Occurrence of Root Rot and Vascular Wilt Diseases in Roselle (*Hibiscus sabdariffa* L.) in Upper Egypt

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Abstract Roselle (*Hibiscus sabdariffa* L.) family *Malvaceae* is an important crop used in food, cosmetics and pharmaceuticals industries. Roselle is cultivated mainly in Upper Egypt (Qena and Aswan governorates) producing 94% of total production. Root rot disease of roselle is one of the most important diseases that attack both seedlings and adult plants causing serious losses in crop productivity and quality. The main objective of the present study is to identify and characterize pathogens associated with root rot and wilt symptoms of roselle in Qena, Upper Egypt and evaluate their pathogenicity under greenhouse and field condition. *Fusarium oxysporum*, *Macrophomina phaseolina*, *Fusarium solani*, *Fusarium equiseti* and *Fusarium semitectum* were isolated from the natural root rot diseases in roselle. All isolated fungi were morphologically characterized and varied in their pathogenic potentialities. They could attack roselle plants causing damping-off and root rot/wilt diseases in different pathogenicity tests. The highest pathogenicity was caused by *F. oxysporum* and *M. phaseolina* followed by *F. solani*. The least pathogenic fungi were *F. equiseti* followed by *F. semitectum*. It obviously noted that Baladi roselle cultivar was more susceptible to infection with all tested fungi than Sobhia 17 under greenhouse and field conditions. This is the first report of fungal pathogens causing root rot and vascular wilt in roselle in Upper Egypt.

Keywords *Fusarium oxysporum*, *F. solani*, *Hibiscus sabdariffa* L., *Macrophomina phaseolina*, Root rot/wilt

Roselle (*Hibiscus sabdariffa* L.) family *Malvaceae* is a summer growing famous medicinal plant. More than 300 species of *Hibiscus* are growing in both tropical and subtropical regions around the world [1]. Most varieties are used as ornamental plants but *Hibiscus sabdariffa* var. *sabdariffa* is an edible type. Another variety, *Hibiscus sabdariffa* var. *altissima* is grown for fiber production [2].

In Egypt, roselle is cultivated mainly in Upper Egypt (especially in Qena and Aswan governorates) representing about 93.9% of the total cultivated area. In Qena, the

cultivated area is about 1,150 ha (45.83%) which either newly reclaimed area (966 ha) or valley old soil (189 ha). About 1,353 out of 2,648 tons (51.1% of the total production) of dry yield of roselle are produced by Qena governorate. Sudani, Masri and White are the most three dominant cultivars in Egypt. Masri cultivars are two types; Baladi and Sobhia 17. Sobhia 17 is produced by Egyptian Ministry of Agriculture and is characterized by early flowering and can be harvested 1.5 month earlier than Baladi type [3]. Most of the research on roselle has so far concerned with its antioxidant activity, health benefits, and nutritional value. But the diseases affecting roselle production are not sufficiently investigated.

One of the most serious obstacles that limit roselle production in many growing areas is the damping-off, wilt and root rot diseases. The most frequent pathogenic soil-borne fungi associated with rotted roots were *Fusarium oxysporum*, *Fusarium solani*, *Macrophomina phaseolina*, *Rhizoctonia solani*, and *Fusarium equiseti* [4, 5].

Although Upper Egypt is considered as the main source of roselle production, the diseases of roselle in Upper Egypt are not investigated yet. Our recent field observation in different localities of Qena governorates has shown that roselle crops suffer from root rot and wilt diseases. The objectives of the present study are isolation and identification

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of the causal pathogens from rotted and wilted plants and evaluation of the pathogenicity of the isolated pathogens on dominant cultivated cultivars of roselle in Upper Egypt under greenhouse and field conditions.

MATERIALS AND METHODS

Source of samples. Samples of the roselle plants suffering from root rot and wilt diseases were collected from different localities in Qena Governorate. Symptoms of naturally infected plants were briefly described and photographed. The infected plants were uprooted and carefully washed in running tap water to remove any soil remains. Affected parts of the infected roots were cut into small pieces (2 mm in length), surface sterilized with 5% of sodium hypochlorite, washed several times with distilled water and then dried between sterilized filter papers. The sterilized pieces were transferred into potato dextrose agar (PDA) medium supplemented with penicillin (20 µL/mL) and incubated at $25 \pm 1^\circ\text{C}$, then examined daily for fungal growth.

Isolation, purification and identification of the causal organism(s). The fungal colonies were purified using single spore or hyphal tip isolation technique suggested by Booth [6] and then identified according to their morphological and microscopical characters as described by Booth [6] and Barnett and Hunter [7]. Identification was confirmed by Assiut University Mycological Centre (AUMC), Assiut University, Assiut, Egypt. The obtained isolates were maintained on PDA slants and kept in a refrigerator at 5°C for further study.

Pathogenicity tests.

Source of roselle seeds: Seeds of roselle were obtained from Crop Research Department, Field Crop Res. Institute, National Agriculture Research Center, Ministry of Agriculture, Egypt. Seeds of two cultivars namely Baladi and Sobhia 17 were surface sterilized and dried in sterilized filter paper. The pathogenicity tests were carried out on pots in greenhouse or in the farm of Plant Pathology Department, Faculty of Agriculture, South Valley University, Qena, Egypt.

Preparation of the fungal inocula. Conical flasks each contained 100 g barely grains and 100 mL of distilled water were autoclaved. These flasks were subsequently inoculated with the fungal isolates using about 5–10 of 1 cm fungal discs, previously grown on PDA for 4 days. The inoculated flasks were incubated at 25°C for 14 days with shake every day.

Preparation of soil and pots. Soil inoculation was performed by mixing of 2% of the inocula with the soil in each pot (100 g/5 kg soil) and then irrigated directly. Sterilized non-inoculated barely grains were used as the

control treatment. Ten sterilized surface seeds of roselle were sown in each pot and a set of three replicates was performed. Data of pre- and post-emergence damping-off were recorded after 4 wk. The severity of the symptoms of root rot/wilt diseases was determined after 90 days post-inoculation. Samples of plants were taken from different treatments for the purpose of re-isolation procedures on PDA. To meet Koch's postulates, the experiments were conducted twice in two successive seasons i.e., 2005 and 2006.

Pathogenicity test for the tested fungi under field conditions.

The inocula of the tested fungi were prepared on barely grains as mentioned above to study their pathogenicity on the 2 cultivars of roselle, Baladi and Sobhia 17. The land used for carrying out the pathogenicity test was under natural solarization. The rows were arranged in a completely randomized design. About 18 rows (2 m in length each and 70 cm apart) were used for this experiment. Each row contained 5 pits with 30 cm apart. Three rows were used for each of the five tested fungi and three rows inoculated with sterilized non-inoculated barely grains as control treatment. Infestation of the soil was performed by applying evenly 200 g of the fungus inoculum in the center of pits. Inoculated pits were covered with soil and irrigated. The rows were irrigated when necessary to avoid dryness and keep sufficient moisture for fungi to survive and multiply. One week later, 10 surface-sterilized seeds of roselle cultivars were sown in each pit. The pits were covered with a thin layer of soil after sowing and then irrigated immediately. Subsequently plants were irrigated when necessary and data of pre- and post-emergence damping-off were recorded after 2 and 4 wk, respectively. Data of wilt and root rot was recorded after 3 months.

Statistical analysis. All experiments were performed twice. Analyses of variance were carried out using MSTAT-C, 1991 program ver. 2.10. Least significant difference was employed to test for significant difference between treatments at $p \leq 0.05$ [8].

RESULTS AND DISCUSSION

Natural disease. Symptoms of naturally infected plants were characterized by cankers in the roots and the basal part of their stems adjacent to soil surface. These areas were brown to black in color. There were no secondary roots as they completely deteriorated and destroyed. In severely infected cases, the adult plants were wilted and dried off from down to up. The leaves of the infected plants lose their turgidity, became flaccid, greenish yellow in color. The young tender shoots also wilted and died. In cross section of infected plant's stem and root, discolored brown areas appear as complete or incomplete ring representing the discolored vascular tissue. Late, the whole plant became brown with dried leaves. Symptoms of

Fig. 1. Natural infection of roselle plants exhibited wilt symptoms (A) and the surrounding area showing absence of some plants indicating post-emergence damping off (B).

Fig. 2. Naturally infected roots of roselle plants showing brown soft rot on the end of the root (centre) and black discoloration of the roots.

naturally infected plants were photographed in Figs. 1 and 2.

Identification of the causal pathogens. Isolation of the fungi from root and stem parts of rotted and wilted plants of roselle on PDA medium resulted in 15 isolates of

Fig. 3. Micrograph of *Fusarium oxysporum* showing ellipsoid and slightly-curved microconidia spores and sickle-shaped, hyaline, mostly 2-3 septated macroconidia spores.

Fig. 4. Micrograph of *Fusarium solani* showing smooth- or rough-walled chlamydospores on the aerial mycelium.

pathogenic fungi and 5 isolates of saprophytic fungi according to their morphological characteristics. All the isolates were tested in a pathogenicity test and only the isolates induced severe lesions were further identified and included in overall pathogenicity tests (data not shown). According to the pathogenicity test the most severe isolates were five pathogenic fungi; *F. oxysporum* (Fig. 3), *F. solani* (Fig. 4), *F. equiseti* (Fig. 5), *Macrophomina phaseolina* (Fig. 6), and *F. semitectum*. Morphological identification of the isolated fungi was achieved according to Booth [6] and Nelson *et al.* [9] and confirmed at AUMC, Assuit University, Assuit, Egypt, depending on their morphological.

Pathogenicity tests.

Pathogenicity under greenhouse condition: All the tested fungi were pathogenic under greenhouse conditions in two successive seasons i.e., 2005, 2006 and the symptoms of pre- and post-emergence damping off and wilt that observed during the pathogenicity test were close to those observed in natural symptom. In pre-emergence damping off, the plant seedlings were absent and no seedlings were emerged in soil surface. In post-emergence damping off, the small

Fig. 5. Micrograph of *Fusarium equiseti* showing formation of microconidia spores inside macroconidia.

Fig. 6. Micrograph of *Macrophomina phaseolina* showing formation of sclerotial bodies.

Fig. 7. Damping off in Baladi roselle plants caused by *Fusarium oxysporum* under greenhouse conditions in 2005 growing season.

seedling started to be yellow from upper part and continued to the above. After one week, the whole seedlings turned to yellow and died. The wilt symptoms started with

Fig. 8. Wilt of Baladi roselle plants caused by *Fusarium oxysporum* under greenhouse conditions in 2005 growing season.

Fig. 9. Damping-off of Baladi roselle plants caused by *Fusarium solani* under greenhouse conditions in 2005 growing season.

yellowing on the upper leaves and upper part of the plant and extended to up. The whole plant turned to yellow and brown and then died (Figs. 7~10).

In season (2005), the highest percentage of pre-emergence and post-emergence damping off and root rot/wilt were caused by *F. oxysporum* (73.3%), *F. semitectum* (13.3%) and *M. phaseolina* (16.7%), respectively in Baladi cultivars. In Sobhia 17 cultivar, the highest percentage of pre-emergence damping off was caused by *M. phaseolina* (63.3%) followed by *F. oxysporum* (60.0%). The highest percentages of post-emergence damping off were caused by *F. oxysporum* and

Fig. 10. Wilt of Sobhia 17 plants caused by *M. phaseolina* under greenhouse conditions in 2006 growing season.

F. equiseti (10.0%), but *F. oxysporum* and *M. phaseolina* (16.7%) caused the highest percentage of wilt.

In season 2006, the highest percentages of pre-emergence and post-emergence damping off and wilt were caused by

F. oxysporum (70.0%), *F. solani* (13.3%), and *F. oxysporum* (20.0%) respectively in Baladi cultivars. In Sobhia 17 cultivars, the highest pre-emergence damping off was caused by *F. oxysporum* (56.0%) and *M. phaseolina* (56.0%). The highest percentage of post-emergence damping off was caused by *F. equiseti* (13.3%). *F. solani* and *M. phaseolina* caused the highest percentages of wilt (23.3% each).

Pathogenicity under field condition: In Baladi cultivar, the highest percentage of pre-emergence damping off was caused by *F. oxysporum* (53.0%) followed by *M. phaseolina* (51.0%). However, *F. semitectum* caused the highest percentage of post-emergence damping off (17.3%) and *M. phaseolina* caused the highest percentage of wilt (41.7%). In Sobhia 17 cultivars, the highest pre-emergence damping off was caused by *F. oxysporum* (49.3%) and that of post-emergence was caused by *F. semitectum* (19.3%) and the highest percentage of wilt was caused by *M. phaseolina* (39.7%).

As shown in Tables 1 and 2, the highest percentage of pre-emergence damping-off was caused by *F. oxysporum* in Baladi cultivar in the two growing seasons. The highest

Table 1. Pathogenicity of the isolated fungi on roselle cultivars in 2005 growing season under greenhouse conditions

Fungi	Pre-emergence damping off (%)			Post emergence damping off (%)			Wilt (%)		
	Baladi	Sobhia 17	Mean	Baladi	Sobhia 17	Mean	Baladi	Sobhia 17	Mean
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Fusarium oxysporum</i>	73.33	60.00	66.66	3.33	10.00	6.66	16.66	13.33	15.00
<i>F. solani</i>	63.33	46.66	55.00	6.66	3.33	5.00	3.33	10.00	6.66
<i>F. equiseti</i>	46.66	36.66	41.66	6.66	10.00	8.33	3.33	6.66	5.00
<i>F. semitectum</i>	33.33	30.00	31.66	13.33	6.66	10.00	13.33	10.00	11.66
<i>Macrophomina phaseolina</i>	66.66	63.34	65.00	3.33	3.33	3.33	16.66	13.33	15.00
Mean	47.22	39.44		5.55	5.55		8.88	8.88	
LSD 5%	A*			A*			A = ns		
	B = 6.59			B = 5.86			B = 7.42		
	AB = ns			AB = ns			AB = ns		

LSD, least significance difference; ns, not significant.

* Significant at $P \leq 0.01$.

ns: non significant.

Table 2. Pathogenicity of the isolated fungi on roselle cultivars in 2006 growing season under greenhouse conditions

Fungi	Pre-emergence damping-off (%)			Post emergence damping off (%)			Wilt (%)		
	Baladi	Sobhia 17	Mean	Baladi	Sobhia 17	Mean	Baladi	Sobhia 17	Mean
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Fusarium oxysporum</i>	70.00	56.66	63.33	3.33	10.00	6.66	20.00	16.66	18.33
<i>F. solani</i>	53.33	43.33	48.33	13.33	3.33	8.33	13.33	23.33	18.33
<i>F. equiseti</i>	46.66	36.66	41.66	10.00	13.33	11.66	16.66	6.66	11.66
<i>F. semitectum</i>	36.66	36.66	36.66	10.00	6.66	8.33	10.00	10.00	10.00
<i>Macrophomina phaseolina</i>	60.00	56.66	58.33	6.66	3.33	5.00	13.33	23.33	18.33
Mean	44.44	38.33		7.22	6.11		12.22	13.33	
LSD 5%	A*			A = ns			A (wilt) = ns		
	B = 9.87			B = ns			B (fungi) = 6.05		
	AB = ns			AB = ns			AB = 8.55		

LSD, least significance difference; ns, not significant.

* Significant at $P \leq 0.01$.

Table 3. Pathogenicity of the isolated fungi on the two roselle cultivars under field conditions

Fungi	Pre-emergence damping off (%)			Post emergence damping off (%)			Wilt (%)		
	Baladi	Sobhia 17	Mean	Baladi	Sobhia 17	Mean	Baladi	Sobhia 17	Mean
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Fusarium oxysporum</i>	53.66	49.33	51.50	16.00	15.66	15.83	25.33	27.00	26.16
<i>F. solani</i>	50.33	43.33	46.83	11.66	17.33	14.50	26.33	26.33	26.33
<i>F. equiseti</i>	42.00	40.33	41.16	14.33	16.00	15.16	29.33	26.33	27.83
<i>F. semitectum</i>	38.33	33.66	36.00	17.33	19.33	18.33	25.66	27.66	26.66
<i>Macrophomina phaseolina</i>	51.66	48.33	50.00	0.00	0.00	0.00	41.66	39.66	40.66
Mean	39.33	35.83		9.88	11.38		24.72	24.50	
LSD 5%	A*			A*			A = ns		
	B = 1.52			B = 1.92			B = 2.58		
	AB = 2.15			AB = 2.71			AB = ns		

LSD, least significance difference; ns, not significant.

* Significant at $P \leq 0.01$.

percentage of post-emergence damping-off was caused by *F. semitectum* and *F. solani* in Baladi cultivar in season 2005 and 2006, respectively. Moreover, *F. equiseti* caused the highest percentage of post-emergence damping-off of Sobhia 17 cultivar in season 2006. It obviously noted that Baladi cultivar was more susceptible to infection with all tested fungi than Sobhia 17 in the two growing seasons.

Under field conditions (Table 3), the highest percentage of pre-emergence damping-off was caused by *F. oxysporum* in Baladi cultivar. The highest percentage of post-emergence damping-off was caused by *F. semitectum* in Sobhia 17 cultivar. The highest percentage of wilt was caused by *M. phaseolina* in both Baladi and Sobhia 17 cultivars. It obviously noted that Baladi cultivar was more susceptible to infection with all tested fungi than Sobhia 17 under field conditions.

In the present study, 5 isolates of pathogenic fungi were collected from different areas in Upper Egypt, Qena. These isolates were identified as *F. oxysporum*, *M. phaseolina*, *F. solani*, *F. equiseti* and *F. semitectum*. Pathogenicity tests of the isolated fungi proved their pathogenicity and virulence for two roselle cultivars; Baladi and Sobhia 17 under greenhouse and field conditions. These isolated fungi were the causal agents of root rot/vascular wilt in the natural roselle disease. Padaganur *et al.* [10] reported for the first time in India that *Hibiscus sabdariffa* L. was a new host to *F. solani* (Mart.). *F. oxysporum* f. spp. *vasinfectum* was found causing severe root rot of roselle crops in Karnataka, Malaysia [4]. The *F. oxysporum* was associated with stem blight of roselle in the tropical forest region of southwest Nigeria causing complete wilting symptoms [11, 12]. Ploetz *et al.* [5] reported the first occurrence of *Fusarium* wilt in roselle caused by *F. oxysporum* in United States to the authors best knowledge this is the first report of root rot and vascular wilt in roselle in Upper Egypt.

The natural symptoms of the affected roselle cultivations were characterized by cankers and/or soft rot in the roots as well as the basal part of their stems adjacent to soil surface. The wilted leaves turned yellow then brown in

color died and defoliated, and the whole plant became brown with dried leaves followed by death of the whole plant. Similar symptoms were described by Agrios [13], Padaganur *et al.* [10], Ooi and Salleh [4], Amusa *et al.* [11, 12] and Ploetz *et al.* [5].

Pathogenicity tests under field conditions confirmed the results obtained by the greenhouse experiment as the severity of the pathogens and susceptibility of the cultivars were the same. The results obtained by field experiment were more pronounced than those of greenhouse condition. The percentages of pre-emergence damping-off, post-emergence damping-off and wilt were varied according to the fungus and the roselle cultivar. As a whole, *F. oxysporum* was the most virulent pathogen followed by *M. phaseolina* and *F. solani*. *F. equiseti* and *F. semitectum* were less pathogenic. Baladi Roselle cultivar assumed more susceptible by any of the five fungi. These differences in susceptibility are probably due to one or more of the following factors; 1) anatomical, physiological and/or biochemical variations, 2) environmental conditions of the experiment such as, temperature, humidity and/or illumination and 3) the ability of the fungus to produce toxins and the ability of secreted cell wall degradation enzymes [13, 14].

Because root rot fungi are soil borne fungi, they can survive in the plant debris thus the infection can be increase every year. The future work has to investigate solving to this problem.

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