First Appearance of Verticillium tricorpus Causing Verticillium Wilt in tested Okra varieties.

Farag, M. Mohamed
Ramadan A. A. Arafa
Mohamed AbdElhalim Abou-Zeid (m.abouzeid@arc.sci.eg)
Agricultural Research Center https://orcid.org/0000-0001-8545-4332
Khairiah M. Alwutayd
Diaa Abd El Moneim
Eman W.R. Ghebrial

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Abstract

Symptoms of Verticillium wilt were observed on okra (*Abelmoschus esculentus* L.) grown widely in Beni Suef Governorate (Nasser, Beba and El-Wasta Counties) in summer 2021. All disease symptoms are externally, infected shoots' leaves turn a light green to yellow colour, lose their turgor, and finally desiccate. Individual shoots in a portion of the plant stem may show symptoms, or the symptoms may emerge over the entire plant. In certain cases, the disease typically progresses over months. According to the morphological characteristics of the isolated fungus, disease symptoms and pathogenicity test, *Verticillium tricorpus* was identified as the causal agent of Verticillium wilt of okra. Identification of this species was confirmed by sequencing of internal transcribed space (ITS region) of ribosomal RNA gene. V. *tricorpus* absolutely has not previously been reported on okra. The sequencing of this fungus showed close ties with *V. tricorpus*, as evidenced by the 99.24–100% identity and 97–100% coverage with several strains of *V. tricorpus*, including the type strain CBS447.54 (NR_126128). The obtained sequences were deposited in the GenBank with accession number MZ936483. Pathogenicity tests confirmed that *V. tricorpus* was pathogenic showing the same disease symptoms previously observed on okra plants in the surveyed areas. The infection sensitivity showed that Iranian red cv. is more sensitive to infection than Balady green cv. This appears to be the first record of *V. tricorpus* associated with Verticillium okra wilt disease.

INTRODUCTION

Okra (*Abelmoschus esculentus* (L.) Moench) is an annual plant in the Malvaceae family, which is widely distributed around the world. Okra's entire plant, including the roots, leaves, flowers, and seeds, has significant economic worth because they are frequently used as a vegetable or for therapeutic purposes (Camciuc et al 1998). Okra was grown in Egypt in 2009 on an area of around 22203 feddans, yielding roughly 134665 tonnes on average (Anein, 2010). Immature fresh pods are cooked or fried like a vegetable, whereas dried pods are highly well-liked for adding to stews and soups. Additionally, they can be powdered and used as flavouring (Tindall, 1983). Fresh pods contain 88% water, 2.1 g. of protein, 0.2 g. of fat, 8 g. of carbohydrates, 1.7 g. of fibre, 1.2 g. of iron, 185 g. of beta-carotene, 0.04 mg of thiamine, 0.08 mg of riboflavin, 0.08 mg of niacin, and 47 mg of ascorbic acid. However, seeds contain protein ranged from 15% and 26% as well as more than 14% of edible oil. (NARP, 1993). Vascular wilt disease is among the most destructive and significant commercial plant diseases in the world, which are brought on by soil-borne pathogens (Tjamos and Beckman 1989). One of the worst and most pervasive vascular diseases that affects vegetables, ornamental plants, and tree crops is known as Verticillium wilt, and it can be brought on by a variety of Verticillium species. A group of fungi known as Verticillium has a lengthy taxonomic history. Up until now, 190 species have been described (Zare, et al., 2004). Major pathogens are involved in the production shortage of okra in several ways. Numerous species of *Alternaria alternata, Aplosporella beaumontiana, Cercospora abelmoschi, Choanephora conjuncta*, and *Fusarium oxysporum* are present in all continents (Kobayashi, 2007). *Verticillium tricorpus* Isaac was first reported as a pathogen on tomatoes (*Lycopersicon esculentum* Mill.) in England (Isaac 1949) and later isolated from melon (*Cucumis melo* D L.) in California (Smith 1965). Keyworth (1952) isolated a *Verticillium* sp. from potato (*Solanum tuberosum* L.) that produced both microsclerotia and dark mycelium and classified the isolate as *V. albo-atrum* Reinke & Berthold. The isolate was subsequently identified as *V. tricorpus* (Smith 1965). *V. tricorpus* has also been isolated from tomatoes, potatoes and cotton (*Gossypium* hirsu*tum* L) in Canada, the Netherlands, Israel and North America (Moukhamedov et al. 1994). In California, *V. tricorpus* is considered a weak pathogen of potato, reducing yield and crop quality and occurring in association with *V. dahliae* Kleb. or *V. albo-atrum* (Smith 1965). Like the latter *Verticillium* spp., *V. tricorpus* can be transmitted on propagating material (Moukhamadov at al.1994).

The present study's goals were (i) Analyses the morphological, cultural, and biometrical characteristics of the *Verticillium* isolates isolated from different hosts in different geographical areas. (ii) To establish the organism's identity and to define its pathogenicity toward the new host in Egypt. (iii) To evaluate which okra types may tolerate the infection with *V. tricorpus.*

MATERIALS AND METHODS

Isolation and identification

To assure the collection of all pathogens responsible for plant wilting, isolates were obtained from the all parts of plants with vascular discolouration. The plant pegments were carefully cleaned with tap water and divided into 0.5 cm^2 pieces. The plant pieces were cleaned on the surface for 3 minutes with 0.5% NaOCl, dried on sterile filter paper, and then resuspended onto PDA medium containing 300 mg/l streptomycin sulphate. Four or five pieces were applied per plate for each plant tissue to isolate the main pathogen on general medium, such as PDA. At 22°C, the plates were incubated for a two-week period (Platt and Bollen, 1995). The isolate was then determined using published descriptions while each culture only contained single spore. (Hawksworth, 1970a and 1970b; Hawksworth. and Talboys 1970a and 1970b and Isaac, I. 1967). All *Verticillium* spp. isolates were maintained in a 25 percent aqueous glycerol solution at –20°C as monoconidial subcultures (Robb, 2000.). Colony parameters (conidia size, size and prevalence of resting structures, and colony color) were assessed following three to four weeks of incubation at 20°C on PDA. The lengths and widths of 100 randomly selected conidia, as well as phialides' morphology was evaluated 21 days after incubation. Whereas after 21–28 days of incubation, the diameters of 50 microsclerotia and chlamydospores per isolate were measured.

Fungal isolate identification using molecular means:

In sterile Petri dishes with autoclaved potato dextrose agar (PDA) media, the fungal isolates were cultured for 7 days at 28°C (Pitt and Hocking, 2009.(A Patho-gene-spin DNA/RNA extraction kit from the Korean company Intron Biotechnology was used to extract the DNA from the cultures at Assiut University's Molecular Biology Research Unit, Egypt. Then, for PCR and rRNA gene sequencing, fungal DNA samples were sent to SolGent Company in Daejeon, South Korea. Using ITS1 (forward) and ITS4 (reverse) primers that were added to the reaction mixture, PCR was carried out. The following composition make up primers: ITS1 (5' - TCC GTA GGT GAA CCT GCG G – 3'), and ITS4 (5'- TCC TCC GCT TAT TGA TAT GC -3'). ddNTPs were added to the reaction mixture together with the identical primers to sequence the purified PCR result (amplicons) (White et al., 1990). The Basic Local Alignment Search Tool (BLAST) on the National

Centre for Biotechnology Information (NCBI) website was used to analyze the obtained sequences. MegAlign (DNA Star) software version 5.05 was used to perform phylogenetic analysis on the sequences.

Pathogenicity trail

On healthy okra cultivar cv. Iranian red, the pathogenicity test was performed. Conidia were obtained from PDA cultures that had been incubated for 20 days at 22 ± 2°C in the dark. Using a hemocytometer, the autoclaved water-created spore suspension's density was adjusted to $1x10^6$ spores/ml. The inoculum was mixed with the soil in plastic pots with a 25 cm diameter at the rate of 3% (v/w), then apparently healthy seeds were sown in the infested soil (5 seeds/pot). Three replicates were used in the experimental pots, along with an uninoculated negative control, in a randomized complete block design.

Thirty days after inoculation, when external symptoms (yellowing, wilting, and leaf fall) and internal symptoms (darkening of the veins) were experienced, a subgroup of particularly susceptible accessions was assessed. Using an ordinal disease modified severity scale (Reis et al., 2004), the plant responses were evaluated. This scale had grades from 0 to 4, with 0- plants with no symptoms, 1- plants without wilt or yellowing symptoms, but with darkened vascular bundles, 2- plants with intensely darkened vascular bundles and with early signs of wilt or yellowed leaves, 3- plants with severe wilt, associated with premature leaf drop, and 4- dead plants. A disease index (DI) was derived from the grades essentially according to McKinney's (1923).: DI (%) = 100.Σ[(f.v)/(n.x)], where DI = disease index; f = number of plants with the same grade; v = observed grade; n = total number of plants evaluated and x = maximum grade on the scale. Data sets were tested for percent infection using the following formula (Uppal et al., 2007):

Infection (%) = (CL/TL) × 100

CL represents the number of chlorotic leaves, while TL is the total number of leaves on a tested okra plant.

Varietal reaction:

At Sids Agricultural Research Station, five okra cultivars were obtained from vegetable research breeding and chosen to test their reactions to infection with V. tricorpus i.e., Balady green, Balady red, Gold cost, Iranian red and Leady finger. With the same method mentioned under pathogenicity test, the seeds of all cultivars were sown in greenhouse. Each cultivar comprised three replicates of 15 seeds, Plants were used as the typical host for the severe vascular wilt symptoms to manifest, and daily observations were made for the development of foliar symptoms like chlorosis, necrosis, and premature defoliation.

Instrument Condition for Salicylic acid:

Agilent 1260 infinity HPLC Series (Agilent, USA), equipped with Quaternary pump cl Column Hyper CloneTM 8DS C 18, 130A 100 mm x 4 6 mm (Phenomenex® USA ! operated at 45°C. The separation is achieved isocratic (A) HPLC grade water 0 1% H:,P04 (\f/v), (Tjamos and Beckman 1989). Acetonitrile 90 10 The injected volume was 20 IJL DetectionVV'/C detector set at 254 nm, Fluorescence detector adjusted at an excitation –vavelenqih = 272 nm excitation, and an emission wavelength = 313 nrn.

Instrument Condition for phenolic compounds:

Agilent1260 infinity HPLC Series (Agilent, USA). equipped with Quaternary pump. The column used: aKinetex®5l-lm EVO C18 100 mm x 4.6 mm, (Phenomenex. USA). operated at 30°C. The separation is achieved using a ternary linear elution gradient with (A) HPLC. grade water 0.2% H3P04 (v/v), (8) methanol and (C) acetonitrile The injected volume was 201-lL. Detection VWD detector set at 284 nm.

Histology

Microtechnique procedures were carried out at Agric. Bot. Depart. Faculty of Agric. Cairo University. For at least 48 hours, materials were killed and repaired. Before embedding in paraffin wax with a melting temperature of 56°C, the sample was placed in F.A.A. (10ml formalin, 5ml glacial acetic acid, 85 ml ethyl alcohol 70%) and dehydrated in a normal butyl alcohol series (Sass 1951). Sections have been cut with a rotary microtome at a thickness of 15–20 microns and stained with crystal violet/erythrosine before mounting in Canada balsam. The slides were examined and taken photos under a microscope.

Statistical Analysis

Using the computer programme Web Agri Stat Package (WASP), the data were statistically analysed using a complete randomised blocks design, and the least significant differences (LSD 0.05) were determined in accordance with Fisher (1948), Snedecor and Cochran (1967), and Duncan's Multiple Range and Multiple F test (1955).

RESULTS

Symptoms:

Externally, infected shoots' leaves turn a light green to yellow color, lose their turgor, and finally desiccate. Individual shoots in a portion of the plant stem may show symptoms, or the symptoms may emerge over the entire plant. In certain cases, the disease typically progresses over months resulting in progressive defoliation, shoot dieback, and final death of the plant. During the hotter parts of summer days, leaves lose turgor and the leaf margins roll upwards; however, when the temperature is lowered near night, some recovery may occur. Stunting is another characteristic of Verticillium infection in plants. Other symptoms of the illness include V-shaped chlorosis of leaflets, or the appearance of a yellow-to-red-brown lesion around the leaf tip, followed by desiccation and abscission.

Such leaves eventually perish and develop a pale reddish-brown color. Internal tissues of the petioles and pedicels of the flower can also become brown in extreme cases, causing the plants to wilt and die. (Fig. 1), The remainder of the plants were badly stunted and produced a poor crop.

Morphological characterization:

On PDA and corn-meal agar at 23^{o} C, growth was quite quickly. Prostrate hyphae were the first to form and were typically orange-yellow in color before turning blackish after two to three weeks. Within one week, the orange-yellow prostrate hyphae began to generate vertical white conidiophores, which in two to three weeks turned the colony white. In the colonies, hyaline sectors commonly surfaced. There were many hyalines, vertically branching conidiophores that were more or less erect, with 3–4 phialides sprouting at each node. Phialides developed conidia apically and ranged in size (12–25 x 2–3 µm). The isolate's morphology was characteristic of *V. tricorpus.*

Pathogenicity

Successful inoculations with *V. tricorpus* were achieved. Twenty-one to twenty-eight days following the inoculation, the inoculated okra plants began to exhibit symptoms. Chlorosis on the lower leaves and typical V-shaped patches in the leaf margins, which finally led to senescence and necrosis after around 1–2 weeks, were observed as typical Verticillium wilt symptoms. Light brown discoloration was visible in longitudinal sections of the basal stems, demonstrating the pathogen's colonization of the vascular tissue. Branching conidiophores and oval conidia (either free or in a verticillate arrangement) were visible under a microscope in infected tissues after kept in a wet chamber for two days and the fungus was recovered from the wilting plants.

PCR identification:

Diseased okra plants (cv. Leady finger) were gathered in Beba county, Beni Suef governorate, during the 2020 growing season. Culture of *V. tricorpus* (AUMC 15110) was isolated from these plants. The typical *V. tricorpus* generated chlamydospores (6.5 to 10 m), round or irregular microsclerotia (58 to 88 m), verticillate conidiophores, dark resting mycelium (3.7 to 7.2 m), and grey to black colonies Fig. (2).

Using the ITS region of rDNA and the primers ITS1/ITS4, this isolate's identification as *V. tricorpus* was verified. The GenBank accession number for the V. tricorpus isolates TASVt1 and TASVt2's sequences is MZ936483. A BLASTn investigation of the 478-bp section of these isolates revealed 99.24% − 100% identity and 97% − 100% coverage with various strains of V. tricorpus, including the type strain CBS447.54 (NR_126128). V. = Verticillium The representative strain was shown to constitute a separate clade from the other by way of phylogenetic analysis, but was found to cluster with recognized *V. dahliae*.

F-2: **Verticillium tricorpusAUMC 15110 (527 letters)**

CTGCGGAGGGATCATTACCGAGTATCTACTCATAACCCTTTGTGAACCATATTGTTGCTTCGGCGGCTCGTCCGCGAGCCCGCCGGTACATCAGTCTCTTTATTTTTACCA
ACTATTAAAACTTTTAACAACGGATCTCTTGGCTCTAGCATCGATGAAGAACGCAGCGAAACGCGATATGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGA
GGGAGGCATGCCTGTCCGAGCGTCGTTTCAACCCTCGAGCCCTAGTGGCCCGGTGTTGGGGATCTACTTCTGTAGGCCCTTAAAAGCAGTGGCGGACCCGCGTGGCCC
ATCGGAGTCCCGCAGGCACCAGCCTCTAAACCCCCTACAAGCCCGCCTCGTGCGGCAACGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATA

Varietal reaction:

Data in Table, 1 show that all tested plants of the five cultivars were significantly infected with *V. tricorpus* when transplanted in soil infested with the pathogen during the two growing seasons, the highest disease incidence and severity were observed on Iranian red cultivar which was the most susceptible and recorded 33.3 and 25%, respectively in the first season (summer 2021) but less than the second season (summer 2022) in these incidence only were 40 and 30%, respectively. On the other hand, Balady green cultivar was the most resistant and recorded the lowest incidence and severity being 6.67 and 3.33% in the first season where in the second season were these figures same. Whereas, Gold cost was moderately of resistant and recorded **13.33** and 10%, respectively disease incidence and severity in the first season where in the second season were 20 and **13.33**% respectively.

Data presented in Table, 2 showed a markedly detrimental effect of *V. tricorpus* colonization on the major growth parameters, including plant height/plant (cm), fruit weight/plant (g) and fruit number/plant in different okra cultivars were detected compared with uninfected control (healthy control) in both seasons. Iranian red cultivar experienced significant decreases in plant height, fruit weight, and fruit number from 65.67 cm to 34.00 cm, 82.33 g to 50.33 g, and from 12.33 to 7.00 on average, respectively in inoculated and non-inoculated during 2021 growing season, and from 71.00 cm to 39.33 cm, 83.33 g to 42.33 g and from 11.67 to 6.00 on average, respectively in the 2022 growing season. On the opposite side, the lowest reduction in plant height (81.00 cm), fruit weight (296.00 g) and fruit number (18.67) were recorded in the most resistant cultivar (Balady green) in inoculated plants compared with non-inoculated plants which gave 87.33 cm, 304.00 g and 22.00, respectively in the first growing season followed by Gold cost cultivar. The same was true in the second growing season (2022). Balady red and Leady finger cultivars showed moderate decrease in this respect.

Results in Table 3 reveal that Balady green-infected plants have much higher levels of the oxidative and hydrolysis enzymes i.e., peroxidase and dehydrogenase, being 12.6 and 25.5 compared with healthy plants, being 10.8 and 8.6 respectively. Meanwhile, infected plants of Iranian red (cv,) showed the lowest activity of the same enzymes, being 8.4 and 28.4 compared with the healthy plants being 6.3 and 22.1 respectively. On the other hand, diminishing of Chlorophyll (a and b) and Carotenoides between leaves of infected and healthy Balady green plants were less than those of Iranian red plants. Whereas, these figures in Gold Cost (cv,) were moderate.

Chromatogram analysis using HPLC: (284nm).

The results of the HPLC analysis of phenols in okra's roots extract are presented in Table 4 and Fig., A. Balady green variety-related marking appeared in the tolerant plants a mixture of different chemically active phenols and flavonoids compounds, i.e., Vanillic acid, Syringic acid, Ferulic acid, Ellagic, o- Coumaric acid, Quercitin, rosemarinic, Myricetin, Kampherol and Salicylic acid recorded 94.24, 9.40, 23.10, 12.60, 2.36181, 9.10, 42.40, 16.46, 3.94 and 309.16

respectively by the total 562.16mg/kg, compared to the susceptible variety (Iranian red) which recorded the lowest amounts of the same phenols and flavonoids were being 202.28 mg/kg.

Anatomy of infection caused by *V. tricorpus* on the tissues of okra cultivars plants (Verticillium wilt): As shown in Fig. 4, for observation of colonization and proliferation in cross sections of roots of three okra cultivars inoculated with *V. tricorpus*. firstly, mycelium was observed in the conducting tissues of diseased roots. The dark occlusions and tyloses in vascular tissue roots of Iranian red cultivar (A, B). This shows that an immune response specific to a cell type may cause the formation of a physical barrier in xylem vessels. Moreover, the xylem parenchyma cells can expand into tyloses (outgrowths of the cell that bulge into the vessel lumen via the pit), and these tyloses can produce substances that resemble gels, potentially preventing the pathogen's vertical spread, where the growth of the mycelium was shown lower in Balady red (cv), so the vessels tissue were infected less with the hyphae. On the other hand, Balady green cultivar showed resistance to the infection and tissues of vessel were been cleared (E, F).

Discussion

Verticillium wilt, which caused by a variety of *Verticillium* species, is a common disease and one of the worst and most pervasive vascular diseases that affect vegetables, ornamental plants, and tree crops. A group of fungus known as *Verticillium* has a lengthy taxonomic history. There have been about 190 species described so far (Zare et al., 2004).

The history of discovering *Verticillium tricorpus* Isaac was reported as the first pathogen on tomatoes *(Lycopersicon esculentum* Mill.) in England (Isaac 1949) and later isolated from melon *(Cucumis melonis* D L.) *V. tricorpus* has also been isolated from tomatoes, potatoes and cotton *(Gossypium hirsutum* L) in Canada, the Netherlands, Israel and North America (Moukhamedov et al., 1994). In California, *V. tricorpus* is considered a weak pathogen of potato, reducing yield and crop quality and occurring in association with *V. dahliae* Kleb. or *V. albo-atrum* (Smith, 1965). Recently, new pathotypes of *V. tricorpus* that attack tomato, eggplant and potato have been discovered in Tunisia.

There is currently general agreement that it is ineffective and unreliable to identify *Verticillium tricorpus* solely based on physical characteristics. In this case, phylogenetic analysis of genomic areas like the ITS has been used as a more trustworthy diagnosis method. Our research, which used ITS data only, gave high resolution to distinguish AUMC15110 (one reference *V. tricorpus* isolate), leading in an unresolved consensus tree in connection to *V. dahliae* or *V. albo-atrum* group. Thus, DNA sequence comparisons based on the ITS region alone would incorrectly identify *V. tricorpus* as *V. dahliae* or *V. albo-atrum* lineages and this is in agreement with Qin et al. (2008) as reported previously.

According to our knowledge, this is the first time that V. tricorpus isolated from okra growing in Egyptian soil has been described.

The aggressiveness of *V. tricorpus* was revealed by the disease severity evaluation done 30 days after inoculation by observations of leaves' symptoms of yellowing, wilting and defoliation as well as vascular discoloration. These symptoms and fungal description are resembling with results of Uys *et al.* (1993).

Our results of varietal reaction confirmed the high levels effect of *V. tricorpus* which were aggressiveness on Iranian red cultivar. The reported here were similar with verticillium wilt that reported by Uys *et al.* (1993).

Once the pathogen is present, the host plant initiates immune defenses, such as reducing the production of some biochemical components such as soluble sugars, phenolic compounds, hormones, or reactive oxygen species (ROS) [Tarkowski, 2019]. Increased phenolic compound level renders pathogens toxic and inhibits the spread of infection. Phenolics promote the lignification of the cell wall, strengthening the structural barrier preventing the disease from spreading throughout the host plant tissue. Lignification may reduce the flow of nutrients from the host plant cell to the pathogen (Nicholson and Hammerschmidt, 1992). Due to their dangerous character, phenolic substances—such as phytoalexins—are thought to activate genes associated with disease resistance and modulate the toxicity of pathogens (Zaynab et al., 2018). Another way of avoiding pathogen invasion entails the synthesis of both enzymatic and non-enzymatic antioxidants as well as the scavenging of reactive oxygen species (ROS) (Walter et al., 2009). In addition to free radicals like superoxide anion ($O2^{\bullet}$) and hydroxyl radical ($OH^{\bullet}$), the ROS also comprises non-radical molecules like hydrogen peroxide (H_2O_2) and singlet oxygen (Das and Roychoudhury, 2014). Three activities may be accomplished by reactive oxygen species:

They can function as molecules that transmit signals, damage cells, and guard against hazardous pathogens (De Gara et al., 2010). An oxidative burst is a common phrase used to describe excessive ROS generation. Overproduction of ROS can cause lipid peroxidation, nucleic acid damage, protein and chlorophyll oxidation, as well as the start of programmed cell death (Foyer & Noctor, 2005 and Zurbriggen et al., 2009). Reactive oxygen species are prevented from accumulating through the activation of enzyme-based antioxidants like catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD), as well as non-enzymatic antioxidants such low molecular weight (LMW) phenolics and carotenoids (De Gara et al., 2010; Barna et al., 2012 and Waśkiewicz et al., 2014). The regulation of the amount of H_2O_2 in plant tissues is carried out by catalase, which also breaks down H_2O_2 into H_2O and O_2. In addition to being engaged in plant development, this enzyme is essential for disease resistance and aging processes (Yang and Poovaiah, 2002). Similar to CAT, peroxidases are implicated in scavenging ROS as a result of pathogen-plant interactions. Additionally, POXs are in responsible of lignin production, suberization, and the expansion of the cell walls of plants (Madadkhah et al., 2012). They also oxidize phenolics, creating them more hazardous to pathogens. In plants under stress, superoxide dismutase is equally important for preserving redox equilibrium and defense mechanisms. Its role is to catalyze the conversion of the hydroperoxide radical ($HO2^{\bullet}$) and oxygen ion ($O2^{\bullet}$) into H2O2 and water. As the first line of defense against pathogen infections, superoxide dismutase protects plants from oxidative stress (Wang et al., 2016). Pathogen defense also significantly relies on hydrogen peroxide. It can help plants develop both local and systemic resistance to pathogen invasion because of its antifungal activities (Gechev and Hille, 2005). The level and activity of chlorophyll pigments can fluctuate in response to the presence of pathogens, altering the effectiveness of photosystem II (PS II) (Hao et al., 2009). Similar results were achieved by other research looking at the photosynthetic pigment concentration in tomato after *F. culmorum* infection (Alwathnani, 2012) and barley (Warzecha et al., 2015).

Wu, Liu, et al. (2009) and Wu, et al. (2008) explored the effect of artificial application of benzoic acid on the soilborne pathogen *F. oxysporum* of watermelon Fusarium wilt. They reported that benzoic acid significantly inhibited the growth, sporulation, and spore germination of the pathogen, which is similar to our results. Wu et al. (2008) and Wu et al. (2009) demonstrated that although benzoic acid decreased the growth and reproduction of *F. oxysporum*, it also caused the synthesis of mycotoxins, which can be thought of as a compensating mechanism. Notably, cinnamic acid stimulate the production of mycotoxins by *F. oxysporum* (Wu et al., 2008). In other words, although the level of *F. oxysporum* decreased, the remaining pathogens triggered a new mycotoxin production pathway to enhance virulence against the benzoic acid-producing host (Wu et al., 2008).

This is consistent and was in harmony with our results. We found that there were ten main phenolic acids, namely., Vanillic acid, Syringic acid, Ferulic acid, Ellagic, o- Coumaric acid, Quercitin, rosemarinic, Myricetin, Kampherol and Salicylic acid. Remarkable, two-fold increase of concentrations of these compounds within in Balady green (cv.) compared with the susceptable cultivar (Iranian Red). In light of the foregoing, the results of the current investigation indicate that, as has already been mentioned in other plant-pathogen interactions, phenolic and separated compounds play a significant role in the defense mechanism of okra (Balady green cultivar) against *V. tricorpus*. (Nicholson and Hammerschmidt, 1992; Roussos et al., 2002; Cayuela et al., 2006 and Baidez et al., 2007). Since discovering the biochemical mechanism behind the reported *V. tricorpus* resistance of 'Balady green' and other resistant okra cultivars would represent a significant advance in the fight against one of the most dangerous okra contemporary diseases, it is obvious that more research is required.

In conclusion, this is the first in-depth investigation of *V. tricorpus* to concentrate on populations from okra plants. Our findings imply that *V. tricorpus* is a very damaging and competitive pathogen of okra, and that a significant inoculum source for the disease may be from established populations in fields that spread along with a variety of prior host plants.

Declarations

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Tables

Table (1): Varietal reaction of okra cultivars against Verticillium wilt caused by *V. tricorpus* during two growing seasons., 2021-2022.

N	Cultivars	Summer 2021		Summer 2022	
		DI%	DS%	DI%	DS%
1	Balady green	6.67	3.33	6.67	3.33
2	Balady red	20.00	10.00	26.67	16.67
3	Gold cost	13.33	10.00	20.00	13.33
4	Iranian red	33.30	25.00	40.00	30.00
5	Leady finger	20.00	11.67	26.67	18.33
	LSD 0.05	7.20	3.04	4.97	4.25

Table 2: Effect of okra verticillium wilt on some plant growth parameters during the two successive growing seasons.

N	Varieties	Season 2021						Season 2022					
		Diseased plants			Control plants			Diseased plants			Control plants		
		Plant height/plant(cm)	Fruit weight /plant (g)	Fruit no. /plant	Plant height(cm)	Fruit weight /plant (g)	Fruit no. /plant	Plant height/plant (cm)	Fruit weight /plant (g)	Fruit no. /plant	Plant height /plant (cm)	Fruit weight /plant (g)	Fruit no. /plant
1	Balady green	81.00	296.00	18.67	87.33	304.00	22.00	96.00	291.00	18.33	103.67	300.00	21.33
2	Balady red	57.33	113.00	10.00	74.33	140.00	14.67	71.00	109.67	10.00	89.33	135.00	15.00
4	Gold cost	38.00	296.33	20.33	50.00	303.67	24.00	35.67	283.00	19.33	45.00	301.00	23.67
3	Iranian red	34.00	50.33	7.00	65.67	82.33	12.33	39.33	42.33	6.00	71.00	83.33	11.67
5	Leady finger	72.33	173.67	16.67	89.00	198.33	21.33	74.67	184.33	15.00	92.67	209.67	19.33
L.S.D. at 0.05		1.774	1.700	2.346	1.009	2.027	1.486	1.326	3.238	1.009	0.821	2.312	1.325

Table (3): Effect of infection with *V. tricorpus* on some plant parameters under greenhouse conditions .

Num.	Treatments		Peroxidase	Chl. a	Chl. b	Carotenoides	Dehydrogenase
1	Balady green	infected	12.6 a	0.673 b	0.207 b	0.305 a	25.5 c
2		Healthy	10.8 ab	0.691 b	0.213 b	0.306 a	8.6 e
3	Gold Cost	infected	10.2 ab	0.668 b	0.21 b	0.291 a	31.6 a
4		Healthy	8.7 bc	0.699 b	0.254 b	0.319 a	24.4 cd
5	Iranian	infected	8.4 bc	0.71 b	0.249 b	0.299 a	28.4 b
6	red	Healthy	6.3 c	0.976 a	0.648 a	0.327 a	22.1 d
LSD			2.71	0.11	0.06	0.11	2.55

Table (4): HPLC chromatogram of okra cultivars extracts after one week of inoculum with *V. tricorpus*, detected on wavelengths: 284 nm

Phenols & Flavonoids	Varieties (phenols and flavonoids amount [mg/kg])				
	Baladygreen	Leady finger	Balady red	Gold cost	Iranian red
3-Hydroxytyrosol	—	–	—	58.34	—
Catechol	20.43	12.36	8.51	9.37	74.99
p- Hydroxy benzoic acid	12.45	22.07	12.22	17.64	18.91
Catechin	—	5.05	4.12	4.29	—
Chlorogenic	4.71	49.48	3.75	—	34.15
Vanillic acid	94.24	12.40	78.66	92.88	—
Caffeic acid	1.81	2.20	—	—	1.55
Syringic acid	9.40	7.04	4.89	2.38	5.44
p- Coumaric acid	–	–	—	—	4.95e-2
Benzoic acid	—	–	—	25.84	—
Ferulic acid	23.10	7.47	2.37e-1	5.86	8.84e-1
Rutin	—	9.43e-1	17.63	31.08	—
Ellagic	12.60	7.95	8.20	8.39	4.04
o- Coumaric acid	2.36	1.97	—	1.87	—
Resvertol	—	–	—	—	—
Cinnamic acid	—	–	—	—	—
Quercitin	9.10	3.06	2.36	—	4.99
rosemarinic	42.40	13.02	—	—	9.31
Neringein	—	–	—	—	20.33
Myricetin	16.46	–	6.37	—	6.85
Kampherol	3.94	2.21	1.64	—	3.29
Salicylic acid	309.16	81.65	59.73	145.11	4.64
Totals	562.16	237.37	210.45	403.05	202.28

Figures

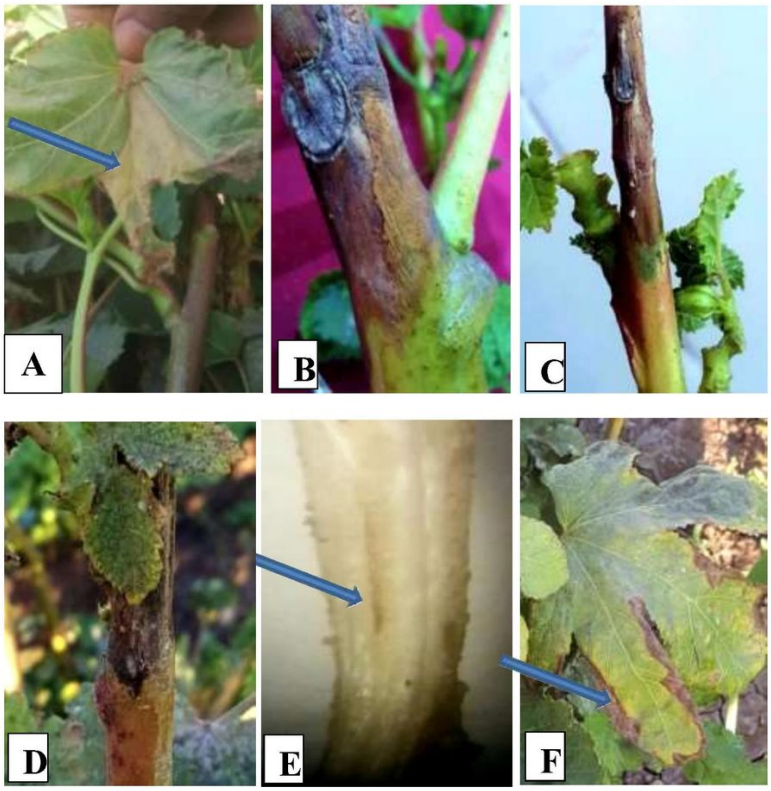

Fig. (1):Symptoms of Verticillium wilt on okra in the field. V shape on an okra leaf seedling (A); Verticillium wilt symptoms on an okra stem in the field. (A, B, C, and D) ; brown Coloring of the vascular bundles of the root (E) The margins of mature leaves are drying off (F).

Figure 1

See image above for figure legend.

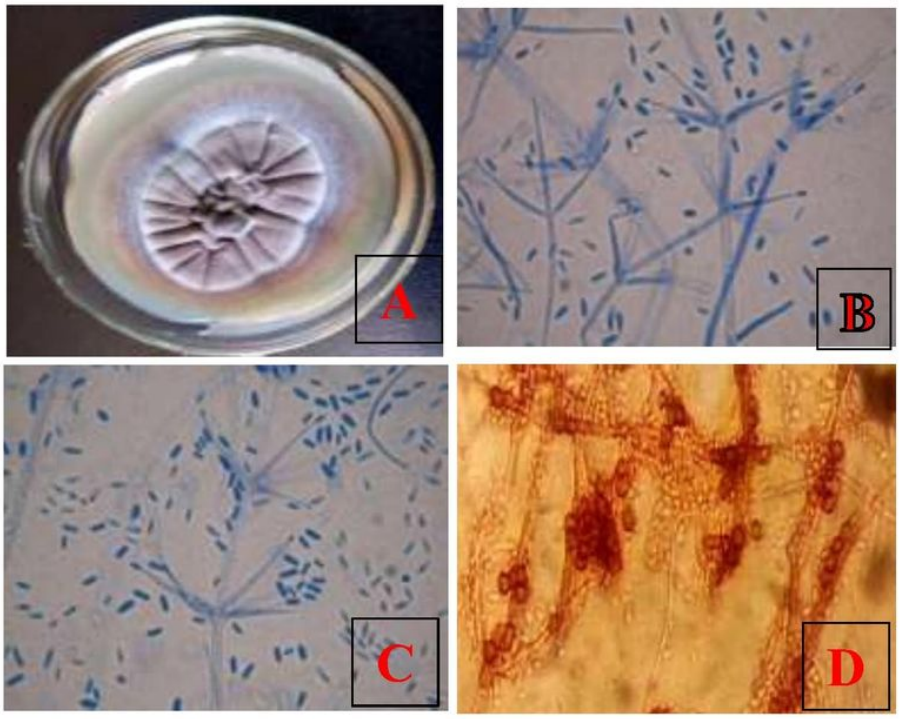

Fig. (2): Cultural characteristics and morphological features of *V. tricorpus*. (A) Colony grown on PDA at 22 °C for 20 days. (B). Mycelium and verticillate conidiophores of the isolate. (C) Conidia of the isolate. Conidiophores and conidia, (D) Resting mycelium and irregular microsclerotia of *V. tricorpus*,

Figure 2

See image above for figure legend.

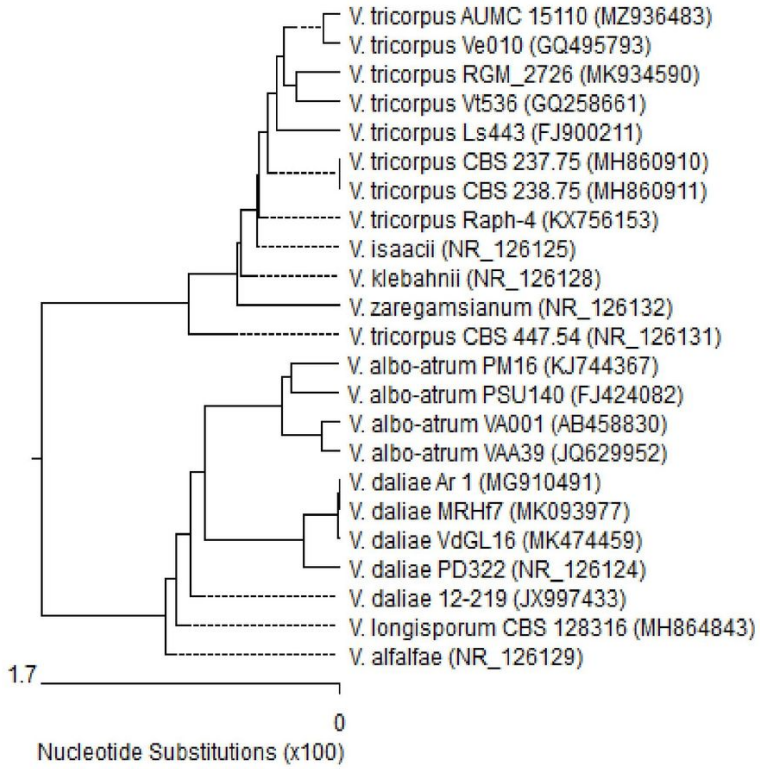

Fig. (3): Phylogenetic tree based on ITS sequences of rDNA of the fungal sample isolated in the present study (*Verticillium tricorpus* AUMC15110 with GenBank accession no. MZ936483, arrowed) aligned with closely related strains accessed from the GenBank (GB). It showed 99.24% - 100% identity and 97% - 100% coverage with several strains of *V. tricorpus* including the type strain CBS447.54 (NR_126128). *V.* = *Verticillium*

Figure 3

See image above for figure legend.

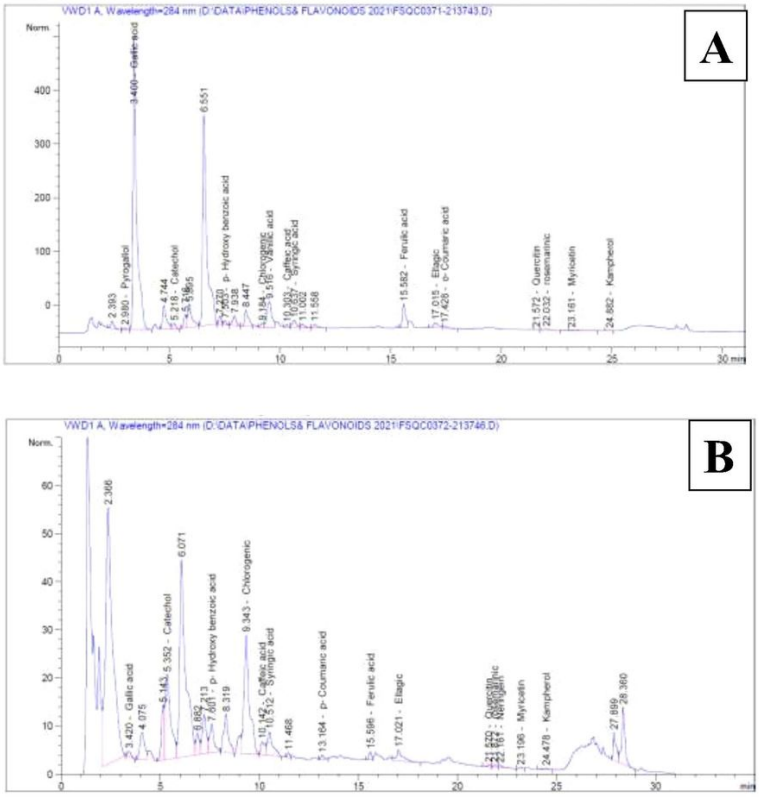

Fig.(4): HPLC chromatogram of extract from okra cultivars (A) Balady green (resistant) and (B) Iranian red (susceptible) one week after Verticillium inoculation detected on wavelengths: 284 nm

Figure 4

See image above for figure legend.

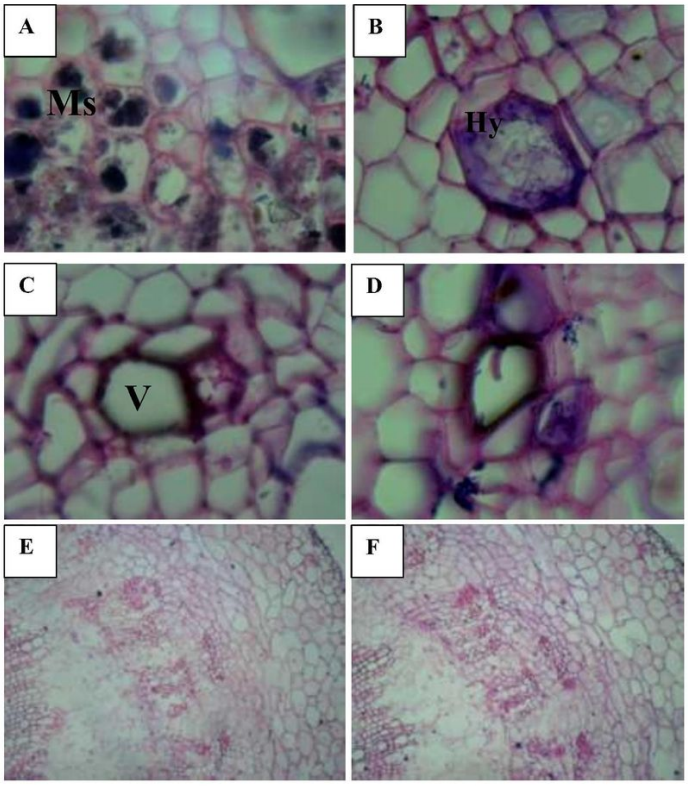

Figure (5): Three varities of okra, (A and B) represent showing Iranian red susceptible (CV) showing hyphae and micro sclerotium in vascular tissues; (C and D) represent Gold cost moderate (CV.)showing low growth of hyphae (Hy) and micro sclerotium (Ms) in the vascular tissues(V); (E and F) represent Balady green resistant (CV.) showing vascular tissues clear and free of hyphal growth.

Figure 5

See image above for figure legend.