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Reaction of certain tomato (*Lycopersicon esculentum* L.) cultivars to damping-off disease

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Abstract

Five fungal isolates were isolated from infected tomato seedlings showing damping-off which collected from several tomato nurseries of Assiut and New Valley Governorates, Egypt. These isolates were *Rhizoctonia solani* Kühn, *Fusarium oxysporum f.sp. lycopersici* Schlechtendahl, *Fusarium semitectum* Berk. & Rav., *Setosphaeria rostrata* Leonard and *Alternaria alternata* (Fries) Keisler. Also, six tomato cultivars and hybred, namely Castle Rock, Super marmande, Super Strain B, Enz 10F1, 0240F1 and Dream Hybrid were evaluated for their susceptibility to damping-off disease. All tested tomato cultivars were susceptible to damping-off disease, Castle Rock cv. showed the highest percentage of pre and post emergence damping-off disease severity followed by Super marmande and Super Strain B cvs. then Dream Hybrid and Enz 10F1 Hybrid. 0240 F1 Hybrid gave the lowest ones. Each tested tomato cultivars showed higher amount of total phenolic and total protein content in infected plants with the tested pathogenic fungal isolates than uninfected plants (control). The highest amount of total phenol and total protein was found in infected plant of tomato Enz 10F1cv. followed by 0240F1cv. while the lowest amount of total phenol and total protein were found in infected plant of castel Roke cv. then super marmande cv.

Keywords: Tomato cultivars and hybred, damping-off, *Rhizoctonia solani, Fusarium oxysporum f.sp. lycopersici, Fusarium semitectum, Setosphaeria rostrata, Alternaria alternata.*

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Introduction

Tomato (*Lycopersicon esculentum* L.) is considered one of the most important economic vegetable crops in Egypt, that attack by several soil borne pathogenic fungi (Morsy Ebtsam et al., 2009). In Egypt, the total cultivated area in 2015 was 187135 feddans and the total production was 3.31 million tons (Anonymos, 2015). There are several diseases on tomato caused by fungi, bacteria, viruses, nematodes and abiotic factors (Balanchard, 1992). Among the fungal diseases, damping-off was causing loss from 19 to 90% (Hadwan & Khara, 1992). The damping-off is a serious disease of vegetables grown in nursery bed. The most common fungi reported to be responsible for damping-off are *Pythium* sp., *Fusarium spp, Sclerotium rolfsii, Phytophthora* sp. and *Rhizoctonia solani*. (Ahmed & Hossain, 1985). The damping-off symptoms can be observed from seeding until the fourth to sixth week post-sowing the disease symptoms can be divided in two phases based on the time of its appearance. They occur when seeds decay prior to emergence. This can occur before seed germination, or when the germinating seeds are killed by biotic stresses while shoot tissues are still below ground in the first case, seeds become soft, rotten, and fail to germinate. In the second case, stems of germinating seeds are affected with characteristic water-soaked lesions formed at or below the soil line. With the progression of the disease, these lesions may darken to reddish-brown, brown, or black. Expanding lesions quickly girdle young and tender stems. Seedlings may wilt and die soon before emergence. In general, random pockets of poor seedling emergence are an indication of pre-emergence damping-off (Horst, 2013). The tomato cultivars were classified into three groups of resistant, tolerant and susceptible according to their reaction to *Fusarium* and *Rhizoctonia* infection (Moustafa & Khafagi, 1992). Matern and kneusel (1988) reported that phenolic compounds are used as precursors for the synthesis of lignin and suberin, which are then incorporated into the cell wall, increasing its mechanical strength and resistance to enzymatic degradation. Brammall (1986) reported that tomato resistant cultivars to *Fusarium oxysporum* were incorporate larger quantities of phenolic compounds in the cell wall more rapidly than tomato susceptible cultivars. Total protein increased in resistant and susceptible melon cultivars roots in response to inoculation with *R. solani* as compared to un-inoculated ones, also inoculated resistant melon cultivar roots had always higher content of total protein than the corresponding inoculated susceptible melon cultivar roots (Salari, et al., 2012). The present investigation was aimed to isolation and identification the causal pathogen of damping off disease of tomato seedling as well as determination of biochemical changes associated with total phenol and total protein in the tested tomato cultivars.

Materials and methods

Isolation of tomato damping-off causal fungi: Naturally infected seedlings of tomato varieties showing damping-off were obtained from several nurseries of Assiut and New valley Governorates, Egypt. Isolation procedures of the caused pathogen were carried out using small pieces of infected roots which were washed in running tap water, surface sterilized with 1% sodium hypochlorite solution for two minutes, washed in three changes of sterilized water and dried between sterilized filter papers. The

samples were placed in Petri plates (9 cm in diameter) contained Potato Dextrose Agar (PDA) medium (200g potato, 20g dextrose, 20g agar and 1 liter water). Petri plates were incubated at 17-30°C. After 5-7 days from incubation period, pure cultures of developing fungi were obtained by hyphal tip technique. The growing fungal culture were stored on PDA slant and kept in refrigerator at 4°C until used (Dhingra & Sinclair, 1985).

Pathogenicity tests: Pathogenic capability of 30 isolated pathogenic fungi was carried out on tomato plants (Castle Rock cv.) under greenhouse conditions, in the Faculty of Agriculture, Assiut University. Inocula of the tested isolates (taken from 7 days old culture grown on PDA medium) were prepared by growing in barley grains medium and incubated at 27°C for 15 days. Sterilized plastic pots (7cm in diameter) were filled with autoclaved (peat moss + Vermiculite 1:1) and infested with each pathogen at the rate 3% v/v of peat moss, mixed well, thoroughly irrigated and left 7 days to ensure establishment of the tested isolates in soil. Non infected soil was used as control. Tomato seeds were surface sterilized by dipping in sodium hypochlorite solution (0.1%) for 2 min, and then the seeds were washed through sterilized water and seeded (3seeds/pot). five replicates were used for each tested isolate.

Disease assessment: The percentage of pre-and post-emergence damping-off was recorded after 21 days and 45 days after cultivated plants, respectively. Percentages of disease incidence were calculated according to the following formula**:**

Pre-emergence % = (No. dead seedlings / No. sown seeds) x 100
Post-emergence % = (No. dead seedlings / No. sown seeds) x 100
Damping-off % = pre-emergence + post emergence.

Disease severity was rated using the scale of 1 to 5 used by Dhanasekaran et al., (2005) as follows:

1= Health seedling, 2= Primary root tip necrotic but firm, 3= Primary root tip soft and rotted, 4= Dead seedling, germinated seed with rotted radical, 5= Dead seed, ungerminated rotted seed.

Disease severity index (DSI) was calculated as the following formula:

$DSI = \Sigma d / (d\ max\ X\ n)\ X100$

Whereas: d is the disease rating of each plant, d max is the maximum disease rating and n is the total number of plants examined in each replicate.

Identification of tomato damping-off causal fungi: The five fungal isolates that showed the highest disease severity in pathogenicity tests were identified according to its morphological characters of mycelia and spores as described by Barnet and Hunter (1977), Domsch et al., (1980) and Moubasher (1993) and confirmed by Assiut University Mycological Center (AUMC), Assiut, Egypt.

Evaluation of certain tomato cultivars and hybrids to damping-off disease under nursery conditions: Six tomato cultivars, namely Castle Rock, Super marmande, Super Strain B, Enz 10F1,

0240F1 and Dream Hybrid were evaluated for their susceptibility to damping-off disease. Inocula of isolates (*R. solani, F. semitectum, F.oxysporum, A. alternata and S. rostrata*) were prepared as mentioned before in pathogenicity tests. Tomato seeds were surface sterilized by dipping in sodium hypochlorite solution (0.1%) for 2 min, and then the seeds were washed through sterilized water and seeded (3seeds/pot). five replicates were used for each tested isolate.

Determination of total phenol on tomato cultivars and hybrids as marker for biochemical changes due to infection: To assess phenolic content, 1 g plant sample was homogenized in 10 ml 80% methanol and agitated for 15 min. at 70^{o}C. One ml of the extract was added to 5 ml of distilled water and 250 µl of 1 N Folin-Ciocalteau reagent and the solution was kept at 25^{o}C. The absorbance was measured with a spectrophotometer (Spectronic 2OD) at 725 nm. Catechol was used as a standard (Saikia et al., 2006).

Determination of total protein on tomato cultivars and hybrids as marker for biochemical changes due to infection: The leaf sample of 0.5g was macerated with 10 mL of phosphate buffer (0.1 M, pH 7.0) using a pestle and mortar. The extract was centrifuged at 10000 rpm at 4℃ for 20 minutes. 0.1 mL of supernatant was taken and 5 ml of dye mixture was added. The solution was mixed well and kept aside for 15 minutes. The color intensity was recorded with a spectrophotometer (Spectronic 2OD) at 595 nm optical density (Bradford, 1976).

Statistical analysis: The obtained data were subjected to statistical analysis using software MSTAT C. Least significant difference (LSD) was employed to test for significant difference between treatment at P=0.05 for comparison between treatments means (Gomez & Gomez, 1984).

Results

Isolation of tomato damping-off causal fungi: Thirty fungal isolates were isolated from infected roots tomato seedling collected from several nurseries of Assiut and New Valley Governorates, Egypt. Twenty tow isolates of fungi belongs to *Fusarium* genera, three isolates of fungi belongs to *Setosphaeria* genera, tow isolates of fungi belongs to *Rhizoctonia* genera, tow isolates of fungi belongs to *Alternaria* genera and one isolate of fungi belong to *Cladosporium* genera. Table (1) show that the source of fungi isolates which isolated from different tomato nurseries located in Assiut and New Valley Governorates, Egypt.

Pathogenicity tests: Pathogenicity tests of 30 isolates were carried out on commercial tomato cultivar (Castle Rock cv.) under nursery conditions. Data in Table (2) show that all the tested fungal isolates were able to infected tomato plants (Castle Rock cv.). All tested isolates significantly caused damping-off disease compared with the control. They differed in their virulence from high to weak. *Rhizoctonia* sp. isolate R1, *Fusarium* sp. isolates F19, F4, *Setosphaeria* sp. isolate S3 then *Alternaria* sp. isolate A2 were the most

pathogenic isolates on the tested tomato (Castle Rock cv.) followed by the other tested ones. The highest of percentage of damping-off and disease severity of fungi was recorded by isolate R1 (97.7%) followed by isolates F19, S3, F4 and A2 which gave 93%, 91%, 90% and 86.6% respectively, while isolates F9, F16 showed that the least disease severity % (52.2%).

Table 1: source of fungi isolates which isolated from different tomato nurseries located in Assiut and New valley Governorates, Egypt.

Isolate No.	Isolate code	Isolate name	Source
1-3	F1,F2,F3		El- Kharga
4	F4		El- Dakhlla
5-11	F_5,F_6,F_7,F_8,F_9,F_{10},F_{11}	*Fusarium sp.*	Badari
12-16	F_{12},F_{13},F_{14},F_{15},F_{16}		Assiut
17-22	F_{17},F_{18},F_{19},F_{20},F_{21},F_{22}		Bni adi
23-25	S_1,S_2,S_3	*Setosphaeria sp.*	El- Dakhlla
26	R_1	*Rhizoctonia sp.*	El- Kharga
27	R_2		El- Dakhlla
28	A_1	*Alternaria sp.*	El- Kharga
29	A_2		Bni adi
30	C_1	*Cladosporium sp.*	El- Kharga

Table 2: Pathogenicity of isolated fungi on castle rook tomato cv. under greenhouse conditions.

Isolates	Isolate genus	Pre %	Post %	Damping-off %	Disease severity %
F1		33.30	16.65	49.95	64.44
F2		66.62	19.98	86.60	85.55
F3		46.62	26.64	73.26	76.66
F4		53.28	33.30	86.58	90.00
F5		53.28	13.32	66.60	68.88
F6		33.30	26.64	59.94	63.33
F7		26.64	26.64	53.28	66.67
F8		19.98	26.64	46.62	68.89
F9		13.32	26.64	39.96	52.22
F10		26.64	19.98	46.62	61.11
F11	*Fusarium*	19.98	26.64	46.62	63.33
F12		26.64	33.30	59.94	70.00
F13		33.30	19.98	53.28	58.88
F14		6.66	39.96	46.62	56.67
F15		13.32	33.30	46.62	58.89
F16		13.32	26.64	39.96	52.22
F17		59.94	19.98	79.92	74.44
F18		73.30	6.66	79.96	80.00
F19		73.30	19.98	93.28	93.33
F20		19.98	26.64	46.62	60.00
F21		33.30	19.98	53.28	63.33
F22		26.64	26.64	53.28	64.44
S1		46.62	19.98	66.60	72.22
S2	*Setosphaeria*	33.30	19.98	53.28	72.22
S3		66.60	26.64	93.24	91.11
R1	*Rhizoctonia*	86.64	13.32	99.96	97.77
R2		73.28	19.98	93.26	94.44
A1	*Alternaria*	46.62	19.98	66.60	76.66
A2		59.94	26.64	86.58	86.67
C1	*Cladosporium*	26.64	33.3	59.94	65.55
Control (non infested soil)		0.00	0.00	0.00	0.00
L.S.D. at 5%		11.20	9.99	9.66	7.80

Identification of tomato damping-off causal fungi: From the thirty isolates of the pathogens, only five isolates (R1, F19, F4, S3 and A2) which gave the highest disease severity were selected for identification. isolates R1, F19, F4, S3 and A2 are *Rhizoctonia solani* Kühn., *Fusarium oxysporum f.sp. lycopersici.* Schlechtendahl., *Fusarium semitectum* Berk. & Rav., *Setosphaeria rostrata* Leonard and *Alternaria alternata* (Fries) Keisler, respectively.

Table 3: Evaluation of certain tomato cultivars and hybrids to incidence of damping-off disease caused by *R. solani* under greenhouse conditions.

Cultivars	Damping-off incidence			
	Pre %	Post %	Total %	Severity %
Castle Rock	86.64	13.32	99.96	85.55
Super marmande	79.96	13.32	93.28	81.11
SuperStrain B	73.28	13.32	86.6	80.00
Enz 10F1	13.32	0.00	13.32	16.66
0 240 F1	6.66	0.00	6.66	13.33
Dream Hybrid	6.66	0.00	6.66	13.33
Main	44.42	6.66	51.08	48.33
L.S.D. at 5%	8.68	7.51	9.37	12.74

Table 4: Evaluation of certain tomato cultivars and hybrids to incidence of damping-off disease caused by *F. semitectum* under greenhouse conditions.

Cultivars	Damping-off incidence			
	Pre %	Post %	Total %	Severity %
Castle Rock	53.28	33.3	86.58	90.00
Super marmande	46.62	26.64	73.26	84.44
SuperStrain B	26.64	33.30	59.94	76.66
Enz 10F1	6.66	6.66	13.32	20.00
0 240 F1	6.66	6.66	13.32	24.44
Dream Hybrid	13.32	6.66	19.98	26.66
Main	25.53	18.87	44.40	53.70
L.S.D. at 5%	9.70	10.62	13.94	11.04

Table 5: Evaluation of certain tomato cultivars and hybrids to incidence of damping-off disease caused by *F. oxysporum* under greenhouse conditions.

Cultivars	Damping-off incidence			
	Pre %	Post %	Total %	Severity %
Castle Rock	73.30	19.98	86.58	93.33
Super marmande	66.60	13.32	73.26	90.00
SuperStrain B	66.60	13.32	59.94	90.00
Enz 10F1	6.66	0.00	13.32	17.77
0 240 F1	6.66	0.00	13.32	13.33
Dream Hybrid	6.66	0.00	19.98	14.44
Main	37.74	7.77	44.40	53.14
L.S.D. at 5%	9.03	7.51	9.37	8.75

Evaluation of certain tomato cultivars and hybrids to incidence of damping-off disease under greenhouse conditions: Data in table (3-7) indicate that all tomato cultivars were susceptible to damping-off disease, Castle Rock cv. showed the highest percentage of pre and post emergence damping-off and severity followed by Super marmande cv. and Super Strain B cv. then Dream Hybrid and Enz 10F1 Hybrid. 0240 F1 Hybrid gave the lowest percentage of pre and post emergence damping-off and disease severity. The main of damping-off severity for the rest pathogens were convergent.

Table 6: Evaluation of certain tomato cultivars and hybrids to incidence of damping-off disease caused by *A. alternata* under greenhouse conditions.

Cultivars	Damping-off incidence			
	Pre %	Post %	Total %	Severity %
Castle Rock	59.94	26.64	86.58	86.66
Super marmande	53.28	26.64	79.92	86.66
SuperStrain B	46.62	33.30	79.92	87.77
Enz 10F1	13.32	0.00	13.32	22.22
0 240 F1	13.32	6.66	19.98	13.33
Dream Hybrid	13.32	0.00	13.32	23.33
Main	33.30	15.54	48.84	53.23
L.S.D. at 5%	10.32	10.01	14.38	9.13

Table 7: Evaluation of certain tomato cultivars and hybrids to incidence of damping-off disease caused by *S. rostrata* under greenhouse conditions.

Cultivars	Damping-off incidence			
	Pre %	Post %	Total %	Severity %
Castle Rock	66.60	26.64	93.24	93.33
Super marmande	59.94	26.64	86.58	88.88
SuperStrain B	6.66	33.30	39.96	88.88
Enz 10F1	6.66	0.00	6.66	7.77
0 240 F1	6.66	0.00	6.66	9.00
Dream Hybrid	6.66	6.66	13.32	15.55
Main	25.53	15.54	41.07	50.56
L.S.D. at 5%	8.30	6.13	11.20	8.16

Determination of total phenol on tomato cultivars and hybrids infected with the pathogens: Data in Table (8) indicated that each tested tomato cultivars showed higher amount of total phenolic content in infected plants with the tested pathogenic fungal isolates than healthy plants (control). The highest amount of total phenol was found in infected plant of tomato Enz 10F1 cv. followed by 0240F1 cv. while the lowest amount of total phenol in infected plant of castel Roke cv. then super marmande cv.

Determination of total protein on tomato cultivars and hybrids infected with the pathogens: Data in Table (9) indicated that each tested tomato cultivars showed higher amount of total protein content in infected plants with the tested pathogenic fungal isolates than healthy plants (control). The highest amount of total protein was found in infected plant of tomato Enz 10F1cv. followed by 0240F1cv. while the lowest amount of total protein in infected plant of Castel Roke cv. then super marmande cv.

Table 8: Determination of total phenol on tomato cultivars and hybrids infected with the pathogens.

Cultivars	Total phenol mg/g dry Weight						Main
	Control	*R. solani*	*F. semitectum*	*F. oxysporum*	*A. alternata*	*S. rostrata*	
Castel Roke	57.99	69.44	70.83	68.75	71.88	70.83	68.40
Super marmande	59.38	70.49	71.88	71.88	71.88	70.83	69.44
Super strain B	60.76	72.22	72.57	70.83	72.92	73.96	70.54
Enz 10F1	72.57	80.21	80.56	81.25	81.25	80.21	79.34
0240F1	72.22	79.86	80.56	80.21	79.17	81.25	78.94
Dream hybred	67.01	75.35	75.69	76.04	76.04	76.04	74.31
Main	64.99	74.59	75.35	74.83	75.52	75.52	-
L.S.D. at 5%	0.78	0.91	0.54	0.89	1.04	0.71	

Table 9: Determination of total protein on tomato cultivars and hybrids infected with the pathogens.

Cultivars	Total phenol mg/g dry Weight						Main
	Control	*R. solani*	*F. semitectum*	*F. oxysporum*	*A. alternata*	*S. rostrata*	
Castel Roke	90.37	92.36	92.69	92.69	92.80	92.91	92.30
Super marmande	91.36	93.02	93.24	93.13	93.36	93.36	92.91
Super strain B	92.47	94.35	72.57	95.02	95.13	95.02	94.50
Enz 10F1	97.56	104.98	105.32	105.54	104.87	105.32	103.93
0240F1	97.34	104.87	105.20	105.54	105.20	104.87	103.84
Dream hybred	95.68	103.32	103.54	103.56	103.43	103.54	102.20
Main	94.13	98.82	95.43	99.25	99.13	99.17	-
L.S.D. at 5%	0.25	0.26	0.30	0.18	0.33	0.16	

Discussion

Damping-off disease is common disease of tomato nurseries in Egypt caused by a number of soil pathogenic fungi **(***Rhizoctonia solani, Fusarium spp*., *Setosphaeria rostrata* and *Alternaria alternata***)** they cause loss in tomato seedling from 19 to 90% (Hadwan & Khara, 1992; Ahmed & Hossain 1985). All tomato cultivars were susceptible to damping-off disease, Castle Rock cv. showed the highest percentage of pre and post emergence damping-off and disease severity followed by Super marmande cv. and Super Strain B cv. then Dream Hybrid and Enz 10F1 Hybrid. 0240 F1 Hybrid gave the lowest percentage of pre and post emergence damping-off and disease severity. The main of disease severity for pathogens were convergent. These results are in agreement with those recorded by Hadwan and Khara (1992). They reported that the incidence of damping-off diseases was ranged from 19 to 90% in two tomato cultivars which infested with *R. solani* in pots. In

addition, *R. solani* was isolated as the predominant damping-off fungus with highest frequency of 60.0 (Jiskani et al., 2007). Each tested tomato cultivars showed higher amount of total phenolic content in infected plants with the tested pathogenic fungal isolates than healthy plants (control). The highest amount of total phenol was found in infected plant of tomato Enz 10F1cv. followed by 0240F1cv. while the lowest amount of total phenol in infected plant of castel Roke cv. then super marmande cv. These results are in line agreement with those recorded by Brammall (1986) who reported that tomato resistant cultivars to *Fusarium oxysporum* were incorporate larger quantities of phenolic compounds in the cell wall more rapidly than tomato susceptible cultivars. Also**,** Matern and kneusel (1988) reported that phenolic compounds are used as precursors for the synthesis of lignin and suberin, which are then incorporated into the cell wall, increasing its mechanical strength and resistance to enzymatic degradation. Each tested tomato cultivars showed higher amount of total protein content in infected plants with the tested pathogenic fungal isolates than healthy plants (control). The highest amount of total protein was found in infected plant of tomato Enz 10F1cv. followed by 0240F1cv. while the lowest amount of total protein in infected plant of Castel Roke cv. then super marmande cv. These results are in accordance with those recorded by (Salari et al., 2012) who reported that total protein increased in resistant and susceptible melon cultivars roots in response to inoculation with *R. solani* as compared to un-inoculated ones, also inoculated resistant melon cultivar roots had always higher content of total protein than the corresponding inoculated susceptible melon cultivar roots.

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