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VERTICILLIUM WILT EFFECT ON FIBER PRODUCTION AND ITS QUALITY TRAITS IN UPLAND COTTON (*GOSSYPIUM HIRSUTUM* L.)

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SUMMARY

Verticillium wilt (*Verticillium dahliae* Kleb.) is a fungal disease that causes significant losses both in fiber yield and its quality traits in upland cotton (*Gossypium hirsutum* L.). Therefore, the following study aimed to determine the effect of verticillium wilt's (VW) impact on lint production and fiber quality of nine upland cotton genotypes grown with *Verticillium dahliae*-infested and non-infested plants under greenhouse conditions. Based on disease ranking results, the lint yield and fiber quality parameters were significantly ($P < 0.05$) higher for the cultivars Bayraktar and Livzara than the genotype Alekberi under fungal pathogen (*V. dahliae*) infestation. Fiber quality traits bore considerable effects from VW infected plants as compared to healthy (H) plants. Fiber length decreased from 29.10 (H) to 26.0 (VW) in the cultivar Alekberi and 29.05 (H) to 26.4 (VW) with cultivar Ganja-110, however, was unchanged in the cultivars Livzara and Barakat. For micronaire, a slight increase emerged in some VW infected cultivars versus their healthy counterparts. For disease severity ranking based on intensity of vascular stem discoloration, the cultivars Livzara, Barakat, Ganja-114, and Bayraktar proved resistant to VW. The selection of cotton cultivars tolerant to *V. dahliae* infection with economically important traits needs further exploration.

Keywords: Upland cotton (*Gossypium hirsutum* L.), cultivars, verticillium wilt, lint production, fiber quality traits, disease ranking, fiber yield and quality losses

Key findings: Verticillium wilt disease considerably affects the fiber quality traits in upland cotton. A slight decrease in fiber uniformity, increase in micronaire, and nonsignificant differences for fiber elongation were notable in the diseased plants compared to healthy ones.

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INTRODUCTION

Cotton (*Gossypium* ssp.) is the main source of natural fiber, coping with 35% of the fiber demand (Alizade *et al*., 2023; Namazov *et al*., 2024). Cotton cultivation occurs in more than 80 countries worldwide, being considered a major industrial crop (Man *et al*., 2022). Compared to synthetic fibers, the cotton fiber is hypoallergenic, with important environmental benefits as a renewable resource of natural fibers (Khalid *et al*., 2023).

Verticillium wilt is one of the most destructive diseases of cotton, with a wide host range encompassing 400 plant species, including cultivated plants and trees (Dhar *et al*., 2020; Wu *et al*., 2022). According to the European Food Safety Authority (EFSA) reports, the pathogen is currently prevalent in most parts of the risk assessment areas, where yield reduction was up to 50% and even more in high-value crops, including cotton. Fungal pathogen *V. dahliae* is soilborne, which has an extensive host plant range, affecting more than 350 plant species (EFSA, 2014). Its microsclerotia can survive up to 14 years in the absence of a host plant under adverse environmental conditions (Short *et al*., 2015). As *V. dahliae* penetrates into the xylem vessels, the mycelium and biomacromolecules produced by parenchyma cells block the vessels, interfering with water and nutrient transport in crop plants (Amrahov *et al*., 2022; Zhang *et al*., 2022). Additionally, the pathogen leads to varying concentrations of free radicals, which alter the activity of antioxidant enzymes (Amrahov *et al*., 2023; Fakhari *et al*., 2025).

The water imbalance occurs in the host plants and causes wilting and yellowing of leaves, and eventually, they die (Song *et al*., 2020). In previous years, verticillium wilt has become a considerably serious problem due to climatic variations, long-term monoculture, and frequent introduction of new cotton cultivars in various regions worldwide (Ranga *et al*., 2020; Amrahov *et al*., 2024). Similarly, chemical treatment and management are ineffective in reducing the fungal inoculum levels in the soil. The only cost-effective and environmentally friendly method of managing the soilborne pathogen is the use of cotton cultivars with resistance or, at least, partial resistance to VW (Ayele *et al*., 2018; Alizada *et al*., 2024).

Based on recent past research, the local cotton cultivars have the inherent tolerance to main diseases affecting cotton crops in Azerbaijan, i.e., verticillium wilt (*V. dahliae* Kleb.) and bacterial blight (*X. malvacearum*) (Prikhodko *et al*., 2019). Along with the highest adaptation to the local environmental condition and resistance to wilting diseases, low fiber quality traits reduce the competitiveness of local cotton cultivars compared with imported genotypes (Mammadova *et al*., 2011). Moreover, an evaluation based on molecular markers can provide valuable insight into the genetic structure of a plant population, which helps in developing improved cultivars in different crops (Jenkins *et al*., 2018; Mammadova *et al*., 2024a, 2024b; Salimov *et al*., 2024).

G. hirsutum L. is the most widely used species grown worldwide, as well as the main target of cotton breeding (Ulloa *et al*., 2013; Alizade *et al*., 2023; Yang *et al*., 2023). The promising study also aimed to analyze the impact of verticillium wilt on fiber production and its quality traits in upland cotton (*G. hirsutum* L.) genotypes based on the following objectives: a) determine the genotypes' specific response to VW inoculation; b) determine the most affected economically important fiber quality traits under VW-infested conditions; and c) find out the tolerant cultivars with economically essential traits in upland cotton. The presented results will provide a base for future breeding programs to improve the VW resistance in upland cotton.

MATERIALS AND METHODS

Procuring the seed material of the examined cotton cultivars came from the National GenBank, Genetic Resources Institute, Ministry of Science and Education, Azerbaijan. The list of cotton cultivars with their GenBank accession numbers and country of origin is available in Table 1.

Table 1. Cotton (*G. hirsutum* L.) cultivars used in the study.

GenBank accession ID	Cultivar name	Species	Country of origin
AzGR-118 37	Alekberi	*G. hirsutum* L.	Azerbaijan
AzGR-11839	Zafer	*G. hirsutum* L.	Azerbaijan
AzGR-10202	Bayraktar	*G. hirsutum* L.	Azerbaijan
AzGR-11839	Karabakh-11	*G. hirsutum* L.	Azerbaijan
AzGR-10139	Agdash-3	*G. hirsutum* L.	Azerbaijan
AzGR-11836	Barakat	*G. hirsutum* L.	Azerbaijan
AZGR-11911	Livzara	*G. hirsutum* L.	Turkey
AzGR-5852	Ganja-110	*G. hirsutum* L.	Azerbaijan
AzGR-7733	Ganja-114	*G. hirsutum* L.	Azerbaijan

The use of the T1 (PYDV6) defoliating pathotype of *V. dahliae* isolated from the cotton served for conidial suspension inoculation. Microsclerotia of *V. dahliae* (defoliating type) isolate growing in the potato dextrose agar (PDA) medium, coming from diseased stem discs, occurred by incubating for 14 days at 25 °C. The microsclerotia, after harvesting from the petri dishes and spores, proceeded their transfer into water from the PDA surface. The collected spore suspension, as filtered with double layers of sterilized cheesecloth, removed the mycelium particles. The spore suspension's adjustment to 4×10^6 spores per ml used the haemocytometer, with 5 ml of suspension poured to each plastic pot. Control plants received treatment with distilled water. Following inoculation, plants' incubation was at 27 °C with light and watering conditions.

The soil used for pots was an Acu loam, fine-loamy, mixed, thermic Aridic Paleustolls (50% sand, 21% silt, and 29% clay) with a pH of 7.8 and 0.5% organic matter (Francl *et al*., 1988). Four seeds of each cotton cultivar succeeded planting into each pot (20 cm tall, 15 cm diameter at the top, and 10 cm diameter at the bottom) and thinning to one seedling per pot after emergence. The seeds sustained inoculation within a day of planting in pots. Pots received watering as needed to keep soil moisture until the establishment of plants. After germination, the seedlings' watering had 2- to 3-day intervals. Each biological replicate consisted of 30 individual plants. Control plants (without wilt inoculation) proceeded to plant in soil previously heated at 60 °C for 40 min (Hu *et al*., 2020).

Verticillium wilt injury evaluation started from 20 to 40 days after planting (DAP). The appearance of the first disease symptoms had recordings of days for individual cotton plants' evaluation. Cotton plants received ratings for foliar disease severity based on the 0–5 scale (Azaddisfani and Zangi, 2007; Zhou *et al*., 2014). The 0–5 ranking scale scoring is as follows: 0 = 0% (no disease symptoms); 1 = 1%–25% (minimum chlorotic lower leaves); 2 = 26%–50% (plants with chlorosis on lower and middle leaves); 3 = 51%–75% (well-developed disease symptoms, such as chlorotic, necrotic leaves and twisted terminal leaflets on branches); 4 = >75% (more than three leaves show severe symptoms of necrosis); and 5 = 100% (a complete plant death). After the harvest of raw cotton, the stem bore cutting on the root neck to evaluate for vascular browning. The rating of stems for vascular cross-section discoloration (0–4 scale), as determined, applied the method suggested by Colella *et al*. (2008) as follows: 0 = 0% with no disease symptoms on the stems; 1 = 25% of stems showing discoloration; 2 = 26%–50% of the stems have turned brown; 3 = 51%–75% of the stems have turned brown; and 4 = 76% of the stems have turned brown (Colella *et al*., 2008).

Fiber quality traits' analysis employed the high-volume instrument (HVI). Using Tukey's HSD test determined the differences among the cotton genotypes for specific traits at the $P < 0.05$ level of significance. Means comparison used Genomics 6 (https://www.jmp.com/en_us/home.html).

RESULTS AND DISCUSSION

The first wilting symptoms associated with verticillium wilt (VW) fungal disease appeared, on average, between 43 and 66 days after planting (DAP) (Table 2). The most resistant upland cotton cultivars, Livzara and Barakat, developed initial symptoms at 52 and 63 DAP, while the cultivar Alekberi was the last to develop wilt symptoms at 66 DAP. Other cotton genotypes had demonstrated intermediate initiation of the symptoms. For comparison, Zhang *et al*. (2012) recorded the first symptoms in cotton at 18 days after inoculation.

According to disease ranking, the highest disease severity appeared for the susceptible cultivar Zafer (3.2), followed by three other genotypes: Alekberi (2.5), Ganja-110 (2.3), and Agdash-3 (2.2). Cultivars Livzara and Bayraktar had a lesser disease severity index (1.0). Tukey's HSD test, as used, determined differences among the cotton genotypes for various traits at the $P < 0.05$ level of significance. Vascular stem discoloration was the highest in cultivars Alekberi (1.8) and Zafer (1.6), followed by the genotype Ganja-110 (1.4). In a study conducted by Kechagia and Xanthopoulos (1998) on four cotton (*G. hirsutum* L.) genotypes, the range of infection severity varied between zero and four.

The cultivars Alekberi and Zafer can be considerably more susceptible genotypes to the verticillium wilt (Figure 1). Results of disease severity and vascular stem discoloration suggested that the cultivars Livzara, Barakat, Ganja-114, and Bayraktar were notably resistant to VW. Cultivars Karabakh-11 and Agdash-3 emerged to be partially resistant. One can note that earliness of the VW symptom initiation was not predictive of disease severity and stem discoloration, which has an association with the defense mechanism of plants after pathogen inoculation. Similar results have also been in past studies' reports in the evaluation of cotton cultivars and lines under greenhouse conditions (Ayele *et al*., 2020).

Fiber traits, especially the fiber length and diameter, are largely dependent on the genetic makeup of cotton genotypes. Fiber maturity properties seemed dependent on the deposition of cellulose in the fiber cell wall and sensitive to environmental variations. Growth environment affects the genetic potential of crop plants and modulates the fiber shape and maturity properties to varying degrees. The fiber quality traits of cotton cultivars underwent studies through the HVI system. Determining the important fiber quality characteristics, such as mean length, upper mean length, strength, micronaire, and fiber elongation, was successful in three replications (Table 3).

Table 2. Mean values for the initial Verticillium wilt development (WILTi), disease severity (DS), vascular cross-section severity disease rate (VCDS), and lint yield per plant (Lint).

Genotypes	$WILT_i$ (DAP)[a]	DS[b]	VCDS[c]	Lint (g)[d]
Alekberi	66 a*	2.5 ab	1.8 A	37 bc
Zafer	55 ab	3.2 a	1.6 B	42 a
Bayraktar	54 ab	1.0 c	0.8 E	37 bc
Karabakh-11	58 ab	1.8 bc	1.0 D	38 bc
Agdash-3	52 ab	2.2 ab	1.0 D	41 ab
Barakat	63 a	1.2 c	0.6 F	38 bc
Livzara	52 ab	1.0 c	0.6 F	45 a
Ganja-110	43 b	2.3 ab	1.4 C	39 c
Ganja-114	54 ab	1.6 bc	0.8 E	43 a

*Means values followed by the same letter are not significantly different at the $p < 0.05$ probability level.
[a]WILTi – time of initial Verticillium wilt disease development, reflecting days after planting (DAP).
[b]DS – Disease Severity
[c]VCDS – is on a 0 to 4 scale, where 0 = no vascular necrosis and 4 = 100% vascular necrosis.
[d]Lint – lint percent

Figure 1. a) Cross section of control and inoculated plant stems of the cultivar Alekberi. Control plants (three stems on the left) has no wilting symptoms; inoculated plants have brown discoloration within the basal part of the stem. b) Verticillium wilt symptoms in roots.

Table 3. Mean values for fiber quality characteristics of the cotton cultivars under VW non-infested and infested conditions.

Cultivars	Upper Mean Length (mm)	Length Uniformity Index (%)	Elongation (%)	Micronaire (unit)	Fineness (m/tex)
No Verticillium wilt (VW)					
Alekberi	29.1 a	93.1 bc	6.6 a	5.0 a	168.0 a
Zafer	27.8 c	91.1 bc	6.2 b	4.9 a	166.0 a
Bayraktar	27.9 bc	95.0 a	6.5 a	4.8 a	158.5 a
Karabakh-11	27.3 c	92.8 b	6.4 a	4.8 a	160.0 b
Agdash-3	28.0 bc	95.6 a	6.7 a	5.1 a	171.8 a
Barakat	29.0 a	93.4 b	6.4 a	5.1 a	173.0 a
Livzara	28.3 b	94.9 a	6.7 a	4.2 b	125.0 c
Ganja-114	28.9 a	91.9 bc	6.4 a	5.2 a	178.2 a
Ganja-110	29.5 a	93.2 b	6.5 a	4.7 a	143.0 b
Verticillium wilt (VW) inoculation					
Alekberi	26.0 b	90.8 bc	6.7 a	5.2 a	172.0 b
Zafer	28.5 a	90.5 bc	7.1 a	4.9 a	155.0 b
Bayraktar	26.2 b	97.0 a	6.2 c	4.6 a	137.0 c
Karabakh-11	26.9 b	89.2 c	6.7 a	4.8 a	150.0 bc
Agdash-3	26.2 b	91.3 bc	6.5 b	4.8 a	150.0 bc
Barakat	29.5 a	93.2 b	6.5 b	4.7 a	143.0 bc
Livzara	28.5 a	94.8 b	6.8 a	4.8 a	149.0 bc
Ganja-114	26.5 b	93.6 b	6.5 b	5.0 a	161.0 b
Ganja-110	26.4 b	92.3 bc	6.4 b	5.0 a	193.0 a

*Means with same letter within a column are not significantly different at the $p < 0.05$ probability level.

The average fiber length is the mean length of all fibers in a cultivar sample. The upper mean length was the average length of the longest fibers and further divided into nine fiber types. Cultivars Ganja-110, Alekberi, and Barakat had the highest mean lengths (29.5, 29.1, and 29.0 mm, respectively) in the variant with no VW inoculation. These results were greatly analogous to past findings in crop plants (Mammadova *et al.,* 2024). In cotton cultivars with the VW inoculation, the said indicator has decreased in all the genotypes except the cultivar Bayraktar, which had the topmost upper mean length (29.5 mm) with VW infestation. Uniformity measurement of cotton fiber occurred by the variations in fiber length. A higher value indicates fewer short fibers surfaced and more regularity in the fiber length. A slight decrease in fiber uniformity was remarkable in all genotypes with the VW inoculation, except for cultivars Bayraktar and Ganja-114. For comparison, Erdogan *et al*. (2006) also found that verticillium wilt significantly reduces fiber length and fiber strength in cotton.

Micronaire is also one of the vital fiber quality traits in cotton, which characterizes the softness and maturity of the fiber together. According to fiber quality classification, the standard interval could be from 3.5 to 4.9 units. Previous studies indicated that more immature fibers with poor elongation may reach growth in the presence of verticillium wilt infestation. In cotton genotypes, the immature fiber has low fineness and mostly breaks during processing in the ginning industries (Abidi *et al*., 2007; Ayele *et al*., 2018). In the presented study, a slight increase in micronaire was evident in some cotton cultivars with VW infestation versus their healthy counterparts.

For fiber elongation, nonsignificant differences appeared among the genotypes in healthy and diseased plants, except for the cultivar Zafer. Zhang *et al*. (2012) determined that in comparison to the healthy plants, plants with VW had a significantly reduced lint percentage, 50% span length, and micronaire. But 2.5% span length and fiber strength emerged unaffected. Moreover, fiber elongation in the infected plants was significantly higher than in the healthy plants. Similar results were apparent in studies conducted on cotton by Avshar *et al*. (2023). In a study evaluating the impact of verticillium wilt on cotton (*Gossypium hirsutum* L.) fiber quality, they found the reduction of micronaire from 5.0 to 3.6 in the susceptible variety and from 4.4 to 4.1 in the resistant variety. Furthermore, all genotypes with verticillium wilt produced substantially higher nep counts and short fiber content than their healthy counterparts (Ayele *et al.,* 2020).

Based on the fiber quality results, the cultivars Zafer and Livzara proved to have complex fiber quality characteristics. Fiber quality analysis revealed wide diversity of cotton cultivars for various fiber quality traits. Cultivars Zafer and Turkish Livzara were evidently capable of reaching full fiber maturity under the climatic conditions of Azerbaijan. Green and Culp (1990) reported that environmental variability can prevent full realization of the fiber quality potential of a cotton genotype. Azerbaijan's location is on the northern borders of the cotton cultivation geography. In Azerbaijan, the existing production window is relatively shorter than in Turkey, and the early rainy season does not allow imported cultivars to reach their maximum yield potential. The dry season is very short in Azerbaijan, lasting only from June to September. In longer dry seasons, cotton has enough time to achieve its full yield potential and fiber maturity. The local cotton cultivars of Azerbaijan can reach full maturity within 90 days of the production window, allowing farmers to harvest raw cotton by the end of September.

In the presented cotton experiment, the healthy genotypes produced better fiber length distribution normally desired by the spinning technology and textile industries. The cultivar Ganja-110 revealed a short fiber length with the presence of VW inoculation. The length distribution revealed such fibers proved below the spinnable quality, and these types of fibers have limited use in yarn processing. The cultivar Alekberi produced well-defined, longer fiber frequencies and thus, can potentially become spinnable in the textile industries.

CONCLUSIONS

The study, based on an evaluation of fiber quality properties with VW-inoculated plants and their healthy counterparts, indicated that the upper mean fiber length has decreased with the VW inoculation. Meanwhile, a slight decrease in fiber uniformity was evident in VW-inoculated plants except for cotton cultivars Bayraktar and Ganja-114. For micronaire, a slight increase resulted in some cultivars with the VW inoculation. Nonsignificant differences were meaningful among the cotton genotypes for fiber elongation in healthy and diseased plants except for cultivar Zafer. The specific differences were notable in some cultivars (Bayraktar, Agdash-3, and Ganja 114), which can be due to their resistance to verticillium wilt. The results indicated that greater losses will refer to the reduction in basic fiber quality properties that will limit the use of cotton fibers in the textile industries. Utilization of VW-resistant cotton genotypes identified based on genetic and morphological assessment can potentially reduce the economic losses.

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