

RELATION BETWEEN FUSARIUM WILT DISEASE AND ACCUMULATION OF PHENOLIC COMPOUNDS WITHIN RESISTANT AND SUSCEPTIBLE TOMATO CULTIVARS

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ABSTRACT: Wilting disease of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*, (FOL) is one of the most destructive diseases. This disease causes significant yield losses in Egypt. Ten *Fusarium* isolates were isolated from wilted tomato plants growing in El-Menoufia Governorate during 2020 growing season. The most virulent isolate (isolate No. 6) was tested for its virulence on ten tomato cultivars under greenhouse conditions. The cultivars were significantly varied in their susceptibility toward the tested virulent isolate. The most susceptible cultivar was Carmen F1cv as it recorded the highest value of infection (100% and 96%) in seeds and seedlings experiments, respectively. On the other hand the results showed that Diamond Arwa cultivar was the most resistant tested cultivar where zero infection percentage was recorded on both seeds and seedling experiments. The chemical analysis using HPLC was conducted with the most susceptible tomato cultivar (Carmen) and the most resistant one (Diamond Arwa) one and three weeks post inoculation with pathogenic isolate of *Fusarium*. The results revealed that the accumulation of total phenolic compounds either in shoots or roots was higher three weeks after inoculation than one week post inoculation within the two tested cultivars. Remarkable that roots and leaves of most resistant cultivar tested recorded more total phenol concentrations rather than the susceptible one. Significant differences were detected among the resistant and the susceptible cultivars tested with the compounds detected at 21.2m; 26.2m and 29.1 minute in both shoots and roots three weeks after pathogen inoculation which suggesting potential role of these particular chemicals in resistance mechanism toward *Fusarium* wilt disease within resistant tomato cultivars.

Key words: *Fusarium* wilt-HPLC-Phenolic compounds- Tomato

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is systematically found in the Solanaceae family. It is considered economically the most important and popular vegetables throughout the world. Tomato is one of the most important produced and consumed agricultural products next to potato in Egypt and all over the world (Hafez *et al.*, 2012). Many diseases affect quality and quantity of tomato and cause substantial economic

loss (Pritesh & Subramanian, 2011). The vascular wilt of tomato caused by *Fusarium oxysporum* f.sp. *lycopersici* (FOL), is one of the most destructive diseases in history, indicating significant yield losses (Anam *et al.*, 2017 and Aleaghae *et al.*, 2018). FOL causing the symptoms of root rot, damping off, wilt diseases and death of the plants (Abu-Taleb *et al.*, 2011). In the last decade, *Fusarium* caused severe damage to tomato cultivars in Egypt (Sagitov *et al.*, 2008). The dangerous of FOL is rising

from its ability to survive for long periods of time under field conditions. Infection of FOL can occur from the germination of soil borne spores in the vicinity of growing roots, attachment to the root surface, penetration of the cortex of root and spreading of mycelium within the root vascular system. The fungus invades and colonizes the parenchyma of the dying tomato plant and sporulates on the plant surface (Michielse and Rep, 2009). Some isolates induce root-rot and vascular diseases on specific cultivars (Olivain *et al.*, 1981 and Olivain *et al.*, 2003) and are classified into 120 formae speciales and races, based on the species of plant and cultivars they infect (Armstrong and Armstrong, 1981 and Alabouvette *et al.*, 2001). Pathogenic isolates of *F. oxysporum* often display a high degree of host specificity (Sakai, 1998). Infection occurs when the pathogen penetrates in roots of the plant. The role of phenolic compounds in disease resistance mechanisms toward *Fusarium* infection is well known (Kalaichelvan and Nagarajan, 1992). The total phenol levels gave surely an increase in the capability of plants to defense against disease infection process and disease development (Ahmed 2005).

MATERIALS AND METHODS

This work was carried out in Faculty of Agriculture, Menoufia University, during 2020 growing season. Tomato seeds were obtained by Arkan agricultural projects development. Ten varieties of tomato plants (Diamond Arwa F1, Hybrid 71, Hybrid Karnak F1, Clara F1, Hybrid Ambarial, Hybrid F16, K-186, Carmen F1, Staffie 409 and Jasper 3709) were investigated to determine the susceptibility and resistance potential to *Fusarium oxysporum* f.sp. *lycopersici* (FOL).

Samples collection and isolation of *Fusarium oxysporum* f.sp. *lycopersici* (FOL):

Fusarium oxysporum f.sp. *lycopersici* (FOL) isolates were isolated from diseased tomato plants (12 weeks old) showing clear wilt symptoms especially, vesicles brown discoloration. Samples were collected from crop field of Sadat City, Menoufia governorate. Samples were taken in sterilized polythene bags (to avoid aerial contamination) separately. Roots and stem bases were first cut into small pieces, gently washed by running tap water to remove soil adhesive particles. The samples were surface sterilized by sodium hypochlorite (3%) for 3 min and rinsed again with sterile distilled water, dried between sterilized filter paper (Mathur and Kongsdal, 2003). The sterilized small pieces were placed in Petri dishes containing Potato Dextrose Agar (PDA) with 300 mg/L of Streptomycin (Sigma). The plates were incubated at 25°C for 5 to 7 days.

Purification and Identification of *Fusarium oxysporum* f.sp. *lycopersici* (FOL):

Identification of fungal cultures was carried out according to cultural and microscopical characteristics described by Booth (1971), Alexopoulos and Mims (1979), Ellis (1971), and Barnett and Hunter (1987). Purification of FOL was carried out, using both single spore and hyphal tip techniques (Hansen, 1926). According to the morphological characteristics of microconidia, macroconidia, phialides and chlamydospores (Summerell *et al.*, 2003) Pure cultures of the obtained isolates were identified in laboratory of Faculty of Agriculture, Menoufia University. Pure cultures transferred to PDA medium slants and kept at 5°C for further studies.

Growth of Tomatoes seed, seedling:

For planting seeds, Pots (20 cm in diameter) were sterilized by dipping in 5% formalin for 7 minutes and left for ten days until formalin evaporation. Pots were filled with the mixture of sterilized sand: clay: peat at ratio (1:1:1), FOL was sub-cultured on potato dextrose agar at 27°C. Each sterilized pot was inoculated with 5 ml of FOL culture suspension (10^7 cfu/ml) for two weeks to allow the fungus distribution into the soil (El-Khallal, 2007). All seeds were surface sterilized with 3% sodium hypochlorite solution for 3 min., rinsed in sterile distilled water, dried between folds of sterilized filter paper. Then all cultivars were planted with rate of five seeds/pot and five replicates for each cultivar were made before incubated at 12:12 hours light: dark cycle, at 25 °C for 15 days. The grown seeds were examined every day for the wilt disease.

For planting seedling, Clay loam soil was autoclaved twice at 121°C for an hour. Inoculum of FOL was prepared using sterilized Barley sand medium (75 g barley + 25 g sand + 100 ml water); using 1000 ml flasks. Flasks were incubated at 25°C for 2 weeks and shaken daily to allow all fungal growth. Sterilized soil was infested with isolate of FOL at the rate of 5% of soil weight. Two seedling plants (21 days old) were planting in each pot with five replicates for each cultivar. Potted soil kept under greenhouse conditions at 65% relative humidity, Three weeks post pathogen inoculation (6 weeks old tomato seedlings) of planted seed and seedling, plants in each variety were individually observed for diseases scoring in each genotype and scored for typical wilt symptoms..

Disease severity (DS) was calculated using 0–4 scale, (0 = asymptomatic, 1 = yellowing, 2 = vascular discoloration, 3 =

wilting, 4 = plant dead) according to Epp (1987) formula equation:

$$DS = [(n_i \times s_i) / (N \times S)] \times 100\%.$$

Where, n_i : number of tomato plants with wilt symptoms, s_i : value of the score of symptoms, N : total number of tested tomato plants, and S : the highest value of score of symptoms (Cachinero *et al.*, 2002). Overall responses of the tested tomato varieties against Fusarium wilt was established using the following criteria: DS = 0%; immune, – DS = 1-20%; resistant, DS = 21-40%; moderately susceptible, DS = 41-70%; susceptible, DS = 71- 100%; very susceptible (Dan Sudarsono, 2004).

Furthermore, percentage of infection (PI) was estimated after 21 in seed experiment and after 60 days for seedling experiment according to this formula:

$$PI = \frac{\text{No. of diseased plants} \times 100}{\text{Total No. of plants}}$$

HPLC analysis:

Chromatogram analysis of shoots and roots of two different tomato cultivars i.e. Diamond Arwa and Carmen was performed using high pressure liquid chromatogram (HPLC) according to modified methods of Selim *et al.*, (2014). The HPLC apparatus, Agilent Technologies 1262 Infinity system, was preceded by an Eclipse plus® C18 reverse-phase guard column (4.6 × 10.0 mm, 3.5 µm). The HPLC system consisted of Pump unit, diode array detector, and auto sampler (1260, Agilent Technologies) which were controlled by Chemo Station for LC 3D system. Before samples were injected, the column had been equilibrated with 90% (v/v) water (solvent A) and 10% acetonitrile (solvent B). After injection, the samples were eluted at a flow rate of 1.0 ml min⁻¹ using an isocratic flow of 90% solvent A and 10% solvent B for 2 min, a linear gradient to 10% solvent A and 90% solvent B for 28 min, followed by an isocratic flow for 5

min with 90% solvent B. For conducting HPLC analysis, 1.5 g of fresh shoots and roots were collected from three replicates of Diamond Arwa and Carmne plants inoculated with *Fusarium oxysporum* one week after inoculation. Fresh shoots and roots were immediately freeze-dried using liquid nitrogen. The freeze-dried materials were mixed thoroughly individually with 15 ml of ethyl acetate in plastic test tubes for 5 min. After separation, the suspension was filtrated into new tubes through two cotton layers and evaporated under vacuum to completion. The extracted compounds were then dissolved in 150 µl absolute methanol and 50 µl of each chemical extraction was injected into HPLC. Spectral analysis was conducted to compare the detected peaks with similar retention times in all extractions. The same procedure was used for the chromatogram analysis of shoots and roots of both two cultivars three weeks after pathogen inoculation.

Statistical analysis:

Data were statistically analyzed according to standard analysis of variance by a one way ANOVA with the software statgraphics (Statistical Graphics. Crop, Rockville, MD), Variance homogeneity for all treatments was confirmed by the Bartlett test. The comparison between means was carried out by Duncan's Multiple Range Test (Duncan, 1955) as given in the figures.

RESULTS AND DISCUSSION

Identification and Morphological Characteristics of the isolates:

The isolated fungi were identified as *Fusarium oxysporum* f. sp. *Lycopersici* (FOL). Ten isolates of (FOL) were observed and isolate No. 6 was chosen for this study as it was considered the

most virulent isolate and it produced more spores.

Determination of Disease severity in tested tomato cultivars:

Data presented in Fig. 1 show that, most of inoculated tested cultivars exhibited wilt symptoms. The highest disease severity (DS) values were observed with Carmen F1, Hybrid 71 and Staffie 409 cvs (100%, 96% and 92% respectively) and (96%, 90.4% and 91.2% respectively) in seed and seedlings experiments, respectively. In contrary, the results showed that both of Diamond Arwa F1 and Clara F1 cvs. recorded the lowest DS values (0%) compared with the other tested cultivars in both seeds and seedlings experiments (fig1). Moreover, moderate DS values ranged from 15-45% were recorded on three different tested tomato cultivars i.e. K-186, Hybrid F16 and Hybrid Am in both seeds and seedlings experiments (Fig.1).

Determination of Percentage of infection within tested tomato cultivars:

Results shown in Fig. 2 clear that most of the tested cultivars had significant susceptibility to *Fusarium oxysporum* pathogen (FOL). The highest infection percentage (100%) was recorded on Carmen F1cv. It was considered the most susceptible tested cultivar either in seed or seedlings experiments among the all 10 tested cultivars (Fig. 3). Moreover, the lowest value of PI (0%) was observed with Jasper3709 and Diamond Arwa cultivars and therefore they were considered the most resistant cultivars toward *Fusarium* pathogen among the all tested cultivars where no yellowing, vascular discoloration, wilting symptoms or plant dead were recorded in either seed or seedlings experiments (Fig. 4).

Relation between fusarium wilt disease and accumulation of phenolic

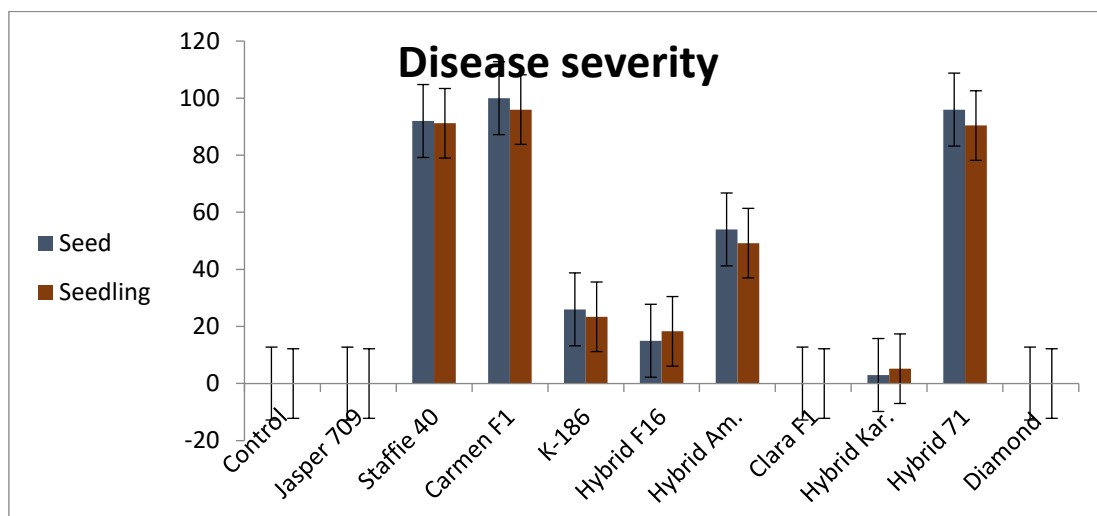


Figure 1: Disease severity of Fusarium wilt disease recorded with 10 tomato cultivars to in seeds and seedlings experiments.

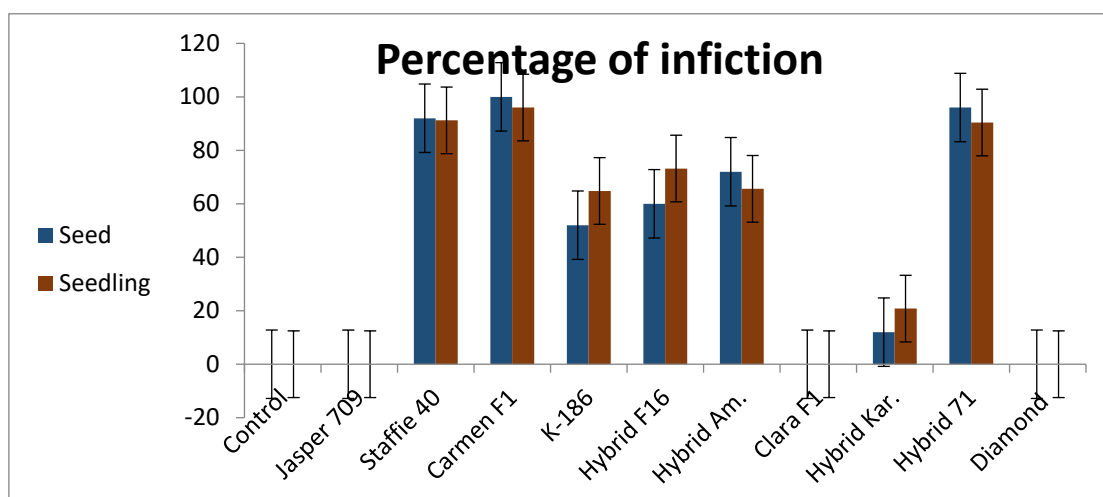


Figure 2: Percentage of infection (PI) in tested tomato cultivars to inoculation with the tested FOL isolate.

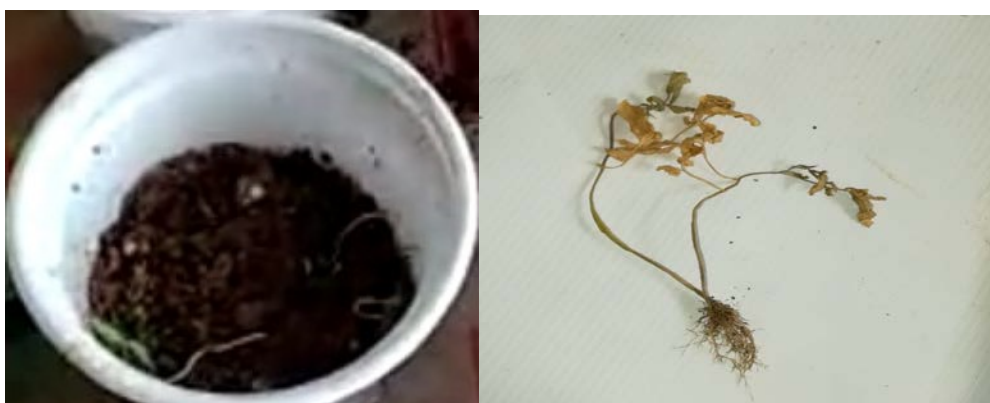


Figure 3: Symptoms of infection with Carmen cultivar in seed and seedlings experiments



Figure 4: Symptoms of infection with Diamond Arwa cultivar in seed and seedlings experiments

Chromatogram analysis using HPLC: (270nm)

In presented study, a mixture of different chemically active compounds, i.e., acetone-DNPH, acrolein-DNPH, 2,5-dimethylbenzaldehyde-DNPH, formaldehyde-DNPH, isovaleraldehyde-DNPH and propionaldehyde-DNPH, were used as references to compare the spectral pattern of obtained peaks one week and three weeks after *Fusarium* inoculation within roots and shoots of the two cultivars tested (Diamond Arwa and Carmen). The chemical analysis using HPLC revealed that either tested susceptible (Carmen) and resistant (Diamond Arwa) tomato cultivars in this study responded chemically to *F. oxysporum* inoculation. Thus, the inoculation of *Fusarium* pathogen resulted in accumulating of different certain compounds in both shoots and roots of infested plants.

The accumulation of chemical compounds in roots and shoots of the most tolerant tomato cultivar tested

(Diamond Arwa F1 cvs.) and the most sensitive tomato cultivar (Carmen F1cv) was determined one week and three weeks post *Fusarium* inoculation using the array detector chromatogram at a wave length of 270nm. The area under the curve of the peaks with retention time of 19.0; 21.2; 24.2; 25.8; 26.2; 28.2; and 29.1 minutes was calculated. Similarity of the peaks detected with similar retention times within the two tested tomato cultivars was also analysed using spectral analysis.

The results show that one week after *Fusarium* inoculation, the accumulation of the chemical compounds generally and particularly with those detected at retention time of 19.0; 21.2; 24.2; 25.8; 26.2; 28.2; and 29.1 minutes was higher (535,85 mAu/g) within shoots and leaves of cultivar Diamond Arwa than within cultivar Carmen (474,2 mAu/g), (Fig. 5). Remarkable, two fold increase of concentration of the compound that detected within retention time of 26.2 minute was recorded with the tolerant cultivar tested (Diamond Arwa), (Fig. 5).

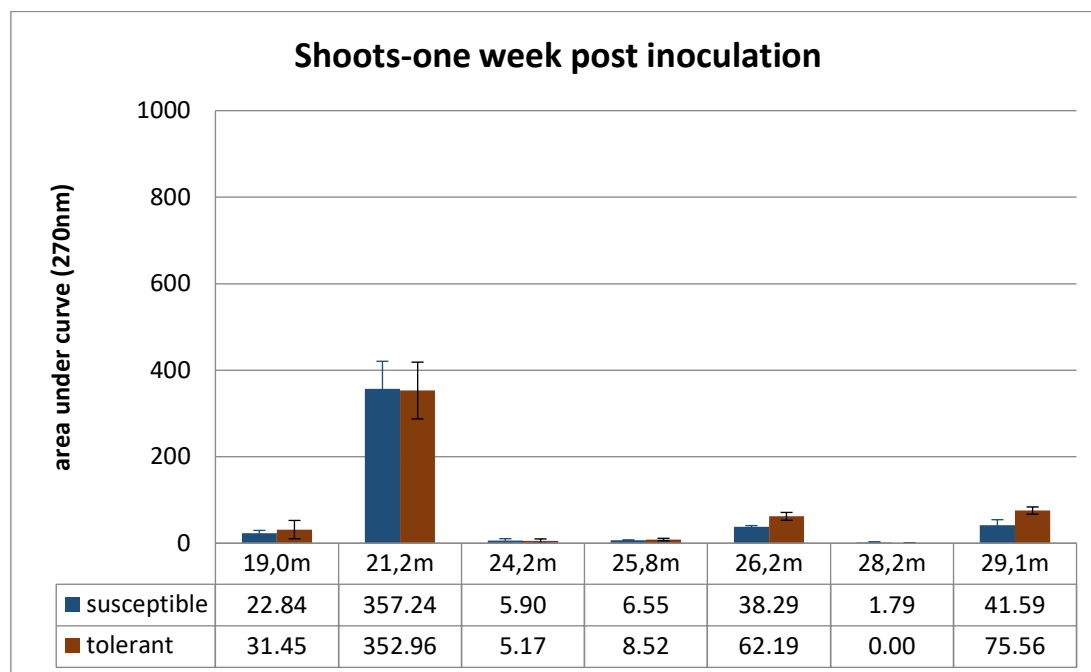


Figure 5: Accumulation of the compounds detected with the retention time of 19.0; 21.2; 24.2; 25.8; 26.2; 28.2; and 29.1 minute at 270nm in leaves and shoots obtained from tomato cultivars Diamond Arwa (resistant) and Carmen (susceptible) one week after *Fusarium* inoculation.

Similar results were recorded again with aspect to the accumulation of these certain compounds in roots of both Cultivars tested (Fig. 6). Thus, the total concentration of the detected chemicals substrates was recorded up to 384 mAu/g and 275 mAu/g within roots of Diamond Arwa and Carmne cultivars, respectively (Fig. 6).

Three weeks after *Fusarium* inoculation, HPLC chromatogram analysis of foliar system of the two tested tomato cultivars showed that the accumulation of the investigated chemical compounds was increased generally comparing to the accumulation of same compounds recorded one week after *Fusarium* application (Fig. 7).

Moreover, significant differences were recorded among cultivar Diamond Arwa and cultivar Carmen with aspect to specific compounds that detected at the

retention time of 21.2; 26.2; and 29.1 minute (Fig 8).

As observed with foliar system analysis, the chemical profiling of root systems three weeks after *Fusarium* inoculation, demonstrated that the accumulation of total screened chemical substrates increased in root system of both two cultivars comparing to the concentration of same chemical substrates recorded one week post inoculation. The total concentrations of detected chemical substances recorded up to 918 and 730 mAu/g with Diamond Arwa and Carmen cultivars, respectively (Fig. 9). Noteworthy, significant differences were detected again among the two tested cultivars with aspect to the accumulation of detected compounds with retention time of 21.2; 26.2 and 29.1 minute at 270 nm array (Fig. 10).

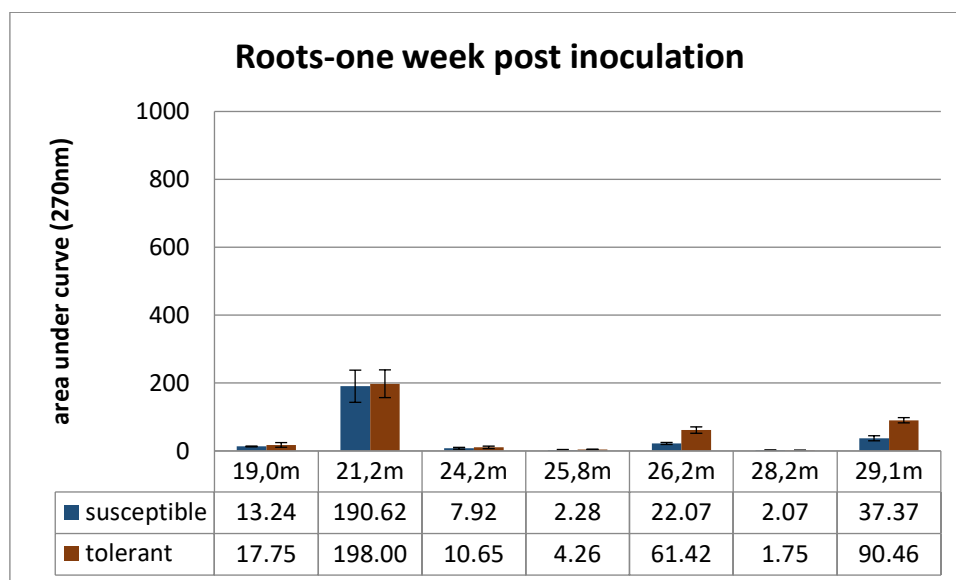


Figure 6: Accumulation of the compounds detected with the retention time of 19.0; 21.2; 24.2; 25.8; 26.2; 28.2; and 29.1 minute at 270nm in roots obtained from tomato cultivars Diamond Arwa (resistant) and Carmen (susceptible) one week after *Fusarium* inoculation.

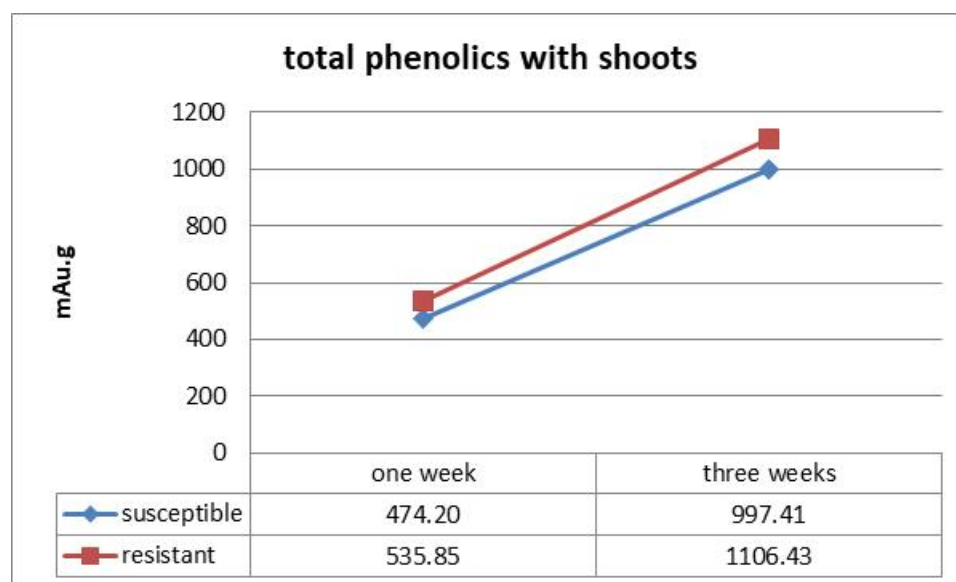


Figure 7: Total phenolic compounds accumulated one and three weeks post inoculation with virulent *Fusarium oxysporum* isolate in shoots of two different tomato cultivars.

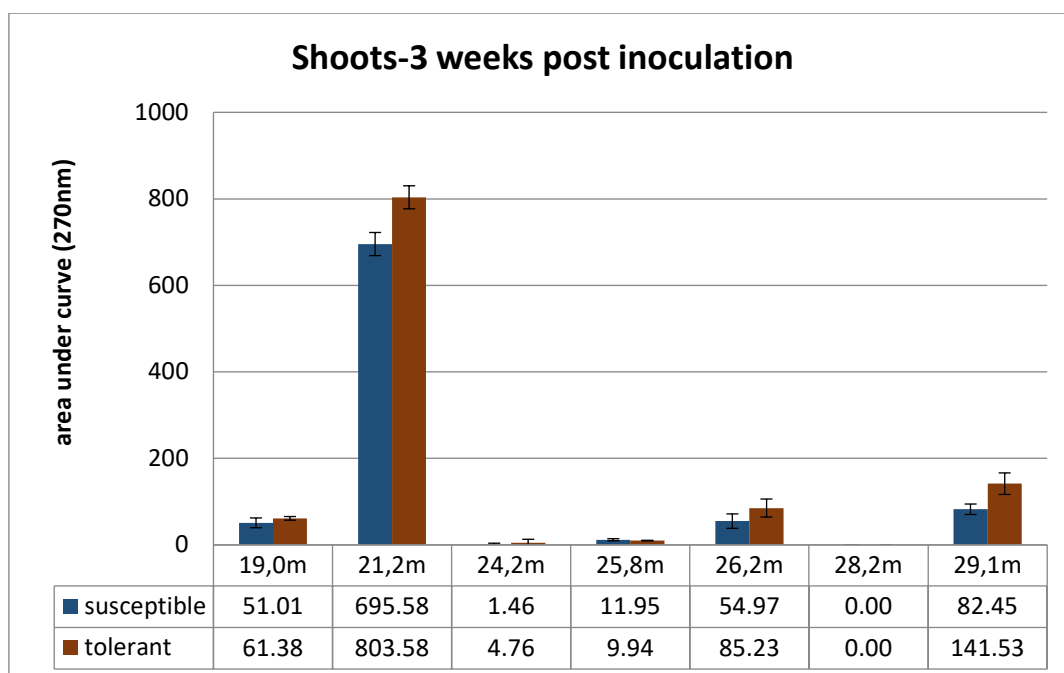


Figure 8: Accumulation of the compounds detected with the retention time of 19.0; 21.2; 24.2; 25.8; 26.2; 28.2; and 29.1 minute at 270nm in leaves and shoots obtained from tomato cultivars Diamond Arwa (resistant) and Carmen (susceptible) three weeks after *Fusarium* inoculation.

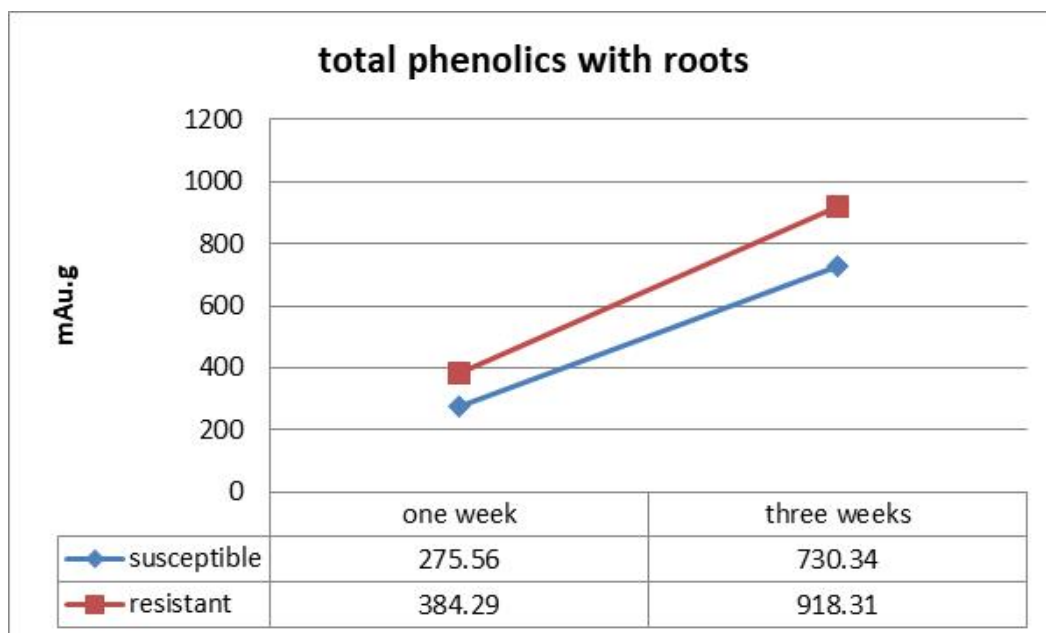


Figure 9. total phenolic compounds accumulated one and three weeks post inoculation with virulent *Fusarium oxysporum* isolate in roots of two different tomato cultivars.

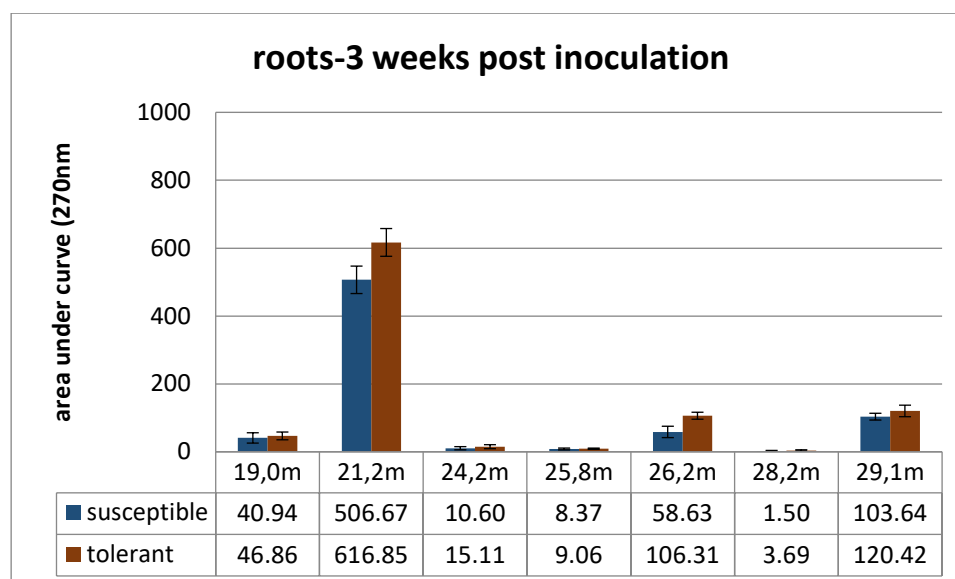


Figure 10: Accumulation of the compounds detected with the retention time of 19.0; 21.2; 24.2; 25.8; 26.2; 28.2; and 29.1 minute at 270nm in roots obtained from tomato cultivars Diamond Arwa (resistant) and Carmen (susceptible) three weeks after *Fusarium* inoculation.

These results are consistence with Zacaes *et al.*, (2007), who surveyed metabolites of the phenylpropanoid pathway using HPLC of extracts from tomato plants challenged with *Pseudomonas syringe* and reported that the bacterial infection induced the systemic plants resistance and at the meanwhile increased significantly the accumulation of a set of four major compounds detected with retention time of (12.5 min, 12.7 min., 13.2 min. and 13.6 min. at 280 nm wave length). They added also not all chemical compounds were be able to identify due to the irregularities in separation process or because these compounds were invisible in the range of the detector detection. Similar results were reported by Selim *et al.*, (2014) who found that increasing the accumulation of different biochemical active compounds, which were alone or in combination, resulted in improving the resistance potential of tomato plants toward bio-trophic pests.

In conclusion, significant differences between resistant and susceptible tomato cultivars toward *Fusarium* wilt disease in accumulation and concentrations of phenolic compounds within roots and shoots system were recorded which pointing to potential role of these chemical substances in controlling *Fusarium* infection on tomato plants. Further studies concerning with isolation, fractionation and identifications of these varied compounds that particularly detected at retention time of 21.2; 26.2 and 29.1 minute are needed to infer size whether these compounds alone or in combinations are involving in interaction between virulent *Fusarium oxysporum* and tomato *Fusarium* wilt resistant cultivars.

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العلاقة بين مرض الذبول الفيوزاريومي وتراكم المركبات الفينولية داخل أصناف الطماطم المقاومة والحساسية

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الملخص العربي

يُعد مرض ذبول الطماطم المتسبب عن الفطر (FOL) (*Fusarium oxysporum* f. sp. *lycopersici*) هو أحد أكثر الأمراض تدميراً. هذا المرض يسبب خسائر كبيرة في المحصول في مصر. تم عزل ١٠ عزلات فيوزاريوم من نباتات الطماطم الذابلة التي تنمو في محافظة المنوفية خلال موسم النمو ٢٠٢٠. تم اختبار العزلة الأكثر ضراوة (العزلة رقم ٦) على عشرة أصناف من الطماطم تحت ظروف الصوبة. اختلفت الأصناف معنوياً في حساسيتها للعزلة شديده الضراوة. كان الصنف Carmen F1cv الأكثر حساسية حيث سجل أعلى نسبة إصابة (١٠٠٪ و ٩٦٪) في تجارب البذور والشتلات على التوالي. من ناحية أخرى أظهرت النتائج أن الصنف Diamond Arwa هو الصنف الأكثر مقاومة حيث تم تسجيل نسبة إصابة صفر في كل من تجارب البذور والشتلات. تم عمل تحليل كيميائي باستخدام HPLC لصنفي الطماطم الأكثر حساسية (Carmen) والأكثر مقاومة (Diamond Arwa) بعد أسبوع وثلاثة أسابيع من العدوي بعزلة رقم ٦ لفطر *Fusarium* المسبب للمرض. أظهرت النتائج زيادة نسبة المركبات الفينولية الكلية في كلا من المجموع الخضري والجذور بعد ثلاثة أسابيع من التلقيح مقارنة بأسبوع واحد بعد التلقيح بالفيوزاريوم في الصنفين المختبرين. وكان واضحاً تراكم الفينولات بتركيز أعلى في المجموع الخضري والجذور للصنف المقاوم مقارنة بالصنف القابل للإصابة. وكان هناك فروق معنوية بين الصنف المقاوم والحساس المختبرين بالمركبات المكتشفة عند الدقة ٢١.٢ و ٢٦.٢ و ٢٩.١ في كل من المجموع الخضري والجذور بعد ثلاثة أسابيع من تلقيح الفيوزاريوم مما يشير إلى الدور المحتمل لهذه المواد الكيميائية في مقاومة مرض الذبول الفيوزاريومي في أصناف الطماطم المقاومة.

أسماء السادة المحكمين

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