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Research Article

Control of Root Rot and Wilt Diseases of Roselle under Field Conditions

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Abstract Roselle (*Hibiscus sabdariffa* L.) is one of the most important medicinal crops in many parts of the world. In this study, the effects of microelements, antioxidants, and bioagents on *Fusarium oxysporum*, *F. solani*, and *Macrophomina phaseolina*, the causal pathogens of root rot and wilt diseases in roselle, were examined under field conditions. Preliminary studies were carried out *in vitro* in order to select the most effective members to be used in field control trials. Our results showed that microelements (copper and manganese), antioxidants (salicylic acid, ascorbic acid, and EDTA), a fungicide (Dithane M45) and biological control agents (*Trichoderma harzianum* and *Bacillus subtilis*) were significantly reduced the linear growth of the causal pathogens. Additionally, application of the previous microelements, antioxidants, a fungicide and biological control agents significantly reduced disease incidence of root rot and wilt diseases under field conditions. Copper, salicylic acid, and *T. harzianum* showed the best results in this respect. In conclusion, microelements, antioxidants, and biocontrol agents could be used as alternative strategies to fungicides for controlling root rot and wilt diseases in roselle.

Keywords Biological control, *Hibiscus*, Root rot disease, Wilt disease

Roselle (*Hibiscus sabdariffa* L.), a famous medicinal plant, is an important crop due to its multitudinous benefit uses such as natural colours materials and in cosmetics. Moreover, it is used as anti-hypertension, cardiac tonic, laxative, diuretic, cough remedy and wound dressing [1].

Root rot and wilt diseases affected seriously roselle production in Egypt [2]. The most frequent pathogenic soil-borne fungi associated with root rot and wilt diseases are *Fusarium oxysporum*, *F. solani*, *F. equiseti*, and *Macrophomina phaseolina* [3-5]. Among them, *Fusarium* wilt caused by *F. oxysporum* f. spp. *vasinfectum* is the most significant disease attacking roselle [6].

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Induction of systemic resistance in the host plant by using micronutrients or antioxidants became a good target for minimizing disease severity with low cost and without environmental hazards [7, 8]. Salicylic acid can improve fungal control when applied jointly or alone. El-Ganaieny *et al.* [9] evaluated the effect of aminobutric acid, potassium salicylate, oxalic acid, salicylic acid, and ascorbic acid on the mycelial growth and spore germination of *F. oxysporum*, *F. solani*, and *F. moniliforme* in onion *in vitro*. They found that the inhibitory effect of antioxidants increased with increased concentrations. Moreover, all the aforementioned antioxidants reduced onion diseases caused by the tested fungi under greenhouse conditions. Potassium salicylate and aminobutyric acid were more effective than other tested antioxidants.

Application of chemical fungicides for controlling root rot and wilt diseases of medicinal plants is improper method. Therefore, using the biocontrol agents, such as *Trichoderma harzianum* and *Bacillus subtilis*, for controlling the soil-borne diseases gave a promising tool which should be tried to avoid the pollution and the imbalance in the rhizosphere microflora [10, 11]. Biological control of root rot and wilt diseases was examined in different hosts using different biological control agents. *Trichoderma*, *Bacillus*, and *Streptomyces* obtained from the rhizosphere soils of sesame plants showed antagonistic effects on *F. oxysporum* and *Rhizoctonia solani* [12]. Moreover, Gabr *et al.* [13] reported that *Bacillus* spp. had great inhibitory effect towards *R.*

solani and *F. solani*, the causal pathogens of lentil damping-off and root rot diseases. The authors added that *Bacillus* spp. had promising effect in reducing the infection with pathogens in pot experiment.

Our recent field observations in different parts in Egypt showed that roselle suffers much from root rot and wilt diseases. Therefore, it was important to perform an extensive study to control these diseases both *in vitro* and *in vivo* conditions.

MATERIALS AND METHODS

Plant and pathogens. Seeds of roselle (cv. Baladi and Sobhia) were obtained from the Ministry of Agriculture Research Institute, Giza, Egypt. Seeds were first disinfected superficially in 0.05% sodium hypochlorite solution for 3 min then washed three times in sterilized water and lastly leaved for air dryness before planting.

Infected roselle plants with root rot and wilt diseases were collected from different localities in Aswan and Qena governorates. The isolated fungi were purified applying single conidial spore method [14]. Identification of the isolated fungi was done in Mycology Center, Assuit University, Assuit, Egypt.

The biocontrol agents were isolated and identified as *Trichoderma harzianum* and *Bacillus subtilis* by Plant Pathology Department, Faculty of Agriculture, South Valley University, Qena, Egypt. Biocontrol agents were kept on nutrient slants for further work.

In vitro experiments.

Effect of microelements on the linear growth of pathogenic fungi: This experiment was performed *in vitro* to study the effect of some microelements on the linear growth of the pathogenic fungi (*Fusarium oxysporum*, *F. solani*, and *Macrophomina phaseolina*). Sterile Czapek's solid medium was amended with serial concentrations of copper, manganese (as sulphate) and molybdenum (as ammonium molybdate) at 50, 100, 200, and 400 ppm. The tested microelements were added to the medium before autoclaving. The tested isolates were inoculated and incubated at 28°C. Three replicates were used for each treatment. The inhibition percentage was calculated according to equation given by Vincent [15]:

$$\text{Inhibition (\%)} = (D1 - D2)/D1 \times 100$$

D1 = Colony diameter in the control.
D2 = Colony diameter in treatment.

Effect of antioxidants and a fungicide on the linear growth of pathogenic fungi: The effect of some antioxidants, i.e., (ascorbic acid, salicylic acid, and ethylenediaminetetraacetic acid (EDTA) and a fungicide (Dithane M45) on the linear growth of *F. oxysporum*, *F. solani*, and *M. phaseolina* using the technique described by Humphery and Fleming [16]. Three replicates were used for each treatment. The percentage of reduction in fungal growth was calculated according to Vincent [15].

The antagonistic effects of biological control agents on pathogenic fungi: The antagonistic effects of the isolated agents (*T. harzianum* and *B. subtilis*) were examined against *F. oxysporum, F. solani*, and *M. phaseolina.* One agar disc (5 mm in diameter) from the advanced zone of mycelial mat of 3-day-old culture of *T. harzianum* was transferred to one side of petri dish containing 15 mL potato dextrose agar medium (PDA). Similar discs from each of the pathogenic fungi were used on the other side of the plates. The inoculated plates were incubated at 28°C and observed daily.

The colonies of *B. subtilis* were streaked at one side of dry nutrient agar plates and incubated for 48 hr at 30°C. Four-day-old culture discs of the pathogenic fungi (5 mm) were transferred into the centre of the bacterial plates then inoculated at 30°C for 4 days until no further growth was observed. Zones of fungal suppression were recorded. In dual culture technique, the inhibition percentage was calculated according to equation given by Vincent [15].

Field experiments.

Effect of microelements in controlling root rot and wilt diseases of roselle: The *in vitro* study showed that copper and manganese were the most effective elements in inhibiting the linear growth of *F. oxysporum*, *F. solani*, and *M. phaseolina*. Therefore, these microelements were used in field trials at concentrations of 100 and 200 ppm. The seeds were treated by soaking the surface-sterilized seeds in the prepared solution of the microelements (500 seeds/100 mL of the solution) for 24 hr. Seed treatments were i.e., copper (100 and 200 ppm) and manganese (100 and 200 ppm).

The rows were arranged in a completely randomized design. About 24 rows, each row was 2 m length and 70 cm wide, were used for this experiment. Two rows were used for each treatment. Two controls were used in this experiment, i.e., 1) two rows inoculated without using any microelements and 2) two rows non-inoculated and without microelements.

The inoculums of the pathogenic fungi were prepared on barely grains. Each conical flask contained about 100 g barely grains and about 100 mL of distilled water was autoclaved then subsequently inoculated with the fungal discs and incubated at 28°C for 14 days.

About 200 g of the cultured grain as fungal inoculum was evenly distributed in the centre of pits (10 cm in depth) for each row then covered with soil and irrigated immediately and when necessary to avoid dryness. One wk later, 10 surface-sterilized treated seeds of roselle cultivars were sown in each pit. The pits were covered with thin layer of soil after sowing and irrigated immediately. Percentages of pre- and post-emergence damping-off were recorded 15 and 30 days after planting, respectively. Severity of root rot/wilt diseases was determined after 90 days using a rating scale of 0 to 5 on the basis of root discoloration and leaf yellowing as follows: 0, neither root discoloration nor leaf yellowing; 1, 1~25% root discoloration or one leaf

yellowing; 2, 26~50% root discoloration or more than one leaf turned yellow; 3, 51~75% root discoloration plus one leaf wilted; 4, up to 76% root discoloration and more than one leaf wilted; and 5, completely dead plants. For each replicate a disease severity index (DSI) was calculated according to Liu *et al.* [17] as following.

$$DSI = \Sigma d/dmax \times n \times 100$$

Where d is the disease rating possible, dmax is the maximum disease rating and n is the total number of plants examined in each replicate.

Effect of antioxidants and a fungicide in controlling root rot and wilt diseases of roselle: Salicylic acid, ascorbic acid, EDTA, and Diathane M45 that proved to be effective in inhibiting fungal growth on PDA medium were used to test their efficiency in controlling root rot and wilt diseases in roselle under field conditions. The seeds were soaked in the prepared solution of the antioxidant (500 seeds/100 mL of the solution) for 24 hr. Seed treatments were i.e., salicylic acid (100 and 200 ppm), ascorbic acid (100 and 200 ppm), EDTA (100 and 200 ppm), and Dithane M45 (5 and 10 ppm).

The inoculums of the tested fungi, soil preparation for planting and soil infestations were prepared as described previously. Data of pre- and post-emergence damping off and wilt /root rot were recorded as described previously.

Biological control of root rot and wilt diseases of roselle by *B. subtilis* and *T. harzianum* under field conditions: *T. harzianum* was grown on PDA and incubated at 28°C for 1 wk. The culture were collected in sterilized distilled water (SDW), counted and adjusted to 1×10^8 propagules/mL [18]. *B. subtilis* was grown on (5%) nutrient sucrose agar medium and incubated at 28°C for 48 hr. Bacterial cells were collected in SDW, counted and adjusted to 1×10^8 colony forming unit/mL (cfu/mL) [18].

The inoculums of *F. oxysporum*, *F. solani*, and *M. phaseolina* were prepared and added to the soil as described previously. Three control treatments were used in this experiment, i.e., 1) normal soil that didn't receive any treatment, 2) soil amended with the antagonistic agents, and 3) soil inoculated with pathogenic fungi without antagonistic agents. Soil infestation and irrigation were performed one week before sowing the seeds in order to give a chance for fungal growth and even distribution. Ten surface-sterilized seeds of roselle cultivars were sown in each pit. The pits were covered with thin layer of soil after sowing and irrigated immediately with 50 mL of the prepared bioagent suspension. Plants were irrigated immediately and when necessary and data of pre- and post-emergence damping-off were recorded after 2 and 4 wk, respectively. Data of wilt was recorded after 2 mon as descried previously.

Statistical analysis. All experiments were repeated twice, analysis of variance was carried out using MSTAT-C program ver. 2.10 (Michigan State University, East Lansing, MI, USA, 1989) [19]. Least significant difference was used to test the significant difference between treatments at $p \leq 0.05$ [20].

RESULTS

In vitro experiments.

Effect of microelements on the linear growth of pathogenic fungi: Copper, manganese and molybdenum had toxic effects on the growth of *F. oxysporum*, *F. solani*, and *M. phaseolina*. The toxic effect increased by increasing

Table 1. Effect of some microelements on the percentage of linear growth (mm) of *Fusarium oxysporum*, *F. solani*, and *Macrophomina phaseolina* grown on potato dextrose agar solid medium

Treatment	Treatment concentration (ppm)	*F. oxysporum*	*F. solani*	*M. phaseolina*	Mean
Control	0	0	0	0	
Cupper	0	0	0	0	0
	50	32.07	35.13	26	31.07
	100	38.71	42.4	32.07	37.73
	200	49.5	50.97	43.57	48.01
	400	51.53	56.33	50	52.62
Molybdenum	0	0	0	0	0
	50	6.3	9.2	0	5.17
	100	15.3	18.57	0	11.29
	200	18.87	20.4	9.31	16.19
	400	20.2	22.5	11.58	18.09
Manganese	0	0	0	0	0
	50	23.83	32.93	3	19.92
	100	31.97	38.5	14.9	28.46
	200	36.33	45.13	22.67	34.71
	400	41.6	51.77	25.33	39.57
X		24.41	28.26	15.89	
LSD 5%	A (fungi), 0.0533; B (minerals), 0.507; AB (interaction), 0.8795				

LSD, least significant difference.

concentrations of elements (Table 1). Manganese reduced the linear growth of the tested fungi at all used concentrations compared to the control. Copper exhibited the highest toxic effect on the tested fungi. Copper at 400 ppm proved to be the most effective inhibitory microelement as it achieved inhibition zones of about 51.53%, 56.33%, and 50.00% for *F. oxysporum*, *F. solani*, and *M. phaseolina,* respectively. On the other hand, molybdenum at concentrations of 50 and 100 ppm had no inhibitory effect on the mycelial growth of *M. phaseolina*.

Effect of antioxidants and a fungicide on the linear growth of pathogenic fungi: Application of ascorbic acid, salicylic acid, EDTA or Dithane M45 to the media inhibited significantly the growth of *F. oxysporum*, *F. solani*, and *M. phaseolina* at all used concentrations compared to the control. Moreover, the linear growth reduction percentage of the pathogenic fungi increased by increasing antioxidant concentrations and reached their maximum reduction at concentration of 100 ppm (Table 2).

Generally, *F. solani* and *F. oxysporum* were highly sensitive to antioxidant compounds and Dithane M45 (56.28% and 38.04% reduction in linear growth, respectively) than *M. phaseolina* (13.05%) which showed high resistance to all treatments. Salicylic acid caused the highest reduction in linear growth of the tested fungi followed by EDTA and ascorbic acid. On the other hand, Dithane M45 was the most effective chemical in this respect only at higher concentrations of 50 and 100 ppm.

***In vitro* study of the antagonistic effects of *B. subtilis* and *T. harzianum* against *F. oxysporum*, *F. solani*, *and M. phaseolina*:** *In vitro*, the antagonistic capabilities of *T. harzianum* and *B. subtilis* obtained from root and rhizosphere of healthy roselle plants were tested against *F. oxysporum*, *F. solani*, and *M. phaseolina,* the causal pathogens of roselle root rot and wilt diseases. Table 3 indicates that *T. harzianum* and *B. subtilis* were able to inhibit the growth of all tested pathogenic fungi.

Field experiments.

Effect of copper and manganese in controlling of root rot and wilt diseases of roselle: The *in vitro* study indicated that copper and manganese were the most effective elements in inhibiting the linear growth of *F. oxysporum*, *F. solani*, and *M. phaseolina*. Therefore, these two microelements were used at concentrations of 100 and 200 ppm to control roselle root rot and wilt diseases under field conditions. Both copper and manganese significantly decreased the percentages of pre- and post-emergence damping-off as well as the percentage of wilted plants compared with the control. The effect of microelements on the infection was variable and differed according to the used mineral and its concentrations as well as the tested fungi. The protection increased by increasing the microelement concentration. Copper was better than manganese in

Table 2. Effect of some antioxidants and a fungicide (ppm) on the reduction percentage of linear growth in millimeter of *Fusarium oxysporum*, *F. solani*, and *Macrophomina phaseolina* grown on potato dextrose agar solid medium

Treatment		Reduction of linear growth (%)			
		F. oxysporum	*F. solani*	*M. phaseolina*	Mean
Control		0	0	0	0
Ascorbic acid (ppm)	5	8.53	30.27	10.3	16.37
	10	12.33	51.53	13.27	25.71
	25	26.8	55.45	21.63	34.63
	50	46.87	66.97	22.53	45.46
	100	65.4	76.87	26.63	56.3
Salicylic acid (ppm)	5	11.4	46.67	8.43	22.16
	10	21.55	55.43	11.71	29.56
	25	41.47	59.53	16.24	39.08
	50	58.33	76.37	22.27	52.32
	100	76.3	84.3	24.98	61.86
EDTA (ppm)	5	16.37	46.53	4.29	22.4
	10	23.6	49.4	5.24	26.08
	25	45.47	61.57	6.37	37.8
	50	63.53	63.67	8.58	45.36
	100	76.97	84.4	8.93	56.76
Dithane M45 (ppm)	5	9.73	16.13	5.3	10.39
	10	23.2	26.4	10.1	19.9
	25	32.8	54.7	11.9	33.13
	50	53.73	85.6	14.9	51.41
	100	84.37	90.23	20.37	64.99
X		38.04	56.28	13.05	
LSD 5%		A (fungi), 0.9274; B (treatment), 0.6826; AB (interaction), 1.1828			

LSD, least significant difference.

Table 3. *In vitro* inhibition ratio of *Fusarium oxysporum, F. solani*, and *Macrophomina phaseolina*

Bioagents	Replicates	Inhibition ratio (%)		
		F. oxysporum	*M. phaseolina*	*F. solani*
Trichoderma harzianum	R1	78	75	76
	R2	80	63	73
	R3	85	76	74
	Control	0	0	0
Bacillus subitilis	R1	88	70	86
	R2	78	85	88
	R3	66	82	73
	Control	0	0	0

reducing the percentage of infected plants in Baladi and Sobhia 17 roselle cultivars and in case of all tested fungi (Tables 4 and 5).

Effect of antioxidants and a fungicide in controlling root rot and wilt diseases of roselle: Salicylic acid, ascorbic acid, EDTA, and Dithane M45 significantly decreased the percentages of pre- and post-emergence damping-off as well as the percentage of wilted plants as compared to the control. The protection increased by increasing the concentration of antioxidant or fungicide. Salicylic acid was the most effective antioxidant in reducing the percentage of infected plants in the two roselle cultivars (Tables 6 and 7). However, EDTA at concentration of 100 ppm was more effective than salicylic acid in reducing infection caused by *F. oxysporum* and *M. phaseolina* to Baladi and Sobhia roselle cultivars, respectively. The least effective antioxidant was ascorbic acid which was better than Dithane M45 in reduction of the percentage of infection at both tested concentrations.

The effects of microelements (copper and manganese) were lower than the effects of antioxidants (salicylic acid, ascorbic acid, and EDTA) in reduction of root rot and wilt diseases of roselle artificially incited by *F. oxysporum, F. solani*, and *M. phaseolina* under field conditions and higher than the effect of Dithane M45 under the same conditions (Tables 4~7).

Table 4. Effect of microelements on the percentage of roselle infected plants of Baladi cultivar artificially infected with *Fusarium oxysporum*, *F. solani*, and *Macrophomina phaseolina* under field conditions

Treatment		Pre-emergence damping off (%)				Post-emergence damping off (%)				Wilt (%)			
		F. oxysporum	*F. solani*	*M. phaseolina*	Mean	*F. oxysporum*	*F. solani*	*M. phaseolina*	Mean	*F. oxysporum*	*F. solani*	*M. phaseolina*	Mean
Control 1		0	0	0	0	0	0	0	0	0	0	0	0
Control 2		44.33	41.66	31.66	39.22	21.66	19.33	3.33	14.77	30.33	31.66	60.66	40.88
Cupper	100	25.66	17.00	21.00	21.22	18.00	13.66	3.33	11.66	28.00	36.66	49.33	38.00
(ppm)	200	22.33	18.66	22.33	21.11	14.33	10.66	0	08.33	27.33	31.66	46.00	35.00
Manganese	100	32.66	20.66	26.66	26.66	16.00	12.66	0	9.55	27.00	39.33	56.00	40.77
(ppm)	200	24.00	16.66	20.00	20.22	18.00	10.00	0	9.33	27.33	44.00	53.00	41.44
Mean		24.83	19.11	20.27		14.66	11.05	1.11		23.33	30.55	44.16	
LSD 5%			A (treatment) 2.32 B (fungi) 1.64 AB 4.02				A (treatment) 2.37 B (fungi) 1.68 AB 4.11				A (treatment) 3.46 B (fungi) 2.45 AB 6.00		

Control 1, two rows inoculated without using any microelements; Control 2, two rows non-inoculated and without microelements; LSD, least significant difference.

Table 5. Effect of microelements on the percentage of roselle infected plants of Sobhia 17 cultivar artificially infected with *Fusarium oxysporum*, *F. solani*, and *Macrophomina phaseolina* under field conditions

Treatment		Pre-emergence damping off (%)				Post-emergence damping off (%)				Wilt (%)			
		F. oxysporum	*F. solani*	*M. phaseolina*	Mean	*F. oxysporum*	*F. solani*	*M. phaseolina*	Mean	*F. oxysporum*	*F. solani*	*M. phaseolina*	Mean
Control 1		0	0	0	0	0	0	0	0	0	0	0	0
Control 2		40.33	34.33	29.66	34.77	20.00	22.33	0	14.11	36.33	33.66	62.00	44.00
Cupper	100	27.33	21.66	18.33	22.44	19.33	13.33	6.66	13.11	27.66	28.66	48.00	34.77
(ppm)	200	25.00	14.33	22.66	20.66	16.00	13.66	0	9.88	22.00	32.66	46.00	33.55
Manganese	100	31.33	28.33	22.33	27.33	13.00	15.00	3.33	10.44	28.33	27.66	55.00	37.00
(ppm)	200	29.66	22.00	23.00	24.88	11.33	18.66	0	10	27.33	24.00	51.66	34.33
Mean		25.61	20.11	19.33		13.27	13.83	1.66		23.61	24.44	43.77	
LSD 5%			A (treatment) 2.11 B (fungi) 1.49 AB 3.65				A (treatment) 2.48 B (fungi) 1.75 AB 4.29				A (treatment) 2.32 B (fungi) 1.64 AB 4.02		

LSD, least significant difference.

Table 6. Effect of antioxidants and a fungicide on the percentage of roselle infected plants of Baladi cultivar artificially infected with *Fusarium oxysporum*, *F. solani*, and *Macrophomina phaseolina* under field conditions

Treatment		Pre-emergence damping off (%)				Post-emergence damping off (%)				Wilt (%)			
		F. oxysporum	*F. solani*	*M. phaseolina*	Mean	*F. oxysporum*	*F. solani*	*M. phaseolina*	Mean	*F. oxysporum*	*F. solani*	*M. phaseolina*	Mean
C1		0	0	0	0	0	0	0	0	0	0	0	0
C2		44.33	41.66	31.66	39.22	21.66	19.33	3.33	14.77	30.33	31.66	60.66	40.88
Salicylic acid	100	21.33	25.33	14.33	20.33	13.33	10.00	9.33	10.88	27.66	20.66	40.00	29.44
(ppm)	200	17.33	22.66	15.33	18.44	10.00	10.33	9.33	9.88	20.33	19.33	32.33	24.00
Ascorbic acid	100	28.66	28.66	19.33	25.55	10.66	10.33	6.66	9.22	26.33	23.33	42.33	30.66
(ppm)	200	25.66	26.33	18.33	23.44	10.33	8.66	8.00	9.00	22.33	21.33	38.00	27.22
EDTA	100	25.33	26.00	20.33	23.88	9.33	11.33	0	6.88	24.33	21.66	42.66	29.55
(ppm)	200	25.33	26.00	19.33	23.55	9.33	11.00	2.66	7.66	21.00	19.33	38.33	26.22
Dithane M45	5	33.00	30.33	27.66	30.33	23.00	20.00	3.33	15.44	30.33	32.00	56.00	39.44
(ppm)	10	30.66	28.66	21.66	27.00	20.33	10.00	8.33	12.88	26.00	29.66	48.33	34.66
Mean		25.16	25.56	18.80		12.80	11.10	5.10		22.86	21.90	39.86	
			A (treatment) 2.20				A (treatment) 2.52				A (treatment) 2.61		
LSD 5%			B (fungi) 1.21				B (fungi) 1.38				B (fungi) 1.43		
			AB 3.82				AB 4.37				AB 4.53		

LSD, least significant difference.

Table 7. Effect of antioxidants and a fungicide on the percentage of roselle infected plants of Sobhia cultivar artificially infected with *Fusarium oxysporum*, *F. solani*, and *Macrophomina phaseolina* under field conditions

Treatment		Pre-emergence damping off (%)				Post-emergence damping off (%)				Wilt (%)			
		F. oxysporum	*F. solani*	*M. phaseolina*	Mean	*F. oxysporum*	*F. solani*	*M. phaseolina*	Mean	*F. oxysporum*	*F. solani*	*M. phaseolina*	Mean
C1		0	0	0	0	0	0	0	0	0	0	0	0
C2		40.33	34.33	29.66	34.77	20.00	22.00	0.00	14.11	36.33	33.66	62.00	44.00
Salicylic acid	100	22.33	21.33	13.00	18.88	11.33	10.00	5.00	8.77	22.00	21.33	42.00	28.44
(ppm)	200	22.00	11.66	10.66	14.77	10.66	10.00	10.00	10.22	11.66	18.33	35.33	21.77
Ascorbic acid	100	27.66	26.00	14.00	22.55	10.66	10.66	0	7.11	24.66	22.66	54.33	33.88
(ppm)	200	25.00	17.00	17.66	19.88	8.00	13.66	10.00	10.55	21.33	20.33	34.66	25.44
EDTA	100	26.00	24.33	17.66	22.66	8.66	10.66	0	6.44	24.66	21.00	41.00	28.88
(ppm)	200	24.33	22.00	17.66	21.33	8.66	10.00	0	6.22	22.33	19.33	38.66	26.77
Dithane M45	5	31.66	30.33	21.66	27.88	20.66	20.00	5.00	15.22	31.33	33.33	56.66	40.44
(ppm)	10	28.66	30.00	20.00	26.22	21.66	20.00	3.33	15.00	30.33	30.00	50.33	36.88
Mean		24.80	21.70	16.20		12.03	12.73	3.33		22.46	22.00	41.50	
			A (treatment) 1.61				A (treatment) 2.36				A (treatment) 1.80		
LSD 5%			B (fungi) 0.88				B (fungi) 1.29				B (fungi) 0.985		
			AB 2.78				AB 4.09				AB 3.11		

LSD, least significant difference.

Biological control of root rot and wilt diseases of roselle by *T. harzianum* and *B. subtilis*: All the tested pathogenic fungi were able to infect both roselle cultivars causing pre- and post-emergence damping-off and wilt symptoms compared with control (Tables 8 and 9). The pathogenicity varied according to the tested pathogenic fungi and the roselle cultivar. *F. oxysporum* caused the highest percentage of roselle infected plants followed *F. solani*. Application of *T. harzianum* and *B. subtilis* individually significantly reduced the incidence of pre- and post-emergence damping-off and wilt diseases. *T. harzianum* was more effective than *B. subtilis* in reducing the pathogenicity since *T. harzianum* produced higher percentage of survival plants.

Application of *T. harzianum* reduced the percentages of infection in Baladi roselle plants with *F. oxysporum*, *F. solani*, and *M. phaseolina* from 32.11~2.22%, 30.89~1.33%, and 32.89~2.67%, respectively. However, application of *B. subtilis* reduced percentage of infection with the same fungi to 7.56%, 5.33%, and 6.67%, respectively (Table 8).

Application of *T. harzianum* reduced percentage of infection in Sobhia roselle plants with *F. oxysporum*, *F. solani*, and *M. phaseolina* from 32.22~0.89%, 30.11~0.89%, and 30.56~2.22%, respectively. However, application of *B. subtilis* reduced the percentages of infection with the same fungi to 6.22%, 3.56%, and 3.11%, respectively (Table 9).

Table 8. Efficiency of certain biocontrol agents on incidence of roselle root rot diseases on Baladi roselle cultivar under field conditions

Treatment	Pre-emergence damping off (%)	Post-emergence damping off (%)	Wilt (%)	Mean
Control	0	0	0	0
Fusarium oxysporum	44.33	21.67	30.33	32.11
Fusarium solani	41.67	19.33	31.67	30.89
Macrophomina phaseolina	31.67	3.33	60.67	31.89
Trichoderma harzianum	0	0	0	0
T. harzianum + F. oxysporum	5.33	1.33	0	2.22
T. harzianum + F. solani	2.67	1.33	0	1.33
T. harzianum + M. phaseolina	2.67	5.33	0	2.67
Bacillus subtilis	0	0	0	0
B. subtilis + F. oxysporum	13.33	9.33	0.00	7.56
B. subtilis + F. solani	10.67	5.33	0.00	5.33
B. subtilis + M. phaseolina	12.00	2.67	5.33	6.67
Mean	14.94	6.6	11.64	
LSD 5%	A (disease), 3.669; B (treatments), 4.0188			

LSD, least significant difference.

Table 9. Efficiency of certain biocontrol agents on incidence of roselle root rot diseases on Sobhia roselle cultivar under field conditions

Treatment	Pre-emergence damping off (%)	Post-emergence damping off (%)	Wilt (%)	Mean
Control	0	0	0	0
Fusarium oxysporum	40.33	20.00	36.33	32.22
Fusarium solani	34.33	22.33	33.67	30.11
Macrophomina phaseolina	29.67	0	62.00	30.56
Trichoderma harzianum	0	0	0	0
T. harzianum + F. oxysporum	2.67	0	0	0.89
T. harzianum + F. solani	1.33	1.33	0	0.89
T. harzianum + M. phaseolina	4.00	0	2.67	2.22
Bacillus subtilis	0	0	0	0
B. subtilis + F. oxysporum	10.67	5.33	2.67	6.22
B. subtilis + F. solani	9.33	1.33	0	3.56
B. subtilis + M. phaseolina	5.33	0.00	4.00	3.11
Mean	12.52	4.58	12.85	
LSD 5%	A (disease), 3.6462; B (treatment), 4.17			

LSD, least significant difference.

DISCUSSION

Fusarium oxysporum, *F. solani* and *M. phaseolina*, the causal pathogens of wilt and root rot diseases in roselle (*Hibiscus sabdariffa* L.), endanger roselle production wherever this crop is cultivated extensively [4-6, 21]. Although synthetic fungicides are often the first line of defence against fungal diseases, the global current trend has converted to safer and environmentally friendly alternative methods to control these organisms. Microelements, antioxidants and biological control could be used as alternative methods to fungicides in controlling fungal diseases [22, 23].

Although micronutrients are essential for microbes, elevation of concentrations above certain threshold rendered them toxic to microrganisms [24, 25]. In the present study, copper and manganese reduced the linear growth of *F. oxysporum*, *F. solani*, and *M. phaseolina* at concentrations of 50, 100, 200, and 400 ppm. Copper at concentration of 400 ppm had the greatest inhibitory effect on the tested fungi, followed by manganese and molybdenum. *M. phaseolina* behaved differently with all of the tested microelements since it has slightly inhibited with microelements than *Fusarium* spp. Zn, Mn, and Cu as sulphates reduced the linear growth and sporulation of *F. oxysprum* f. spp. *cepea* [26, 27]. Under field conditions, copper showed the best control of root rot caused by *M. phaseolina* followed by manganese and zinc [28].

Application of these compounds to the media inhibited significantly the growth of *F. oxysporum*, *F. solani*, and *M. phaseolina* at 5, 10, 25, 50, and 100 ppm compared to the control. Moreover, the percentage of reduction of linear growth of all tested fungi increased by increasing the concentration of salicylic acid, ascorbic acid, EDTA, and Dithane M45 and reached their maximum reduction at concentration of 100 ppm. *F. solani* was found to be the most sensitive fungus to antioxidants than *F. oxysporum*

and *F. moniliforme* [9]. Salicylic acid caused the highest reduction in the linear growth of the pathogenic fungi followed by EDTA and ascorbic acid. Under field conditions, salicylic acid, ascorbic acid, and EDTA significantly decreased the percentages of root rot and wilt diseases incited by each of the pathogenic fungi as compared with the control. Results are in accordance with those performed *in vitro*. Salicylic acid and ascorbic acid were used to control damping-off; wilt and root rot diseases of several crops such as cowpea [29], sesame [8, 30], sesame and sunflower [31], onion [9], cotton [32], and sugar beet [33]. Most of previous studies were done *in vitro*. The antagonistic capabilities of *Trichoderma harzianum* and *Bacillus subtilis* against *F. oxysporum*, *F. solani*, and *M. phaseolina* revealed that they were able to inhibit the growth of all the tested pathogenic fungi *in vitro*. Such antagonistic effect may be due to direct influence of antagonistic fungi against the pathogens through coiling the hyphae of *T. harzianum* around the hyphae of the pathogens to prevent their continued growth [34]. Additionally, production of antagonistic substances which can play an important role in lysis of the cell wall components of the pathogenic fungi [32, 35, 36]. This antagonistic substance may also prevent the spore germination of the pathogens and the mycelial growth [37, 38]. Biological control has been used to control soil borne fungal pathogens [39-42]. In conclusion, this study suggests that microelements, antioxidants and biological control could be effectively used in Integrated Pest Management (IPM) strategies in roselle plants.

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