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OCCURRENCE OF STRAWBERRY CROWN AND ROOT ROTS UNDER PLASTIC-HOUSE CONDITIONS

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ABSTRACT

Fusarium solani (Mart.) Sacc. and *Rhizoctonia solani* Kühn are considered the most serious pathogenic soil borne fungi causing strawberry crown and root rot diseases distributed in several cultivated districts in El-Sharkia Governorate. The investigated cultivar "Festival" showed the highest significant disease infection at Abo-Hammad with the highest disease severity, however, "Sweet Charlie" cultivar recorded less infection. The infected strawberry plants showed black discoloration where the infected roots seemed to be black and small in size. The entire root and the core often darken, unhealthy and lack new growth. In addition, foliage is reduced in size, has poor colour and wilted. Both isolated pathogenic fungi at the investigated districts are frequently differed in their ability to cause the disease. *Rhizoctonia solani* recorded the least survived plants under plastic-house conditions and proved to be the most virulent fungal pathogen. The sandy soil under investigation was superior in decreasing infection percentage for both strawberry cultivars comparing with the other soil types (clay and sandy clay soil).

Key words: Strawberry, root and crown rots, *Fusarium solani, Rhizoctonia solani,* plastic-house, soil types.

INTRODUCTION

Strawberry, (*Fragaria x ananassa* Duch.) has become one of the most economic vegetable crop in Egypt. It is one of the most important members of the family Rosaceae. It is cultivated in El-Gharbia, El-Ismaelia, El-Kalubia, El- Behera, El-Sharkia and El-Giza Governorates. Strawberry plants in the field are liable to be attacked by several diseases which are responsible for considerable losses in the yield and fruit quality. Crown and root rot diseases are widespread of perennial matted-row strawberries (Crane, 2007; Koike 2008 and Aviles *et al.,* 2009).

The affected strawberry roots have one or more of the following symptoms : 1) much smaller root systems than normal, 2) main roots are spotted with dark patches or zones, 3) most feeder roots are lacking and 4) all or part of the main root is dead and blackened throughout. Affected plants are less vigorous and produce few runners. Severely infected plants might die (Manici and Patrizia, 2007 and Particka and Hancock, 2008). Discoloration of root and crown tissues resulted in wilting of foliage and fruit infection might occur.

The most frequently isolated pathogens from affected crown and roots are *Fusarium solani* (Mart.) Sacc. and *Rhizoctonia* spp. (especially *R. solani* Kühn and *Rhizoctonia fragariae* Husain & McKeen), *Macrophomina phaseolina* (Tassi) Goid. Other fungi were also isolated (Zveibil and Freeman, 2005; Abdel-Sattar *et al.,* 2008 and Baino *et al.,* 2011).

The role of the isolated fungi genera proved to be pathogenic as expressed of crown and root rot of strawberry plant cultivars (Basurto-Cadena *et al.,* 1995; Koike 2008 and Aviles *et al.,* 2008).

Thus, this work was conducted to study the presence of strawberry crown and root rot diseases in different districts of El-Sharkia Governorate. Isolation, purification and identification of the causal organisms as well as their pathogenic

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potentiality were also undertaken. Strawberry varietal reactions and effect of soil types on disease incidence, were also investigated.

MATERIALS AND METHODS

Sample Collections

Diseased samples were collected from various strawberry cultivars (*Fragaria* × *ananassa* Duch.) cultivated area in different districts at El-Sharkia Governorate (Abo-Hammad, Belbas, Fakous and El Salihiyah El-Kademah). Collected naturally infected samples exhibit typical symptoms doubted to be due to root and crown rots of strawberry disease, were investigated. The deteriorated naturally infected strawberry plants showing damaged of the adventitious roots, browning in the crown area in addition to scarcity of feeder rootlets were subjected to isolation trials for the causal organisms.

Detection of the Pathogenic Microorganisms

The infected roots were thoroughly washed, cut into small pieces and surface sterilized in 1% sodium hypochlorite solution for 3 min_s., then washed several times with sterile distilled water and dried between sterilized filter paper. Sterilized samples were placed onto water agar (WA) medium in Petri dishes using the methods suggested by Booth (1971) and Nelson *et al.* (1983). The developed fungi were recorded as percentage of frequency for all of the isolated microorganisms grown on PDA at 27 ± 1°C for 7 days. The isolated fungi were purified using the hyphal tip technique and/ or single spore isolate technique described by Dhingra and Sinclair (1995).

The isolated microorganisms were microscopically identified using the description of Barnett & Hunter (2003). Identification of the selected isolates was confirmed by the Fungal Taxonomy Department, Plant Pathology Research Institute, Agricultural Research Centre, Giza.

Disease Assessment

Disease assessment was carried out through determination of both percentage of infection and disease severity. The infection percentages was calculated according to the following formula:

$$\text{Infection}\,\% = \frac{\text{number of infected plants}}{\text{total number of plants}} \times 100$$

The disease severity percentage was determined according to a numerical rating score to facilitate visual determination of discoloration of both infected roots and crowns which reflects the damage caused by both fungal genera .The rating score involves conversion of estimated percentages to score was determined according to Smith and Black (1987).

Pathogenicity Tests

Pathogenic potentiality of the three fungi (*F. solani, R. solani and M. phaseolina*) isolated from crown and root rots of two strawberry cultivars namely Sweet Charlie and Festival, were carried out under the plastic-house conditions at Fac. of Agric. Zagazig Univ.

Inoculum was prepared by growing each of the isolated fungi in 500 ml conical flasks containing 200 ml of autoclaved potato dextrose broth (PDB) medium and incubated at 27±1 °C for 10–15 days. The fungal growth was blended in a blender for two minutes using sterilized distilled water to homogenize the inocula.

Plastic pots (20 cm in diameter) were sterilized using 5% formalin solution for 15 minutes then left to dry for 7 days to ensure getting-rid off and evaporation of the excess poisonous formalin. The sterilized pots were then filled with sterilized sandy clay soil obtained from Abo-Hammad. Chemical and physical analyses of the investigated soil types presented in Table 1. Sandy clay soil was sterilized using formaline solution (5%) for one week. After sterilization period, sterilized soil was left for 15 days to get red off formaline toxicity.

Soil infestation was carried out by adding the blended homogenized fungal inoculum to the sterilized soil at the rate of 3-5% of soil weight (v/w). The infested soil then watered and left for 10 – 15 days before cultivation to stimulate the fungal growth. Control pots were inoculated with fungal free sterilized potato broth medium at the same rate and watered.

Fifty six pots were used (28 pots/ cultivar, 7 pots/ fungus) while 7 pots were used as a control. Each replicate was planted using five strawberry transplants (cv. Festival).

Table 1. Physical and chemical properties of soils, under investigation

Character	Soil 1	Soil 2	Soil 3
	Physical analysis		
Clay (%)	36.80	2.10	12.10
Silt (%)	16.70	4.03	10.30
Sand (%)	46.50	91.87	77.60
Textural class	**Clay**	**sandy**	**Sandy clay**
$CaCO_3$ (%)	0.61	0.50	0.60
	Chemical analysis		
pH (1:2.5)	7.8	8.02	7.90
EC dSm^{-1}	2.00	0.51	0.42
Na^+ mg/l	12.4	2.67	0.39
K^+ mg/l	0.90	0.69	0.14
Ca^{++} mg/l	5.90	1.52	1.82
Mg^{++} mg/l	2.30	0.38	2.80
CO_3^{--} mg/l	--	--	--
HCO_3^- mg/l	1.40	1.60	2.10
Cl^- mg/l	6.80	2.80	0.45
SO_4^{--} mg/l	13.3	0.86	2.60
Organic matter (%)	1.62	0.48	1.10
	Available contents (mg/kg soil)		
N	198	49.70	149.10
P	194.5	17.36	54.6
K	266	80	80

Four pots were used for each particular treatment. All plants were observed daily and watered as needed. Crown and root rots were recorded 30 days after inoculation (Smith and Black, 1987). Healthy survived plants were recorded as usual after 50 days from inoculation.

Effect of Soil Types

The effect of soil types on disease incidence caused by either *R. solani* and/or *F. solani*, the most pathogenic fungi, has been carried out using two strawberry cultivars (cv. Sweet Charlie and Festival) under plastic-house conditions at Fac. Agric., Zagazig Univ.

According to the physical and chemical analyses, three soil types were investigated *i.e.*, clay, sandy and sandy clay (Table 1). Soil samples were obtained from Abo-Hammad district. The previously mentioned physical and chemical analyses has been carried out by the aid of Soil Analysis Lab. of Soil Sci. Dept. Fac. of Agric. Zagazig Univ.

Pots (20 cm in diam.) were surface sterilized as mentioned above. The three soil types were separately sterilized as mentioned under pathogenicity tests. Each pot was filled with the soil and inoculated individually with each pathogen at a rate of 5 ml/100 g (v/w) soil. Seventy two pots were used, 36 pots for each cultivar, 12 pots for each particular soil type (four pots were inoculated with *F. solani*, another four pots for *R. solani* and four pots were left without inoculation to serve as a control). Four replicates were used for each particular treatment. One transplant of each cultivar obtained from Abo-Hammad, Al Mollak, was cultivated in each pot. Pots were left under plastic-house conditions for 30 days. Treatments were irrigated as needed. Infection percentage and disease severity, were determined as mentioned before.

RESULTS AND DISCUSSION

Samples of strawberry plants seemed to be naturally infected with root and crown rots collected from different districts at El-Sharkia Governorate, were investigated.

Data presented in Table 2 indicates the disease assessment of two strawberry cultivars (Sweet Charlie and Festival). The investigated cultivar Festival recorded the highest infection percentage (60%) at Abo-Hammad with the highest disease severity (71.3%). Percentage of infection and disease severity of cultivated strawberry at El-Salihiya El-kadema exhibited the least results being (31.11%) and consequently revealed the least disease severity being (45.66%). Moderate percentages were recorded for those cultivated at Belbase and Fakous districts.

The investigated cultivar Sweet Charlie revealed the highest infection percentage (57%) at Abo-Hammad district with the highest disease severity (66.35%). The percentage of infection and disease severity of cultivated strawberry at El-Salihiya El-kadema exhibited the least infection percentage recorded 27% and severity percentage 41.66%, respectively. Moderate percentages were recorded for those cultivated at Belbase and Fakous districts.

Thus, variations were recorded in both disease infection and severity percentages in the inspected districts. This might be due to one or more of the following factors: i) pathogen frequency differed between the districts either qualitatively or quantitatively. ii) climatic conditions that considerably vary between regions. iii) varietal sensitivity iv) dissemination factors available in the region. v) the cultural practices. Similar results were obtained by Chen *et al.* (2011).

The isolated causal organisms purified and microscopically examined and found to be identical to those described by Kohmoto *et al.* (1981); Tanaka *et al.* (1995) and Benlioglu *et al.* (2004).

Isolated and purified fungi were identified as *Fusarium solani* (Mart.) Sacc., *Rhizoctonia solani* Kuhn. and *Macrophomina phaseolina* (Tassi) Goid.

Data presented in Table 3 show that *F. solani* (42.75) and *R. solani* (47.25) were the most frequent isolated fungi of all the collected diseased samples.

The frequency% of *F. solani* was higher at El-Salihiya El-kadema being 54 followed by Fakous 47. However, the frequency of *R. solani* was significantly the highest at Belbase (57) followed by Abo-Hammad bieng (53), respectively. *Macrophomina phaseoliana* was the least frequent one at all the investigated districts. The different results especially of both *Fusarium solani* (Mart.) Sacc. and *R. solani* Kühn might be due to the differences between the investigated districts, irrigation water, salinity, relative humidity, the underground water level, soil type and the usual agricultural practices that might appropriate the needs of one organism rather than the others. Similar conclusion was obtained by Jimenez (2011).

Results of pathogenicity tests as shown in Table 4 reveals that the isolated fungi varied in thier pathogenic potentiality causing damping-off and root rot of strawberry plants. *R. solani* was highly pathogenic causing post emergence damping-off and root rot for both Festival and Sweet Charlie cultivars followed by *F. solani*, whereas *M. phaseolina* was less virulent.

The two strawberry cultivars differed in their reactions to the infection. The lowest percentage of survived plants was recorded with cv. Festival when *R. solani* was examined (0.0%). Results also indicate that, Festival cultivar was more affected with both damping-off and root rot diseases than Sweet Charlie one.

The obtained results might be attributed to one or more of soil mechanical or physical factors, variability in genetic make-up of the tested cultivars, the inoculum potentiality of the tested pathogens and/or degree of virulence.

Differences in pathogenic potentiality of the investigated fungi genera might be due to the differences between fungi in their ability to produce enzymes and toxins essential for their pathogenicity and might be also due to host-parasitic interactions (Manici and Patrizia, 2007; Abdel-Sattar *et al.*, 2008; Poling, 2008; Aviles *et al.*, 2009 and Nam *et al.*, 2011).

Table 2. Disease assessment at different investigated districts of El-Sharkia Governorate on two strawberry cultivars (Sweet Charlie and Festival)

Districts	Disease Infection (DI%)		Disease Severity (DS%)	
	Strawberry cultivars			
	Sweet Charlie	Festival	Sweet Charlie	Festival
Abo- Hammad	57.00	60.00	66.35	71.30
Belbase	42.00	55.50	60.70	65.60
Fakous	41.25	53.30	50.00	56.00
El-Salihiya El-kadema	27.00	31.11	41.66	45.66
Mean	41.81	49.97	54.67	59.64
$L.S.D._{0.05}$				
Cultivar	3.017		3.936	
Districts	4.267		4.567	
Interaction	6.034		7.873	

Table 3. Frequency of the isolated fungi associated with root and crown rot of strawberry at four districts in El- Sharkia Governorate

Isolated fungi	Abo-Hammad	Belbase	Fakous	El-Salihiya El-kadema	Average
Fusarium solani	27	43	47	54	42.75
Rhizoctonia solani	53	57	40	39	47.25
Macrophomina phaseolina	20	0	13	17	10.00
Total	100	100	100	100	100
L.S.D. at $_{0.05}$					
Fungi			2.398		
Districts			2.769		
Interaction			4.796		

Table 4. Pathogenicity tests of the three isolated fungi from rotted roots and crown rot of strawberry (cv. Festival and Sweet Charlie), under plastic-house conditions

Fungi	The percentage of infection								
	Root rot			Post emergence damping off			Healthy survivals		
	Festival	Sweet Charlie	Mean	Festival	Sweet Charlie	Mean	Festival	Sweet Charlie	Mean
F. solani	42.85	28.57	35.71	42.85	42.85	42.85	14.28	28.57	21.42
R. solani	42.85	42.85	42.85	57.14	42.85	49.99	0	14.28	7.14
M. phaseolian	28.57	28.57	28.75	28.57	14.28	21.51	42.85	57.14	49.99
Control	0	0	0	0	0	0	100	100	100
Mean	28.56	24.99	26.82	32.14	24.99	28.58	39.28	49.99	44.63
L.S.D.at $_{0.05}$									
Cultivar	2.785			2.522				1.761	
Fungi	3.938			3.567				2.490	
Interaction	5.569			5.045				3.521	

Samples collected during survery study indicate symptoms include black discolorations on strawberry (Fig. 1) where the infected roots seemed to be black and smaller in size rather than the healthy ones (Fig.1 A). The entire root or all but the core often darken, look unhealthy, and lack new growth (Fig.1 B,C,D). Foliage is reduced in size, has poor colour, and wilts in warm weather (Fig.1 E,F).

Typical symptoms of damping-off and root rot diseases showed severe necrosis on roots and hypocotyle, stunting of plants, yellowing of foliage, death of lower leaves and eventually death of all plants (Fig. 2). The re-isolation trials proved that these pathogens were readily isolated from such plants, showing typical damping-off and root rot disease symptoms.

The physiological factors leading to different virulence levels among isolates are still unclear. Although synthesis and secretion of cell wall degrading enzymes as well as the production of mycotoxins have been assumed to play an important role in this respect (Desjardins *et al.*, 1992).

Data in Table 5 show that the disease infection and severity differed significantly in all examined soil types. The investigated sandy soil was superior in decreasing infection percentage for both examined strawberry cultivars. Besides, reduction percentages of infection were obtained for Sweet Charlie one (50%) equality with sandy loam soil. The highest value of infection percentage were obtained when clay soil and sandy loam soil was investigated especially when cultivated with Festival cultivar (75%).

Disease severity percentage reveal that the highest values were detected when both cultivars (Festival and Sweet Charlie) were cultivated in clay soil (65.3% and 58.3%, respectively). This might be due to the high humidic values around strawberry roots which favoured the fungal infections .On the other hand, the least severity percentages were realized when either Sweet Charlie or Festival strawberry cultivars were cultivated in sandy soil (43.0% and 49.9%, respectively) followed by sandy loam and sandy soils. However, clay soil was the highest soil type in affecting the disease severity.

Accordingly, it might be suggested that the growth and distribution of the pathogens hyphae and/or propagules were effective in soils having high amount of clay keep high levels of organic matter and enough compact water, nutrients that enough for the growth and more favourable for both *F. solani* and *R. solani* as compared with the other soil types. Apparently clay soil seemed to be more suitable for disease severity exhibited by the tested pathogens. However, the relatively lower values of organic materials and nutrients in both sandy and sandy clay soil could be restricting factors for fungal growth or the outcome of disease reactions. The contribution of soil nutrients and water potential in disease reactions was reported by Misaghi (1982). Jimenez (2011) reported that there was variation in the population across soil types but this variation was significantly different in the sandy soil texture in which populations were reduced. Also sandy as well as loamy sand could have a negative impact upon inoculum survival.

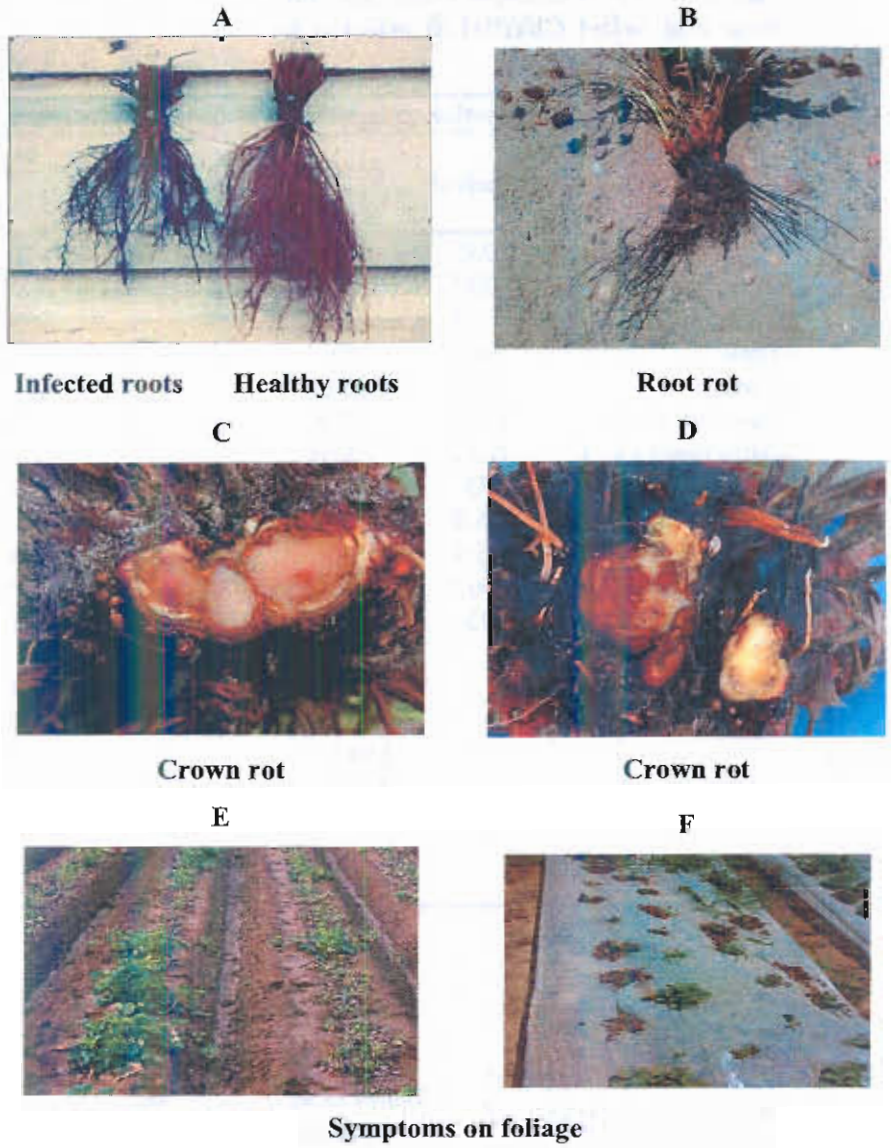

Fig. 1. Symptoms of strawberry root and crown rots

Fig. 2. Symptoms of damping-off and rot diseases as induced by pathogenic fungi, *Fusarium solani* and *R. solani*

Table 5. Disease incidence caused by *Fusarium solani* and *Rhizoctonia solani* on two strawberry cultivars (Festival and Sweet Charlie) as affected by different soil types, investigated under plastic-house conditions

Soil type	Cultivar / Fungi	Infection percentage (IF %)			Disease severity (DS %)		
		Festival	Sweet Charlie	Mean	Festival	Sweet Charlie	Mean
Sandy soil	**Control**	0.0	0.0	0.0	0.0	0.0	0.0
	Fusarium solani	100.0	75.0	87.5	79.16	66.6	72.9
	Rhizoctonia solani	75.0	75.0	75.0	70.8	62.5	66.6
	Mean	58.3	50	54.2	49.9	43.0	46.5
Claly soil	**Control**	25.0	25.0	25.0	16.6	12.5	14.55
	Fusarium solani	100.0	75.0	87.5	87.5	79.16	83.33
	Rhizoctonia solani	100.0	100.0	100.0	91.66	83.3	87.48
	Mean	75	66.7	70.8	65.3	58.3	61.8
Sandy clay soil	**Control**	25.0	0.0	12.5	12.3	0.0	6.15
	Fusarium solani	75.0	75.0	75.0	87.5	79.16	83.33
	Rhizoctonia solani	100.0	75.0	87.5	83.3	75.0	79.15
	Mean	75	50	62.5	61.0	51.4	56.2
L.S.D.$_{0.05}$							
Cultivar			1.721			2.120	
Soil type			2.108			2.597	
Cultivar x Soil type			2.981			N.S.	
Fungi			2.108			2.597	
Cultivar x Fungi			2.981			N.S.	
Soil type x Fungi			3.651			4.498	
Cultivar x Soil type x Fungi			5.163			N.S.	

*N.S. = Not significant

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ظهور أعفان جذور وتيجان نباتات الفراولة تحت ظروف الصوبة البلاستيكية

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يعتبر كل من فطرى الفيوزاريوم سولانى والرايزوكتونيا سولانى أكثر فطريات التربة خطورة على نباتات الفراولة حيث تسبب لها أمراض أعفان الجذور والتيجان وذلك فى عديد من المناطق المنزرعة بها فى محافظة الشرقية، أظهر الصنف فستيفال اكبر نسبة إصابة وكذلك أكثر شدة إصابة معنوية فى أبو حماد، بينما كانت أقل منها فى الصنف سويت شارلى، وأظهرت نباتات الفراولة المصابة أعراض تغير فى لون الجذور إلى اللون الأسود مع صغر حجمها كما تلونت من الداخل (القلب) بلون داكن مع نقص فى تكوين النموات الجديدة، بالإضافة إلى صغر حجم المجموع الخضرى وشحوب لونها وذبول أوراقها، أظهر كلاً من الفطرين المعزولين اختلافاً فى نسبة تكرارهما فى المناطق المختلفة بمحافظة الشرقية وكذلك فى قدرتهما على إحداث الإصابة، حيث كان الفطر رايزوكتونيا سولانى الأكثر قدرة على إحداث الإصابة تحت ظروف الصوبة حيث أعطى أقل نسبة نباتات سليمة باقية. وكانت التربة الرملية هى أفضل أنواع التربة المستخدمة فى تقليل نسبة الإصابة وشدة الإصابة مع كلا من الصنفين (فستيفال وسويت شارلى) مقارنة بأنواع التربة الأخرى تحت الدراسة.

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