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Research Article

Diagnosis of *Fusarium* Species in Garlic Cultivation Areas in Gaziantep Province

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ABSTRACT

Garlic (*Allium sativum* L.) is an important crop for Gaziantep and Turkey. The aim of this study was to identify the *Fusarium* species that caused serious crop losses in the garlic cultivation areas of Gaziantep. A sampling was conducted in garlic cultivation areas in April of 2018 and 2019. *Fusarium* spp. were diagnosed according to their cultural and morphological characteristics as *Fusarium oxysporum*, *F. poae*, *F. proliferatum*, *F. sporotrichoioides*, *F. semitectum* and *F. verticillioides*. From pathogenicity test, all identified species were found to be pathogenic to the host plant garlic.

Keywords: *Fusarium*, garlic, Gaziantep

Gaziantep İli Sarımsak Ekiliş Alanlarındaki *Fusarium* Türlerinin Teşhisi

ÖZ

Sarımsak (*Allium sativum* L.), Gaziantep ve Türkiye için önemli bir üründür. Bu çalışmanın amacı, Gaziantep ili sarımsak ekiliş alanlarında ciddi ürün kayıplarına neden olan *Fusarium* türlerinin belirlenmesidir. 2018 ve 2019 yılı Nisan aylarında sarımsak ekim alanlarında örnekleme yapılmıştır. *Fusarium* türleri, kültürel ve morfolojik özelliklerine göre *Fusarium oxysporum, F. poae, F. proliferatum, F. sporotrichoioides, F. semitectum* ve *F. verticillioides* olarak belirlendi. Patojenisite testinden, tanımlanan tüm türlerin konukçu bitki sarımsakta patojen olduğu belirlendi.

Anahtar kelimeler: *Fusarium*, sarımsak, Gaziantep

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Introduction

Garlic (*Allium sativum* L.) is produced in temperate regions all over the world and is used not only for feed, but also for medical purposes. At the same time, it has antimicrobial, antimutagenic, and anticarcinogenic properties (Lopez-Bellido et al., 2016; Oh and Kim, 2016). Globally, 1.5 million hectares were employed by the garlic in 2018 (FAO-STAT 2018).

There are many types of garlic grown in the world. The most widely used of these are white garlic, which is grown to obtain clove garlic (Taskaya, 2003). The variety preferred by the producers in the areas where garlic is grown is the Chinese garlic variety in Gaziantep. Kastamonu is first province that comes to mind when garlic is mentioned in our country, but Gaziantep province ranked first with a production of 21.5 thousand tons in 2019. In Gaziantep province, an average of 20 thousand tons of garlic is produced annually, but a part of the production is lost due to fungal diseases, with farmers facing enormous economic losses.

Plant protection problems emerge frequently in agricultural areas and fungal plant diseases cause critical economic losses in crop production (Atakan et al., 2018; Atakan et al., 2020). *Allium* genus members, such as onions and garlic, are attacked by soil-borne pathogens (Stankovic et al., 2007; Bayraktar et al., 2014). Worldwide, root diseases in garlic caused by *Fusarium* species are a serious problem and, in case of the most severe attacks the garlic bulbs are entirely softened and emptied (Mondani et al. 2021a). It has disrupted garlic production, causing up to 30 % of yield losses (Tonti et al., 2012). Sowing healthy plant materials is important to grow healthy plants, as diseases caused by *Fusarium* species are often detected in the field during early crop periods (Mondani et al., 2021b). Therefore, it is very important to determine the *Fusarium* spp. that cause economic losses in garlic cultivation.

Garlic is a plant that is usually propagated with cloves. Therefore, the selection of healthy plant material is important to reduce pathogen distribution. Recently, in a study conducted by Dugan et al. (2019), it has been stated that healthy-visible bulbs of garlic show disease symptoms during the storage period. Plant materials used as seeds can be infected by *Fusarium* spp., even if apparently healthy.

The objectives of this study were to (I) diagnose the *Fusarium* spp. that caused economic losses in garlic cultivation areas in the Gaziantep province, (II) determine the pathogenicity of these species in Chinese garlic cultivar.

Materials and methods

A sampling of diseased plant and pathogen isolation

Random sampling from diseased plants were performed from garlic cultivation areas in April 2018 and April 2019. A total of 420 diseased plants were collected according to the randomize sampling method from Gaziantep. These samples were kept in plastic bags and moved to the laboratory. For the isolation process, the roots and stem of affected plants were washed thoroughly under tap water and dried. The infected tissues were cut into small pieces (approximately 4-5 mm in length) from the plant parts forming from diseased and healthy tissues. All pieces were surface-sterilized in sodium hypochlorite (2% NaOCl, for 2 min) solution and washed twice in sterile distilled water and dried again. After drying, sterilized tissues were plated on PDA (potato dextrose agar; Difco Laboratories, USA). After incubation, fungi started to grow from the tissue and were transferred to the other Petri dishes. In addition to PDA, other mediums used to identify *Fusarium* species were SNA (synthetic nutrition agar) and CLA (carnation leaf agar).

Diagnosis of *Fusarium* Species

Fusarium isolates were cultured on above mentioned medium. After ten days of incubation at 25°C, the isolates were identified using morphological characteristics including hyphal branching, phialides, micro and macroconidia sizes, sporodochium and chlamydospore formation, according to The *Fusarium* Laboratory Manual (Leslie and Summerell, 2006). The lam culture technique was used for identification (Booth, 1971). The hyphae and reproductive structures of *Fusarium* species were examined a light microscope and photographed with the Progress Mac Pro Capture program. To determine each isolate colony growth rate, radial mycelial growth was measured on 4th days after the initial of incubation.

Pathogenicity Tests

The pathogenicity of identified *Fusarium* spp. were determined under controlled conditions with pot trials in a climate room. In pathogenicity tests, a Chinese garlic cultivar was used as plant material. The growth medium was sterilized twice by autoclaving at 121° C for 60 minutes. Seedlings were transferred to 10 cm diameter pots including autoclaved soil: sand: peat (v:v:v;1:1:1). All seedlings were placed in the climate room at 25±2°C, 16:8 photoperiod and 65% moisture. An isolate of each *Fusarium* species was cultured on wheat culture in 9 cm diameter petri and incubated at 25°C for 10 days. Plants were inoculated with four grams of wheat culture piece by placing around roots. Plants used as control were inoculated similarly without pathogens in the experiments. Three replicates were made for each isolate.

Disease severity was determined after 20 days of inoculation. The wilting symptoms were scored into five scales; 0: healthy plant; 1: chlorosis in the bottom parts of the plant; 2: bottom parts of the plant and 1/3 of the chlorosis or wiltness; 3: wiltness in the upper parts of the plant; 4: dead plant (Prados-Ligero et al., 2007). Scala values were evaluated by Townsend-Heuberger (1943) formula, and disease severity rates (%) were calculated.

Statistical Analysis

The data were subject to Analysis of Variance. Tukey's multiple comparison test ($p < 0.05$) was used to determine the differences among the means.

Results and Discussion

In the present study, six species belonging to *Fusarium* genus were diagnosed considering their morphological and cultural characteristics as *F. oxysporum, F. proliferatum, F. semitectum, F. sporotrichioides, F. verticillioides* and *F. poae* (Figure 1).

Distinguishing characteristics of all species mentioned-above were parallel with specifications 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.

Four weeks after pathogen inoculation, pathogenicity tests of all species above-mentioned were performed using a Chinese garlic cultivar. *Fusarium* species produced typical yellowing and wilting symptoms on host. These observed symptoms were quite similar to those in the field. In this study, the result obviously showed that identified all species were pathogenic in the garlic plant (Table 2). Tests under controlled conditions demonstrated the virulence of these pathogens.

Table 2. Disease severity of identified *Fusarium* species in Chinese garlic cultivar

	Disease severity (%)
Control	0[b**]
F. oxysporum	80[a]
F. poae	72[a]
F.proliferatum	68[a]
F. sporotrichoioides	68[a]
F. semitectum	72[a]
F. verticillioides	76[a]

** Means that do not share a letter are significantly different ($p < 0.05$ based on Tukey's test).

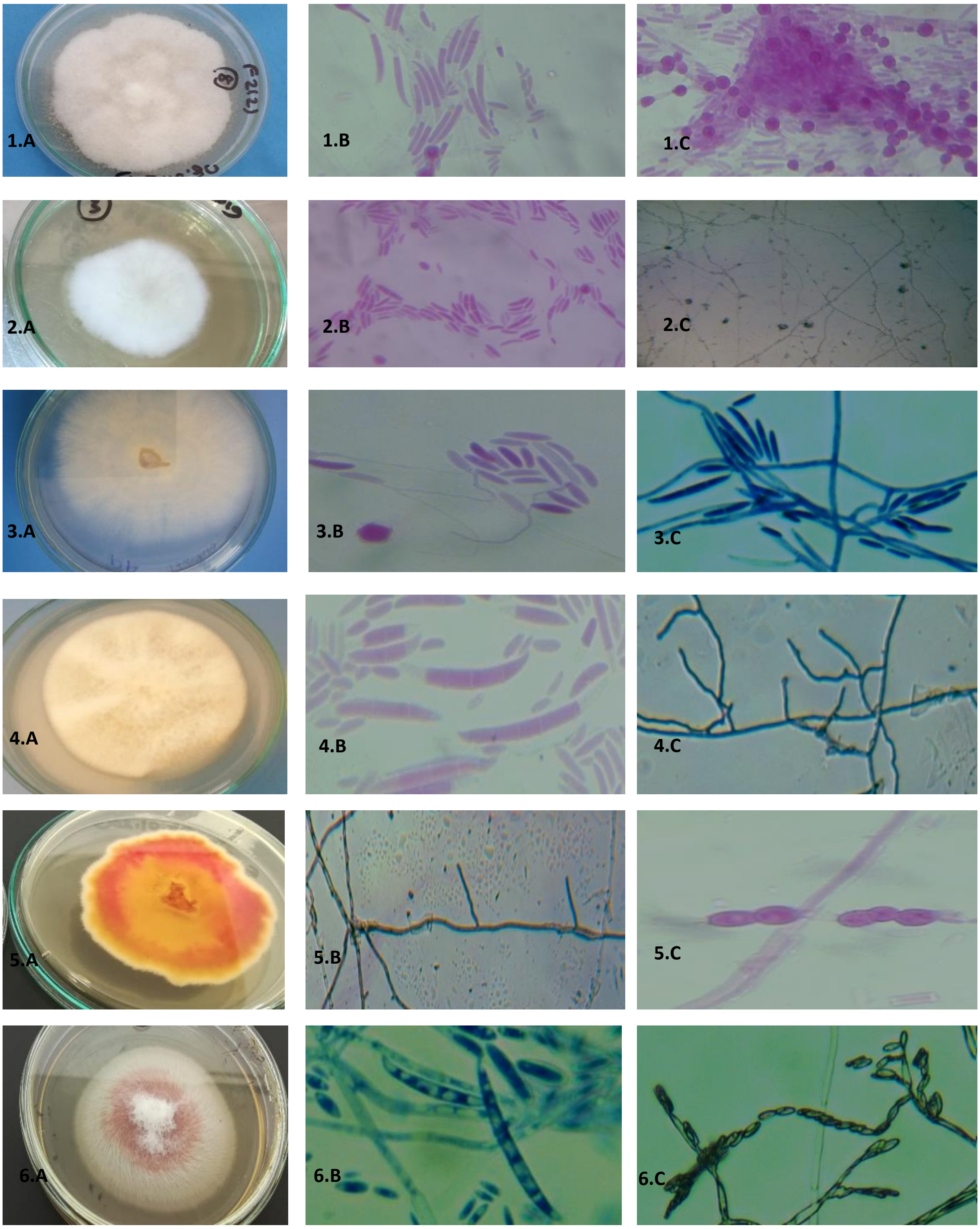

Figure. 1. Macroscopic and microscopic characteristics of *Fusarium* species. ***Fusarium oxysporum***: Upper surface of colony (1.A); Macroconidia (1.B); Chlamydospore (1.C); ***Fusarium poae***: Upper surface of colony (2.A); Macroconidia (2.B);Phialide (2.C); ***Fusarium proliferatum***: Upper surface of colony (3.A); Microconidia (3.B); Macro and microconidia (3.C); ***Fusarium semitectum:*** Upper surface of colony (4.A); Macro and microconida (4.B); Phialide (4.C); ***Fusarium sporotrichioides***: Upper surface of colony (5.A); Phialid (5.B); Chlamydospore (5.C); ***Fusarium verticillioides:*** Upper surface of colony (6.A); Macroconidia (6.B); Microconidia chain(6.C); (40X magnification).

Table 1. Cultural and morphological characteristic of *Fusarium* species

	The daily growth rate 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	20-50 x 3-6
F. poae	1.5	Yellow-Red	-	Napiform Pyriform	0-1	2-3	+	-	Curved Tapered	Foot	18-38 x 3.8-7
F. proliferatum	1.1	Cream-Violet	-	Clavate Pyriform	0-1	3-5	+	+	Curved Tapered	Foot	30-58 x 3.3-4.4
F. semitectum	1.1	Orange-Brown	+	Clavate	0-2	3-5	+	+	Curved	Curved Foot	22-40 × 3-4.5
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 Nothed	30-58 x 2.7-3.6

*(+) Present, (-) Absent

The diseases caused by soil-borne fungi devastate a significant proportion of agroecosystems and these fungi are the most destructive class of plant pathogens (Summerell et al., 2003). The cultural and morphological characteristics of *Fusarium* species have been utilized from past to present as the preferred methods for species identification (Fisher et al., 2012).

Fusarium species, which are plant pathogen, cause vascular wilts and root rots on various types of plants (Mohamad Saseetharan and Zakaria, 2014). In recent years, root rots of garlic caused by *Fusarium* species have emerged as an important disease and cause vigorous yield and quality losses during the period from field to storage (Leyronas et al., 2018; Quesada Ocampo et al., 2014). This study focused on the diagnosis of *Fusarium* spp. and their pathogenicity in garlic growing areas in Gaziantep, Turkey. The species identified were *F. oxysporum, F. proliferatum, F. semitectum, F. sporotrichioides*, *F. verticillioides* and *F. poae*. From pathogenicity test, all identified species were found to be pathogenic to the host plant garlic.

In many studies conducted on garlic and onion plants in different parts of the world, it has been reported that *Fusarium* species are responsible for wilt and root rot in the life processes of these plants. (Bayraktar and Dolar, 2011; Sankar and Babu, 2012; Moharam et al., 2013). In a study conducted on onion cultivation areas in Turkey, seven *Fusarium* species were identified (Bayraktar and Dolar, 2011). It was reported that the virulence of *F. oxysporum* was quite high, and the virulence of *F. solani* and *F. verticillioides* was lower in onion plants (Dissanayake et al., 2009). In order to determine the pathogenicity of *Fusarium* species isolated in garlic and onion cultivation areas in Serbia, garlic and onion plants were artificially inoculated. According to the results of pathogenicity tests, it was revealed that onions are more sensitive to *Fusarium* species than garlic. Stankovic et al., (2007) reported that the identified species were *F. proliferatum, F. oxysporum, F. solani, F. acuminatum* and *F. equiseti*. In a study performed in Italy, the dominant genus isolated from garlic samples was *Fusarium*. In addition, the most prevalent of these were identified as *F. proliferatum* and *F. oxysporum*, respectively (Mondani et.al., 2021c).

Conclusion

Garlic is an important crop for Gaziantep and Turkey. Soil borne pathogens negatively affect garlic cultivation, especially with their effects on yield and quality. The present study showed that the *Fusarium* species are prevalent on garlic cultivation areas in Gaziantep.

According to pathogenicity tests, these species constantly pose a serious problem in garlic cultivation. Although much research has been performed in different garlic and onion cultivation areas in Turkey, no research has been conducted in Gaziantep. Therefore, this study forms the basis of many future studies.

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