Egypt. J. Phytopathol., Vol. **44**, No. 2, pp. 261-282 (2016)

Olive Cultivar Reaction and Biochemical Changes Due to Infection by Root Rot Pathogens

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Root rot disease was studied in several olive nurseries and orchards in Ismailia, Behera, Giza and Fayoum governorate. Observed disease symptoms were leaves internal rolling, partial wilt, yellowing or browning of leaves, drying of branches and leaves, twig dieback and severe root rot. *Fusarium konzum* was the most pathogenic fungus, followed by *F. solani*. All evaluated olive cultivars were susceptible to the tested fungi however, cultivar Picual was the most susceptible while Koratina was the least one. The infection of Picual, Maraki, Kroneiki and Koratina with *F. konzum* increased the total sugars and decreased total sugar contents and non-reducing sugars in Toffahi. Also, the infection of olive transplants of cultivar Kroneiki with *F. konzum* or *F. solani* increased the total phenols however, decreased the total phenols in cvs. Toffahi, Maraki and Koratina. Oleuropin, Pyrogallol and E-vanillic increased in infected olive transplants of cv. Picual by *F. konzum* or *F. solan*. The highest amounts of flavonoids in both of healthy and infected tissues of cvs. Koratina and Picual were Luteo.6-arbinose8-glucose and Apig.6-glucose8-rhamnose. The oxidative enzymes peroxidase, poly-phenoloxidase and catalase recorded an increment in infected tissues of the tested olive cultivars compared to the control and catalase was the highest activity however, poly-phenoloxidase was the least one.

Keywords: Biochemical analysis, Fusarium, olive, oxidative enzymes and root rot.

Olive-tree (*Olea europaea* L.) is one of the most ancient domesticated fruit trees and the most extensively cultivated fruit crop worldwide (Fabbri *et al*., 2009). The Mediterranean region is the native of olive tree (97% of the global cultivation area is located in the Mediterranean Basin).

The total area planted with olive trees in Egypt was about 150,000 feddans according to Anonymous, (2011) in the following governorates: Fayoum, Behera, Ismailia, Marsa Matrouh, El Arish, New Valley, El-Sharkya and El-Giza.

Olive plants are liable to be attacked by several soil borne pathogens causing severe losses in yield and quality due to death of young olive trees or transplants (Ghoneim *et al*., 1996 and Barreto *et al*., 2001). Root rot disease of olive is caused by *Fusarium oxysporum*, *F. solani*, *F. moniliforme*, *F. equiseti*, *Rhizoctonia solani*, *Pythium spp.*, *Phytophthora spp.* or *Macrophomina phaseolina* (Saied, 1986; Radwan *et al*., 1995; Ghoneim *et al*., 1996; Sanchez Hernandez *et al*., 1996 & 1998 and Mousa *et al*., 2006).

The present investigation was planned to identify the causal agents of olive root rots in some Egyptian governorates and study their pathogenic capabilities in infected roots of olive transplants. In addition, the varietal susceptibility of olive transplants against the root rot disease was evaluated. Also, the relationship between olive root contents (sugars, phenolic compounds and oxidative enzymes) and the varietal susceptibility was investigated.

Materials and Methods

Isolation, purification and identification of the causal organisms:

Diseased root samples collected from different olive orchards were washed with tap water then cut into small pieces and surface sterilized in 0.5% sodium hypochloride for 5 min. then pieces were dried and put in sterilized Petri dishes containing potato dextrose agar medium (PDA). The plates were incubated at 27°C for seven days then the developed fungi were purified (using hyphal tip technique) and identified microscopically according to morphological characters, either to the generic or to the species level using the description of Booth and Waterston (1964). This identification was done in fruit and wood trees Dis. Res. Dept., Plant Pathol. Res. Instit., ARC., Giza, Egypt and confirmed by Mycol. and Dis. Survey Dept., Plant Pathol. Res. Instit., ARC., Giza, Egypt. Moreover, identification of *Fusarium konzum* and *F. solani* was carried out by Mycol. Center, Fac. of Science, Assuit Univ.

Pathogenicity test:

The sterilized pots (20 cm in diameter) were filled with autoclaved soil (2kg/pot). The tested fungi were grown on autoclaved corn meal sandy medium (100g corn meal, 50g sand and 100ml distilled water) in glass bottles. The bottles were inoculated with discs (6 mm in diameter taken from 7 days-old cultures of each tested fungi) then incubated at 27°C for 15 days. The autoclaved soil was individually infested with the tested fungi at the rate of 5% of soil weight. Three olive transplants (six-month-old) were used for each treatment and pots contained uninoculated medium were used as a control. The transplants were examined after 30, 60 and 90 days from transplanting by calculating the percentage of infected olive transplants. Re-isolation was carried out from roots of the infected transplants showing disease symptoms and the isolated fungi were compared with the original fungal cultures.

Cultivar reaction:

Seven olive cultivars (six-month-old) Picual (P), Maraky (M), Ogizi (O), Manzanillo (Mn), Toffahy (T), Koratina (Ko) and Kroneiki (Kr) were used to test their reaction against two isolated pathogenic fungi causing root rot disease *i.e. Fusarium konzum* and *F. solani*. The above mentioned fungi were previously grown on corn meal sand medium and the sterilized plastic bags (20 cm in diameter) were filled with autoclaved soil (2kg/bag) then infested with each particular tested fungi as mentioned before. Three transplants were used as replicates for each treatment. The pots were kept under greenhouse conditions. The percentage of infected transplants was calculated after 60 days from transplanting.

Biochemical changes in olive transplants due to infection by the tested fungi:
Determination of phenolic compounds:

Inoculated and non-inoculated olive roots samples with the tested fungi were collected from five cultivars, Maraki, Picual, Koratina, Kroneiki and Toffahi, and extracted in 80% methanol, the total phenolics were determined by the Folin-Ciocalteu method described by Slinkard and Singleton (1977) and their phenolic and flavonoid profiles were examined by HPLC Agilant (series 1200) according to the method described by Goupy *et al*. (1999) and Mattila *et al.* (2000), respectively. The analysis of phenolic, flavonoid compounds and sugars were conducted at Food Technology Research Institute, ARC.

Phenol content determination:

The total phenol content was determined using the colorimetric technique at 765 nm according to Ivanova *et al*. (2010), using Folin-Ciocalteu reagent. 1 ml of methanol solution of olive roots extract was added to a 10 ml volumetric flask containing 5 ml of distilled water then 0.5 ml of Folin-Ciocalteu reagent was added and the contents mixed at the shaker for 2 min. then 1.5 ml Na_2CO_3 solution of concentration 0.5% was added and made up to total volume of 10 ml of distilled water. After keeping the samples at 50°C (water bath) for 16 min. in sealed flasks and subsequent cooling, their absorbance was read at 765 nm against distilled water as the blank. A calibration curve was constructed using gallic acid standard solutions (0-100 mg/L).

Determination of phenolic compounds and flavonoids by HPLC:

Phenolic compounds were determined by HPLC according to the method of Goupy *et al*. (1999) as follow: 5 gm of olive roots sample were mixed with methanol and centrifuged at 10000 rpm for 10 min. and the supernatant was filtered through a 0.2 µm Millipore membrane filter then 1-3 ml was collected in a vial for injection into HPLC Agilent, (series 1200) equipped with auto-sampling injector, solvent degasser, ultraviolet (UV) detector set at 280 nm and quaternary HP pump (series 1100). The column temperature was maintained at 35°C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/ min. Phenolic acid standard from sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used to calculation of phenolic compounds concentration by the data analysis of Agilent software.

Determination of sugar contents:

Total and reducing sugars were extracted from Inoculated and non-inoculated olive roots samples with tested fungi and collected from olive tested cultivars, by water clarified by late acetate. Sodium oxalate was used to precipitate the excess of late acetate. Total and reducing sugars were determined in the clarified solution by Somogyi (1952) and Nelson (1944).

Determination of enzymes activity:

All steps of enzyme extraction were carried out at 4°C. Five grams of the homogenized olive roots pericarp were extracted with 0.1 M phosphate buffer pH 7 containing 5 g of polyvinylpyrrolidone using magnetic stirrer for 15 min. The homogenate was filtered through Whatman No.41 filter paper and then centrifuged

at 2,500 rpm (1000 series centifugal, England) for 20 min. The supernatant was filtered through Whatman No.42 filter paper and collected as an enzyme extract.
For enzyme assays polyphenol oxidase (PPO) activity was determined using a spectrophotometric method based on an initial rate of increase in absorbance at 410 nm (Soliva *et al*., 2001). Phosphate buffer solution pH 7 (0.1 M, 1.95 mL), 1 mL of 0.1 M catechol as a substrate and 50 µL of the enzyme extract were pipetted into a test tube and mixed thoroughly. Then the mixture was rapidly transferred to a 1-cm path length cuvette. The absorbance at 410 nm was recorded continuously at 25°C for 5 min using ultraviolet-visible spectrophotometer, Agilent, Germany. Peroxidase (POD) activity was assayed spectrophotometrically at 470 nm using guaiacol as a phenolic substrate with hydrogen peroxide (Díaz *et al*., 2001). The reaction mixture contained 0.15 mL of 4% (v/v) guaiacol, 0.15 mL of 1% (v/v) H_2O_2, 2.66 mL of 0.1 M phosphate buffer pH 7 and 40 µL of the enzyme extract. The blank sample contained the same mixture solution without the enzyme extract. Catalase activity was measured at 60°C in 100 mM sodium phosphate buffer (pH 7) using 10 mM H_2O_2 as substrate. The decrease in absorbance at 240 nm was monitored. Enzyme activity was determined using the initial rate of the reaction and the extinction coefficient for H_2O_2, was taken as 39.4 M^{-1} cm^{-1}. One enzyme unit was defined as the amount of enzyme that catalyzes the decomposition of 1 µmol H_2O_2 per min. The assays of enzymes activity were conducted at Food Technology Research Institute, Agricultural Res. Center, according to Arnnok *et al*. (2010) and Yuzugullu *et al*. (2011).

Statistical analysis:

The analysis of variance (ANOVA) of the data that performed with the software WASP. The least significant difference (LSD) at 5% level of significant was used to compare treatment means.

Results

Isolation, purification and identification of the causal organisms:

Data presented in Table (1) show that nine different fungi were isolated from diseased olive trees. According to their morphological features, the isolated fungi were identified as *Pythium* sp., *Fusarium solani*, *Trichoderma sp.*, *Fusarium oxysporum*, *Fusarium konzum*, *Penicillium sp.*, *Aspergillus sp.* and *Fusarium moniliforme*.

Frequency of isolated fungi:

Table (1) exhibited that the isolated fungi differed in their frequency. *Pythium* sp. recorded the highest frequency percentage (66.4%) while *Aspergillus* sp. had the least frequency (1.38%).

Table 1. Frequency of isolated fungi from olive trees in four governorates in Egypt

Fungi	Ismailia	Fayoum	Giza	Beheira	Total
Pythium sp.	17.33	12.41	13.33	23.33	66.4
Fusarium solani	6.67	15.17	4.44	3.33	29.61
Trichoderma sp.	0.00	2.76	0.00	0.00	2.76
Fusarium konzum	0.00	2.69	0.00	0.00	2.69
F. oxysporum	4.00	3.45	0.00	6.67	14.12
Penicillium sp.	0.00	2.07	0.00	10.00	12.07
Aspergillus sp.	0.00	1.38	0.00	0.00	1.38
F. moniliforme	6.67	0.69	0.00	0.00	7.36
Total	41.34	42	51.1	53.33	

Pathogenicity tests:

Table (2) show that all the tested fungi were pathogenic to Picual and Ogizi cultivars however, they differed in their pathogenic capability. *Fusarium konzum* was the most pathogenic fungus, followed by *F. solani*. On the other hand, *Pythium* sp. followed by *Fusarium moniliforme* and *Fusarium oxysporum* were the least pathogenic ones, respectively. Also, data in Table (2) show that there were significant differences between Picual and Ogizi cultivars in their reaction to the tested fungi. Picual was more susceptible than the cultivar Ogizi and the infection percentages were significantly increased with increasing of the incubation period from 30 to 90 days. *Fusarium konzum* and *F. solani* were the most virulent pathogens and they were selected for the subsequent studies.

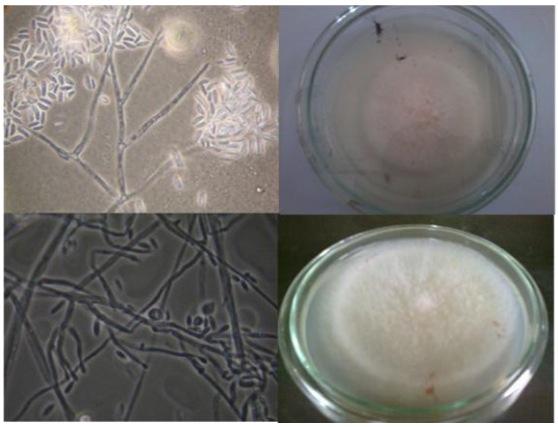

Figure 1. (a) *Fusarium solani*, (b) *Fusarium konzum*

Table 2. Pathogenicity of some isolated fungi from diseased olive orchards

Fungi	Cultivars/ Days					
	Picual			Ogizi		
	30 days	60 days	90 days	30 days	60 days	90 days
Fusarium konzum	54.72	82.18	94.3	12.99	54.07	82.6
F. solani	17.63	71.3	85.83	10.66	36.95	52.1
F. oxysporum	12.6	41.3	50.77	12.73	26.14	48.2
F. monilforme	8.33	20.27	43.6	7.83	14.17	24.33
Pythium sp.	3.83	16.17	26.63	0.73	12.28	19.85
Control	0	0	0	0	0	9
L.S.D. $_{0.05}$ for: Fungi (F)= 0.479 Cultivar (C)= 0.276 Days (D)= 0.331 (C x D x F) = 1.16						

Cultivar reaction:

All tested cultivars were susceptible to infection by the tested fungi but there was variation between them in their reaction. Results presented in Table (3) show that there are significant differences in susceptibility of tested cultivars. *Fusarium konzum* and *F. solani* caused the highest percentages of infection on Picual, 96.15% and 67.25%, respectively, followed by 80% and 75.62%, respectively on Kroneiki. On the other hand, Koratina exhibited the least infection percentages with *F. konzum* and *F. solani*, 23.35% and 21.74%, respectively. Also, data reveal that *F. konzum* was more pathogenic on all tested olive cultivars than *F. solani*. All the untreated transplants of each cultivar remained disease free.

Biochemical changes in olive transplants due to infection:

Total, reducing and non-reducing sugars:

Data presented in Table (4) show that the highest content of total sugars in healthy olive transplants of the tested olive cultivars, Picual, Toffahi, Maraki, Kroneiki and Koratina, was observed in cultivar Koratina (1.01 mg/gm fresh weight) while the least one was in Kroneiki (0.024 mg/gm fresh weight).

The infection of Picual, Maraki, Kroneiki and Koratina with *F. konzum* increased the amount of total sugars while decreased total sugar contents and non-reducing sugars in Toffahi. Also, reducing sugars decreased in Koratina and Picual cultivars and it was observed that, the highest content of total sugars was in Koratina cultivar (1.87 mg/gm fresh weight) however, the least one was in Maraki cv. (0.356 mg/gm fresh weight). The highest content of non-reducing sugars was in Koratina (1.661 mg/gm fresh weight) however, the least one was in Toffahi cv. (0.042 mg/gm fresh weight). On the other hand, reducing sugars was the highest in Koratina cultivar (0.209 mg/gm fresh weight) and it was the least in Picual cv. (0.102 mg/gm fresh weight). On the other hand, the infection with *Fusarium solani* increased the reducing sugars and total sugars in all tested olive cultivars however, decreased total sugar contents and non-reducing sugars in Toffahi. Also, it decreased the non-

reducing sugar contents in Koratina cultivar. Table (5) show that the highest content of total sugars and reducing sugars in inoculated olive transplants of all tested cultivars by *F. solani* was in Koratina cultivar (0.992 and 0.428 mg/gm fresh weight, respectively) however, the least content of total sugars, reducing sugars and non-reducing sugars was in Toffahi cv. (0.079, 0.018 and 0.061 mg/gm fresh weight, respectively). It was found that, Picual recorded the highest content of non-reducing sugars (0.694 mg/gm fresh weight).

Table 3. Reaction of seven olive cultivars to infection with *Fusarium konzum* and *F. solani*

% infection percentage			
Variety	*F. konzum*	*F. solani*	Control
Toffahi	45.58	45.18	0.00
Manzanillo	60	4.35	0.00
Ogizi	49.77	36.06	0.00
Maraki	51.06	48.71	0.00
Kroneiki	80	75.63	0.00
Koratina	23.85	21.74	0.00
Picual	96.15	67.25	0.00
LSD $_{0.05}$ for: Fungi (F) = 0.772 Cultivar (C) = 1.192 (C x F) = 2.066			

Total Phenols:

Data in Table (6) indicate that the highest content of total phenols, in healthy olive transplants of tested olive cultivars, was observed in cultivar Koratina (2.242 mg/gm fresh weight) while, the least one was in Kroneiki and Picual (0.331 and 0.339 mg/gm fresh weight) of total phenols, respectively.

However, the infection with *F. konzum* decreased the total phenols in the tested cultivars Toffahi, Maraki and Koratina cultivars to (0.331, 0.543 and 1.8 mg/gm fresh weight) respectively, and the highest level of phenolic compounds was observed in Koratina however, the least one was in Toffahi.

Also, data in Table (7) exhibit that the infection with *F. solani* decreased the total phenols in Toffahi, Maraki and Koratina cultivars to (0.319, 0.712 and 1.285

mg/gm fresh weight) respectively, and the cultivar Koratina recorded the highest level of total phenols while Toffahi recorded the least one.

On the other hand, the infection of olive transplants of cultivar Kroneiki with *F. konzum* or *F. solani* increased the total phenols to (0.339 and 0.781 mg/gm fresh weight) respectively. It could be concluded that the infection with root rot pathogens, *F. konzum* or *F. solani*, led to an accumulation of total phenols in Kroneiki more than the other tested olive cultivars.

Table 4. Reducing and non-reducing sugars (mg/g fresh weight) in five olive cultivars inoculated by *F. konzum*

Cultivars	Sugar Contents					
	Uninoculated			Inoculated		
	Reducing sugar	Total sugar	Non-reducing sugar	Reducing sugar	Total sugar	Non-reducing sugar
T	0.104	0.782	0.678	0.014	0.056	0.042
Kr	0.006	0.024	0.018	0.149	0.408	0.259
M	0.014	0.165	0.151	0.185	0.356	0.171
Ko	0.299	1.01	0.711	0.209	1.87	1.661
P	0.015	0.078	0.063	0.012	0.366	0.354

Table 5. Reducing and non-reducing sugars (mg/g fresh weight) in five olive cultivars inoculated by *F. solani*

Cultivars	Sugar Contents					
	Uninoculated			Inoculated		
	Reducing sugar	Total sugar	Non-reducing sugar	Reducing sugar	Total sugar	Non-reducing sugar
T	0.104	0.782	0.678	0.018	0.079	0.061
Kr	0.006	0.024	0.018	0.023	0.157	0.134
M	0.014	0.165	0.151	0.02	0.592	0.572
Ko	0.299	1.010	0.711	0.428	0.992	0.564
P	0.015	0.078	0.063	0.256	0.95	0.694

Phenolic compounds:

Twenty-four phenolic compounds were determined in the highly susceptible olive cultivar (Picual) and the less susceptible olive cultivar (Koratina) inoculated by *Fusarium konzum* or *Fusarium solani*. Table (8) shows that the infected tissues of cv. Koratina with *F. konzum*, *F. solani* had higher content of oleuropin (2923.57, 4090.69 ppm), respectively, E-vanillic (168.533, 289.413 ppm), Pyrogallo (87.427, 170.18 ppm) and catechol (41.704, 55.872 ppm) compared to (509.605, 83.092 ppm), (110.836, 66.567 ppm), (55.375, 32.586 ppm) and (21.765, 14.66 ppm) of oleuropin, E-vanillic, Pyrogallo and catechol, respectively in the highly susceptible olive cultivar (Picual) which contained higher level of Benzoic acid (43.234, 108.582 ppm) compared to cv. Koratina (42.168, 56.356 ppm).

However, lower content of Gallic acid was found in infected tissues of cv. Picual with *F. konzum*, *F. solani* (0.349, 0.279 ppm), respectively compared to cv. Koratina (0.418, 0.588 ppm). Also, the lowest phenolic compounds in the infected plants of cv. Picual with *F. konzum*, *F. solani* were 4-Aminobenzoic acid (0.154, 0.525 ppm), respectively and Gallic acid (0.349, 0.279 ppm), however, oleuropin and E-vanillic were the highest ones in tissues infected with *F. konzum* while, Ellagic acid (288.212 ppm) and Benzoic acid (108.582 ppm) were the most in tissues infected with *F. solani*. On the other hand, oleuropin and E-vanillic were the highest level of phenolic compounds in tissues of cv. Koratina infected with *F. konzum* or *F. solani*, however, Ellagic acid was not found.

Table 6. Total phenols (mg/g fresh weight) in five olive cultivars inoculated with *Fusarium konzum*

Cultivars	Total phenols (mg/g fresh weight)		
	Uninoculated	Inoculated	Mean
T	1.273	0.331	0.802
Kr	0.331	0.339	0.335
M	1.927	0.543	1.235
Ko	2.242	1.8	2.021
P	0.339	0.339	0.339
Mean	1.222	0.6704	

Table 7. Total phenols (mg/g fresh weight) in five olive cultivars inoculated with *Fusarium solani*

Cultivars	Total phenols (mg/g fresh weight)		
	Uninoculated	Inoculated	Mean
T	1.273	0.319	0.796
Kr	0.331	0.781	0.556
M	1.927	0.712	1.32
Ko	2.242	1.285	1.764
P	0.339	0.339	0.339
Mean	1.2224	0.6872	

Table 8. Phenolic profile (ppm) in highly susceptible olive cultivar (Picual) and less susceptible olive cultivar (Koratina) inoculated by *F. konzum* and *F. solani*

Phenolic compound	Phenolic compounds (ppm)					
	(Koratina) Less susceptible			highly susceptible (Picual)		
	F.Konzum	*F.Solani*	control	*F.Konzum*	*F.Solani*	control
Gallic	0.418	0.588	1.02	0.349	0.279	0.369
Pyrogallol	87.427	170.18	27.79	55.375	32.586	6.905
4-Amino-benzonic	0.366	0.495	0.858	0.154	0.525	0.231
Protocatchuic	10.336	18.640	8.919	12.765	1.615	2.418
Catechein	9.374	16.710	20.37	5.976	5.509	3.631
Chlorogenic	8.614	7.248	20.37	4.916	7.460	4.976
Catechol	41.704	55.872	22.31	21.765	14.66	2.311
Epicatechein	4.285	3.445	25.5	3.439	0.704	2.841
Caffein	6.139	8.472	6.27	0.43	1.983	0.63
Caffeic	2.661	0.840	1.77	2.2023	1.108	0.894
Vanillic	5.807	7.411	6.696	2.181	1.252	1.47
P=coumaric	2.567	6.908	8.602	1.642	1.55	3.038
Ferulic	16.408	26.993	9.804	5.369	4.187	1.604
Iso-ferulic	14.121	14.907	10.35	15.212	2.352	2.651
Reversetrol	2.419	4.019	36.28	4.342	0.743	1.319
Oleuropin	2923.57	4090.69	4361	509.605	83.092	81.32
Ellagic	-------	----------	32.09	-----	288.212	13.44
E-vanillic	168.533	289.413	121.8	110.836	66.567	30.58
Alpha=coumaric	14.717	27.432	10.65	9.645	5.713	1.347
Benzoic	42.168	56.356	158.7	43.234	108.582	18.19
3,4,5-methoxy-cinnamic	16.513	18,513	6.547	8.522	3.860	0.907
Coumarin	4.885	5.564	13.97	8.004	4.555	1.013
Salycilic	36.342	12.692	46.09	17.893	5.447	1.94
cinnamic	2.640	1.652	1.637	3.359	1.913	0.17

Flavonoid content:

Data in Table (9) exhibit twenty flavonoids which were determined in tissues of the less susceptible olive cultivar (Koratina) and tissues of the highly susceptible olive cultivar (Picual) infected by *Fusarium konzum* or *Fusarium solani* and it was observed that, the highest flavonoids in tissues of cv. Koratina were Luteo.6-arbinose8-glucose (682.62, 300.78 ppm) followed by Apig.6-glucose8-rhamnose (480.44, 112.85 ppm), however, the lowest ones were Apegnin (1.51, 0.83 ppm) followed by Rhamnetin (3.96, 1.9 ppm) and Kampferol (2.85, 3.53 ppm). On the other hand, the highest flavonoid in tissues of cv. Picual inoculated by F. konzum was Luteo.6-arbinose8-glucose (466.94 ppm), followed by Apig.6-glucose8-rhamnose (223.49 ppm) while, Acacetin (102.32 ppm) and Luteo.6-arbinose8-glucose (42.29 ppm) were the most flavonoids in the tissues inoculated by *F. solani*, however, Apegnin was the lowest one in tissues infected with *F. solan*i or *F. konzum* (1.57, 0.91 ppm), respectively.

Also, it was found that, the infected tissues of cv. Picual with F. konzum or F. solani had higher content of Acacetin (75.15, 102.32 ppm), respectively compared to (11.75, 13.46 ppm) of Acacetin in the less susceptible olive cultivar (Koratina) which contained higher level of Kampferol 3,7-dirhamoside (36.11,73.49 ppm), Narengin (31.29, 36.37 ppm) and Hespiridin (27.1, 50.05 ppm) compared to (18.97,

7.88 ppm), (15.12, 15.63 ppm) and (16.34, 14.78 ppm) of Kampferol 3,7-dirhamoside, Narengin and Hespiridin in infected tissues of cv. Picual.

Oxidative enzymes activities:

Results of this research recorded an increment in the tested oxidative enzymes in the five olive cultivars as a result of inoculation with *Fusarium konzum* or *F. solani* compared to the control.

Data in Table (10) show that the infection of all tested olive cultivars with *F. konzum* increased the activity of catalase enzyme compared to the control ones while, the highest activity of catalase enzyme was in cv. Koratina followed by cv. Picual (0.33 and 0.30 u/gm fresh weight), respectively. However, the least activity was in cv. Kroneiki (0.19 u/gm fresh weight). On the other hand, Table (11) exhibit that the activity of catalase enzyme was the highest in cv. Picual inoculated with *F. solani* (0.53 u/gm fresh weight) however, it was the least in cv. Toffahi (0.33 u/gm fresh weight).

Also, Table (12) and Table (13) show that the highest activity of polyphenoloxidase was in cv. Picual inoculated with *F. konzum* (0.025 u/gm fresh weight) or inoculated with *F. solani* (0.030 u/gm fresh weight), however, it was the least in cvs. Maraki and Kroneiki inoculated with *F. konzum* (0.010 u/gm fresh weight) or inoculated with *F. solani* (0.020 u/gm fresh weight).

Table 9. Flavonoids profile (ppm) in highly susceptible olive cultivar (Picual) and less susceptible olive cultivar (Koratina) inoculated by *F. konzum* and *F. solani*

Flavonoids	Flavonoides (ppm)					
	(Koratina) Less susceptible			highly susceptible (Picual)		
	F.konzum	*F.solani*	control	*F.konzum*	*F.solani*	control
Luteo.6-arbinose 8-glucose	682.62	300.78	105	466.94	42.29	152
Luteo.6-glucose 8-arbinose	23.83	40.94	52.7	37.29	11.82	14.6
A Pig.6-arbinose 8-glactose	14.98	13.63	4.8	16.74	7.17	1.56
A Pig.6-rhamnose 8-glucose	39.14	26.78	18	7.03	9.19	9.09
A Pig.6-glucose 8-rhamnose	480.44	112.85	492	223.49	17.87	96.5
Luteol.7-glucose	10.1	20.27	12.3	3.22	3.57	4.11
Narengin	31.29	36.37	44.5	15.12	15.63	50.4
Rutin	6.42	6.92	4.18	2.99	6.02	2.18
Hespirdin	27.1	50.05	95.4	16.34	14.78	36.9
Rosmarinic	10.14	11.91	36.5	1.77	3.39	7.77
A Pig.7-o-neohespirosid e	7.09	11.84	16.1	4.67	3.44	6.34
Kamp.3,7-dirhamoside	36.11	73.49	13.5	18.97	7.88	4.58
Apig.7-glucose	5.52	8.93	13.4	6.9	2.18	4.87
Quercetrin	2.55	9.06	4.29	4.99	1.93	2.7
Quercetin	3.62	9.04	7.1	5.46	3.97	2.05
Naringenin	4.8	8.97	8.29	3.15	1.58	1.48
Hespirtin	23.13	9.89	10.7	26.41	8.83	2.15
Kampferol	2.85	3.53	2.95	3.94	2.32	1.47
Rhamnetin	3.96	1.9	1.05	3.29	2.03	0.72
Apegnin	1.51	0.83	0.44	1.57	0.91	0.43
Acacetin	11.75	13.46	5.16	75.15	102.32	13.5

Table (14) exhibits that all tested olive cultivars infected with *F. konzum* recorded an increase in the activity of peroxidase enzyme compared to the control ones while, the highest cultivar in the activity of peroxidase enzyme was Koratina (0.15 u/gm fresh weight). However, the least one was Picual (0.11 u/gm fresh weight). Also, Table (15) shows that the activity of peroxidase enzyme increased in the five olive cultivars infected with *F. solani* compared to the control ones and it was the highest in cv. Koratina (0.16 u/gm fresh weight) however, it was the least in cv. Picual (0.13 u/gm fresh weight).

Table 10. Catalase activity in five olive cultivars inoculated by *F. konzum*

Enzyme	Catalase activity u/gm fresh weight		
Cultivars	Uninoculated	Inoculated	Mean
T	0.18	0.22	0.2
Kr	0.16	0.19	0.175
M	0.19	0.28	0.235
Ko	0.15	0.33	0.24
P	0.12	0.3	0.21
Mean	0.16	0.264	

Table 11. Catalase activity in five olive cultivars inoculated by *F. solani*

Enzyme	Catalase activity u/gm fresh weight		
Cultivars	Uninoculated	Inoculated	Mean
T	0.18	0.33	0.255
Kr	0.16	0.35	0.255
M	0.19	0.44	0.315
Ko	0.15	0.42	0.285
P	0.12	0.53	0.325
Mean	0.16	0.414	

Table 12. Poly-phenoloxidase activity in five olive cultivars inoculated by *F. konzum*

Enzyme	Poly-phenol oxidase activity u/gm fresh weight		
Cultivars	Uninoculated	Inoculated	Mean
T	0.008	0.015	0.012
Kr	0.006	0.010	0.008
M	0.005	0.010	0.008
Ko	0.006	0.020	0.013
P	0.007	0.025	0.016
Mean	0.0064	0.04	

Table 13. Poly-phenoloxidase activity in five olive cultivars inoculated by *F. solani*

Enzyme	Poly-phenol oxidase activity u/gm fresh weight		
Cultivars	Uninoculated	Inoculated	Mean
T	0.008	0.025	0.017
Kr	0.006	0.020	0.013
M	0.005	0.020	0.013
Ko	0.006	0.025	0.016
P	0.007	0.030	0.019
Mean	0.0064	0.024	

Table 14. Peroxidase activity in five olive cultivars inoculated by *F. konzum*

Enzyme	Peroxidase activity u/gm fresh weight		
Cultivars	Uninoculated	Inoculated	Mean
T	0.07	0.12	0.095
Kr	0.08	0.14	0.11
M	0.07	0.13	0.1
Ko	0.08	0.15	0.005
P	0.09	0.11	0.1
Mean	0.078	0.13	

Table 15. Peroxidase activity in five olive cultivars inoculated by *F solani*

Enzyme	Peroxidase activity u/gm fresh weight		
Cultivars	Uninoculated	Inoculated	Mean
T	0.07	0.14	0.105
Kr	0.08	0.15	0.115
M	0.07	0.14	0.105
Ko	0.08	0.16	0.12
P	0.09	0.13	0.11
Mean	0.078	0.144	

Discussion

Olive trees and transplants are attacked by several soil-borne pathogens, causing diseases and loss in olive yield in orchards in different governorates in Egypt. Root rot disease was studied in several olive nurseries and orchards in Ismailia, Behera, Giza and Fayoum governorate. Common disease symptoms were observed, leaves internal rolling, partial wilt, yellowing or browning of leaves, drying of branches and leaves, twig dieback and severe root rot. Finally, these symptoms caused decline and tree death. The high incidence of root rot disease observed is due to the establishment of new olive tree plantations on land previously cropped with plants susceptible to soil-borne pathogens, and the probable use of infested soil or infected planting material in olive nurseries (Rodriguez Jurado *et al*., 1993 and Thanassoulopoulos, 1993). The isolated fungi from rotted roots at the tested locations were *Pythium* sp., *Fusarium solani*, *Trichoderma* sp., *Fusarium oxysporum*, *Fusarium konzum*, *Penicillium* sp., *Aspergillus* sp. and *Fusarium moniliforme*. These results are in agreement with Radwan *et al*. (1995); Sanchez Hernandez *et al*. (1998); Barreto *et al*. (2001), Mousa *et al*. (2006) and El-Morsi *et al*. (2009). The results indicate that *Pythium* sp. was the most frequent fungi (66.4 %) while, *Aspergillus* sp. was the least one (1.38%).

Pathogenicity tests indicated that, all the tested fungi were pathogenic and able to cause typical symptoms of root rot in olive transplants of two cvs. Picual and Ogizi. *Fusarium konzum* and *F. solani* caused the highest percentages of infection in both tested cultivars. Variation in pathogenicity of different isolates of Fusarium spp. isolated from infected olive trees also have been reported by (Radwan *et al*., 1995; Sanchez-Hernandez *et al.*, 1998; Barreto *et al*., 2002 and Mousa *et al*., 2006). Picual was more susceptible than the cultivar Ogizi. Increasing time of infection from 30 days to 90 days caused increase in the percentage of infection with the tested fungi. All uninoculated transplants remained healthy. These results agree with Abdel Hafeez (1991) and Abdel Ghany (2001).

In the present study, it was found that, all tested cultivars were susceptible to infection by *Fusarium konzum* and *F. solani*, but they varied in their reactions against tested Fungi. Cultivars Picual followed by Kroneiky were the most susceptible cultivars while, Koratina was the least one. Similar results were obtained by Mousa *et al*. (2006). However, Ghoneim *et al*. (1996) found that olive cultivars Krygula and Picual were less susceptible to different soil borne fungi than other tested cultivars.

Results showed that the healthy olive cultivar Koratina contains the highest amount of total phenols compared to the rest of cultivars. The infection with *Fusarium konzum* or *F. solani* decreased the total phenols of cvs. Toffahi, Maraki and Koratina and increased them in Kroneiki. Abdel Hafeez (1991**)** recorded that the infection of mango variety Alphonse with *Fusarium oxysporum*, *F. moniliforme* and *F. moniliforme* var. *subglutinans* caused an increase in total and free phenols and a decrease in conjugated phenols. This result would suggest that conjugated phenols are responsible for resistance in olive cultivar. Similar results were obtained by Hussain (1975) and Abdel Hafeez (1982).

The total phenols were higher in all inoculated transplants compared to healthy ones. Ammar (2003) and Sabet *et al*. (2006) also found that the contents of free and conjugated phenols in the inoculated tissues with *Fusarium moniliforme* were higher than that determined in the non-inoculated ones.

Root infection led to an increase in reducing sugar contents of all tested olive cultivars compared to the control transplants. Many authors had similar results (Nafea, 1995 and Ammar, 2003). This result may be correlated with disappearance of starch granules from pith cells or high activity of the pathogen in degradation of cellulose components. Results showed also a decrease in the non-reducing sugar contents of the tested olive cvs. Toffahi and Koratina infected with *Fusarium konzum* or *F. solani*. Similar results were obtained by Hussain (1975); Pandey *et al*. (1977) and Sabet *et al*. (2006). Menoufi *et al*. (1987) argued the reduction in sugar contents to the sugar consumption during fungal growth and disease development.

It was observed that the infected tissues of cv. Picual with *F. konzum* or *F. solani* had higher content of Acacetin compared to Acacetin in the less susceptible cultivar (Koratina) which contained higher level of Kampferol 3,7-dirhamoside, Narengin and Hespiridin. Also, it was recorded that the concentration of flavonoids, Acacetin and Kampferol 3,7-dirhamoside was higher in infected olive transplants compared to the uninfected ones. Similar results were obtained by Bensalah *et al*. (2014). Also, similar results were found in potato plants inoculated with *V. dahliae*, which induced a production of flavonol glycosides two to three times higher than in the uninoculated plant (El Hadrami *et al*., 2011). On the other hand, it was noticed that, some of flavonoids like Narengin and Hespiridin increased in uninfected olive transplants compared to the infected ones. It was suggested that, the tannin content of the uninfected sample was higher than that of the infected one. This explains that tannins, which are constitutive substances, mainly present in the bark, were synthesized and used initially by the olive plant in its defense against pathogens before transforming into flavonoids. Tannins were found in tropical plants at high concentrations, by Makkar and Becker (1998), because their synthesis is promoted by light, whereas flavonoids and alkaloids are inducible compounds, since they are not produced directly during the photosynthesis, but result from further chemical reactions.

Oleuropin, Pyrogallol and E-vanillic were the highest level of phenolic compounds in tissues of both cvs. Koratina, the less susceptible cultivar, and Picual, the highly susceptible cultivar. Also, it was observed that the concentration of phenols, Oleuropin, Pyrogallol and E-vanillic was higher in infected olive transplants of cv. Picual with *F. konzum* or *F. solani* compared to the uninfected ones while E-vanillic and Pyrogallol recorded an increament in infected transplants of Koratina. Similar results were obtained by (Bensalah *et al*., 2014) show that total polyphenols were present in infected olive trees at higher levels than in uninfected ones and the HPLC analysis revealed the presence of three new phenolic compounds in infected olive stems with *Verticillium dahliae*, namely verbascoside, apigenine- 7-glycoside and hydroxycinnamic derivatives.

Biochemical changes associated with the inoculation of olive transplants of five olive cultivars by *Fusarium konzum* or *F. solani* were investigated and data showed

an increment in the activity of catalase (CA), peoxidase (POX) and poly-phenoloxidase (PPO) enzymes compared to the non-inoculated ones.
These results are in harmony with Narayanasamy (2011) and Saber *et al*. (2013) but the largest increase was observed in case of catalase more than the peroxidase and poly-phenoloxidase enzymes in inoculated transplants.

The role of PPO enzyme in disease resistance was postulated by many authors (Lozovaya *et al*., 2006 and Narayanasamy, 2011). Lozovaya *et al*. (2006) reported that, resistance levels of crops to fungi could be increased by genetically manipulating metabolic events that lead to production of antimicrobial compounds that are toxic to pathogens or that can strengthen the barriers of plant cells to pathogen entry. In several crops, resistant cultivars produce higher quantities of specific peroxide isoenzymes upon infection than the susceptible cultivars (Mohan and Kolattukudy, 1990).

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(Received 15/11/2016;
in revised form 15/12/2016)

رد فعل بعض أصناف الزيتون تجاه الإصابة ببعض مسببات أعفان الجذور والتغيرات البيوكيميائية الناتجة عنها

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تم عزل وتعريف عدة فطريات، بيثيوم، فيوزاريوم سولانى، فيوزاريوم اكسيسبورم، فيوزاريوم مونيليفورم، ترايكودرما، بنيسيليوم، فيوزاريوم كونزم واسبرجيللس والتى تصاحب مرض عفن الجذور الذى يصيب أشجار الزيتون والتى تظهر عليها الأعراض النموذجية للمرض ولقد عزلت هذه الفطريات من أربعة محافظات فى مصر وهى: البحيرة، الاسماعيلية، الجيزة والفيوم وقدرت نسبة تكرار ظهورها وكانت أعلى نسبة للفطريات المعزولة من محافظة البحيرة وأقل نسبة من محافظة الاسماعيلية وكان أكثر الفطريات تكرارا على مستوى المحافظات الفطر بيثيوم. و أوضحت اختبارات القدرة المرضية على شتلات الزيتون من الصنفين عجيزى و بيكوال أن كل الفطريات المختبرة كانت ممرضة ولكن اكثرها قدرة على أحداث المرض الفطر فيوزاريوم كونزم يليه الفطر فيوزاريوم سولانى والتى استخدمت فى اختبارات تقييم الاصناف وتقدير التغيرات البيوكيميائية فى أنسجة الزيتون الناتجة عن الإصابة، كما أثبتت الدراسة أن زيادة فترة الحضانة من 30 إلى 60 ثم 90 يوم تزيد من نسب الإصابة.

تم اختبار سبعة أصناف من الزيتون لدراسة مدى قابليتها للإصابة أو مقاومتها لأعفان الجذور. وأظهرت النتائج أن كل الاصناف كانت قابلة للاصابة و أن الصنف بيكوال كان اكثرها قابلية للإصابة بينما الصنف كوراتينا كان اقلها قابلية للاصابة و تباينت باقى الأصناف فى مدى قابليتها للإصابة. أظهرت نتائج التحليل الكيماوى لأنسجة جذور الزيتون لخمسة أصناف مختلفة فى مدى قابليتها للإصابة ب أعفان الجذور ان محتوى الشتلات المعداه بالفطرين محل الدراسة لجميع الاصناف من السكريات الكلية كان أعلى منه فى الأنسجة السليمة عدا الصنف تفاحى الذى قلت فيه السكريات الكلية وغير المختزلة . كما أظهرت نتائج تقدير محتوى أنسجة جذور الزيتون للخمسة أصناف محل الدراسة من الفينولات الكلية أن إصابة هذه الجذور بالفطرين المختبرين أدى إلى زيادة الفينولات الكلية فى الصنف كروناكى فقط لكن يقللها فى باقى الأصناف. وقد أوضحت أيضا نتائج التحليل الكيميائى أن أعلى الفينولات تركيزا فى الشتلات السليمة لأعلى صنف قابل للاصابة (بيكوال) و أقل صنف قابل للاصابة (كوراتينا) هى اوليوروبين، اي فانيليك وبيروجالول. ولوحظ أن تركيزها زاد فى انسجة الصنف بيكوال المصابة مقارنة بالسليمة.

كما لوحظ أن أعلى الفلافونويدات في الأنسجة السليمة للصنفين محل الدراسة هى لوتيو- 6-اربينوز8- جلوكوز وابيج 6- جلوكوز- 8رامنوز، بينما كان الفلافونويد اكاسيتان هو الأعلى فى الصنف بيكوال فقط أما كامبفيرول 7,3دايراموساید، نارنجين وهيسبريدين فكانت هى الأعلى فى صنف كوراتينا فقط، ولكن لوحظ زيادة تركيز الفلافونويدات كامبفيرول 7,3 دايراموساید واكاسيتان فى الأنسجة المصابة للصنفين المختبرين عنها ف ي السليمة .

تم تقدير النشاط الانزيمى لكل من الكتاليز، البيروكسيديز، البولى فينول اكسيديز في الخمسة اصناف محل الدراسة. وقد أوضحت النتائج زيادة نشاط الانزيمات الثلاثة مقارنة بالكونترول، ولوحظ زيادة لنشاط الانزيمات الثلاثة فـي الصنف الأقل قابلية للإصابة مقارنة بباقي الأصناف.