chain (8.d); (40X magnification)

Table 1. Morphological and cultural characteristics of *Fusarium* species

	The DGR on PDA(cm)	Pigmentation on PDA	Chlamydospor formation	Microconidia		Number of septa in macroconidia	Types of conidiogenous cells		Apical cell shape	Basal cell shape	Macroconidia sizes (µm)
				Shape	Number of septae		Monophialide	Polyphialide			
F. oxysporum	1.3	White-Violet	+	Oval Ellipsoid Cylindrical	0-2	3-5	+	-	Curved	Foot	20-50x3-6
F. poae	1.5	Yellow-Red	-	Napiform Pyriform	0-1	2-3	+	-	Curved Tapered	Foot	18-38x3.8-7
F. proliferatum	1.1	Cream-Violet	-	Clavate Pyriform	0-1	3-5	+	+	Curved	Foot	30-58x3.3-4.4
F. sambucinum	1.1	Cream Brown Red	+	-	-	3-5	-	+	Needle tipped	Foot	22-50x4-5.6
F. semitectum	1.1	White Orange Brown	+	Clavate	0-2	3-5	+	+	Curved	Curved Foot	22-40×3-4.5
F. solani	0.9	Brown, Orange	+	Clavate Ellipsoid	0-2	3-5	+	+	Elongated Curved	Foot Notched	27-65x4.4-6.8
F. sporotrichiodes	1.6	White Pink Violet-Brown	+	Oval Pyriform Napiform	0-1	3-5	+	+	Curved	Foot	21-36×3.6-4.0
F. verticilloides	1.2	Greyish cream Violet	-	Oval Clavate	0-2	3-7	+	-	Tapered Curved Needle tipped	Foot Notched	30-58x2.7-3.6

*(+) Present, (-) Absent, DGR: Daily Growth Rate

F. oxysporum and *F. solani* have been reported to cause wilting and plant death of *Cucumis* in different countries of the world (Palti and Joffe, 1971; Palodhi and Sen, 1979; Zitter et al., 1996; Bruton and Miller, 1997; Pivonia et al., 1997; Akbar et al., 2018). In another study conducted in Turkey, it was reported that *F. solani* and *F. oxysporum* caused wilting and plant death in cucumber plants (Özkan et al., 2002; Ozan and Aşkın, 2006).

Pathogenicity tests

In the pathogenicity tests, *Fusarium* species produced typical yellowing and wilting symptoms on their hosts. These observed symptoms were similar to yellowing and wilting symptoms at the greenhouses. The disease severity of *Fusarium* species varied from 48% to 92% rates (Table 2).

Table 2. Disease severity of *Fusarium* species used in pathogenicity tests

Species	Host plant	DS(%)
F. oxysporum	Tomato (cv. Bestona)	72 ^{ab*}
	Cucumber (cv. Aspendos)	84 ^{a}
	Melon (cv. Balkan)	72 ^{ab}
	Eggplant (cv. Sicilia)	80 ^{a}
	Pepper (cv. D-36)	76 ^{ab}
F. solani	Tomato (cv. Bestona)	48 ^{c}
	Eggplant (cv. Sicilia	80 ^{a}
F. sambucinum	Tomato (cv. Bestona)	60 ^{b}
F. sporotrichoioides	Cucumber (cv. Aspendos)	84 ^{a}
F. proliferatum	Pepper (cv. D-36)	84 ^{a}
F. semitectum	Tomato (cv. Bestona)	80 ^{a}
	Pepper (cv. D-36)	76 ^{b}
F. poae	Pepper (cv. D-36)	76 ^{b}
F. verticillioides		92 ^{a}

*Means that the different letters are significantly different from each other ($p<0.05$ based on Tukey's test). DS: Disease Severity.

Results showed that the most virulent species was determined in pepper plants (cv. D-36) as *F. verticillioides*. In addition to this, *F. solani* had the lowest disease severity with 48% in tomato plants (cv. Bestona).

In the current research, the disease severity of *F. solani* in eggplant (cv. Sicilia) was higher than in tomato (cv. Bestona). This may be explained by the fact that the pathogen is forma specialis (Edel-Hermann and Lecomte, 2019). In a study conducted in Malaysia, it was reported that *F. solani, F. oxysporum* and *F. proliferatum* were pathogenic in Solanacea crops such as tomato and pepper. Moreover, the disease severity of these species ranged from 60-90% on their host (Saseetharan and Zakaria, 2014).

Consequently, in the current investigation, a total of eight *Fusarium* species were identified in vegetable greenhouses in different host plants. The findings of this study indicate that the *Fusarium* species are a potential problem for vegetable crops.

Conclusion

Kumluca is an important district where many kinds of vegetables are produced and exported to other countries in Turkey. In the present study, species belonging to the genus *Fusarium* were morphologically identified. Pathogenicity tests were conducted on their host plants under controlled conditions. These tests identified virulence differences among the species identified as being the causal agents of the wilting disease on their hosts in the Kumluca district. Since no study has been conducted in this region, the findings of this investigation will contribute to disease survey studies both in Turkey and globally.

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