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Efficiency of some organic acids as safe control mean against root and stem rot disease of *Coleus forskohlii*

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Abstract

Coleus (*Coleus forskohlii*) is an important medicinal crop containing forskolin in their roots. Root rot is considered the most important disease in coleus, causing serious losses affecting growth and yield. Infected coleus plants showing root and stem rots were collected from Giza gardens and nurseries. The pathogenicity tests of the isolated fungi (*Fusarium oxysporum, F. roseum, F. semitectum, F. solani, Macrophomina phaseolina, Pythium splendens* and *Rhizoctonia solani*) revealed the ability of these fungi to cause infection. Application of three organic acids (Ascorbic, oxalic and salicylic acid) each at 250 and 500 ppm, against *R. solani, F. semitectum, F. oxysporum* and *F. solani* was performed in the greenhouse. All the tested organic acids reduced significantly disease incidence when used as dipping unrooted cuttings and/or spray plants 30 days after planting or soil drenching under greenhouse conditions particularly at 500 ppm compared with untreated plants. In general, oxalic acid at 500 ppm was more efficient in reducing infection with root and stem rot diseases. As for plant growth parameters, soil drenching with oxalic acid was the best treatment in increasing plant height and branch number compared with the other treatments. However, ascorbic acid was the least effective treatment compared with the other treatments. On the other hand, dual combination of three organic acids was superior on the other treatments and also recorded superiority in peroxidase and polyphenol oxidase enzyme activities.

Keywords: *Coleus forskohlii*, root and stem rot, ascorbic acid, oxalic acid, salicylic acid.

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Introduction

Coleus forskohlii Briq. belonging to family lamiaceae is one of the commercial ornamental and medicinal plants grown extensively in Egypt; it is used as a bedding plant for public gardens. *C. forskohlii* is cultivated mainly for its medicinal uses in some countries. Forskolin, a labdane diterpene compound, originates from coleus roots, has vasodilatory properties and considered a potent hypotensive agent (Shah & Kalakoti, 1991; Seamon, 1984). Its ethnomedicinal uses for a remedy for cough, eczema, epidermal infections, tumors and furuncles have been recorded (Rupp et al., 1986). *C. forskohlii* is susceptible to many diseases, of which root rot disease is a major constraint throughout the world (Srivastava et al., 2001; Mihail, 1992).The disease is caused by soil-borne pathogens; it infects plant through root causing discoloration and foliar chlorosis and the disease cause huge loss (Kamalakannan et al., 2006). Regarding management of medicinal plant diseases, organic acids are being considered because fungicides can result in the accumulation of harmful residues which may lead to health risks and serious ecological effects. The use of organic acids (antioxidants) to control the fungal diseases was reported by several researchers (Zaky & Mohamed, 2009; Khan et al., 2001; Prusky et al., 1995). The main objective of this study was to evaluate some organic acids (Ascorbic, oxalic and salicylic acid) for controlling root and stem rot of *C. forskohlii.*

Materials and methods

Isolation and identification of fungi: Samples of *C. forskohlii* plants showing root and stem rot disease symptoms were collected from different locations of Giza governorate, Egypt during 2017. Infected tissues were washed in tap water, and cut into sections with sterilized scalpel. The sections were surface sterilized in 1% sodium hypochlorite for 2 minutes and rinsed with sterilized distilled water. The sterilized tissues were then transferred into plates containing potato dextrose agar (PDA) medium. The plated petri dishes were incubated at 27^{o}C for seven days and checked daily for fungal development. The growing fungi were purified and identified according to the description given by Gilman (1957), Barnett & Hunter (1972) and Nelson et al. (1983). The identification was also kindly confirmed by department of mycology and plant disease survey research, Plant Pathology Research Institute., ARC, Giza, Egypt. Isolation frequency of the developing colonies of each fungus was calculated as percentage of the total developing colonies.

Inoculum preparation: The isolated fungi were cultured on PDA medium and incubated at 27°C. Inoculum of each fungus (*Fusarium oxysporum, F. roseum, F. semitectum, F. solani, Macrophomina phaseolina, Pythium splendens* and *Rhizoctonia solani*) was grown on maize meal-sand medium in 500 ml glass bottles. Bottles were incubated at 27°C for 20 days.

Pathogenicity tests: The experiment of pathogenicity tests was carried out under greenhouse conditions. Formalin sterilized pots (20-cm diameter) packed with sterilized clay sand soil (1:1 w/w) were infested with each fungal inoculum at the rates of 0.5 and 1% (w/w). Each pot was planted with four unrooted stem tip cuttings. Three replicates were used for each treatment. Results were recorded as percentage of dead plants 30 and 60 days after sowing as follows:

$$\text{Dead plants (\%)} = \frac{\text{No. of dead plants}}{\text{Total No. of plants}} \times 100$$

Effect of three organic acids on the percentage of disease incidence under greenhouse conditions: Ascorbic acid (AA), oxalic acid (OA) and salicylic acid (SA) and dual combination of them, were tested for controlling *C. forskohlii* root and stem rot disease caused by *Fusarium oxysporum, F. semitectum, F. solani* and *Rhizoctonia solani* at two concentrations (250 and 500 ppm) in pot experiments compared with treatment of carbendazim 50% WP (2g/l) [Common name: Carbendazim, Chemical composition: 2-(Methoxycarbomylsmino-benzimidazole) and Manufacture: Agriphar S.A., Ballgium.] and untreated plants. Formalin sterilized pots (20-cm diam.) packed with sterilized clay sand soil (1:1 w/w) were infested separately with each fungal inoculum at 1% (w/w). The infested soils were watered for 7 days to improve distribution and development of the fungal inoculum. The organic acids were applied by different methods as follows: (1) Dipping unrooted cuttings: Healthy unrooted cuttings were dipped in the tested solutions for 15 min. before planting (96 unrooted cuttings per 1000 ml tested solution), then four cuttings were planted in each pot (20 cm in diameters). Three pots for each test were used as replicates. The Percentages of dead plants were recorded 60 days after planting. The plant height and number of branches per plant were also recorded at the end of the experiment. (2) Dipping unrooted cuttings before planting and then spraying with different treatments 30 days after planting: Healthy unrooted cuttings were dipped in the tested solutions for 15 minutes before planting (96 unrooted cuttings per 1000 ml tested solution), then four cuttings were planted in each pot (20 cm in diameters), and they were spread 30 days after planting date with the tested solutions. Three pots were used as replicates for each test. Percent of dead plants incidence and growth parameters were recorded as mentioned before. (3) Soil drenching: Cuttings unrooted and non-treated were planted in pots (four per pot). Pots were irrigated (50 ml/pot) with (AA), (OA) and (SA) solutions at two concentrations (250, 500 ppm) when they were planted. Three replicates were used for each test. Percent of dead plants incidence and growth parameters were recorded as mentioned before.

Effect of three organic acids and Carbendazim 50% WP as dipping unrooted cuttings of coleus on some defense-related enzymes: Coleus leaves samples (about 10 gm/treatment) were obtained from each greenhouse treatment and control to determine peroxidase and polyphenol oxidase enzymes in plants72 h after each treatment. Using a mortar and pestle, the samples were grinded in 10 ml of 0.1 M sodium phosphate buffer (pH 6.8). The samples were then strained through four layers of cheese-cloth and the filtrates were centrifuged at 3000 rpm for 20 min. at 6^{o}C (Aina et al., 2012). The supernatant was then taken and used for enzyme assay.

Peroxidase: The activity of peroxidase enzyme was determined by measuring the oxidation of pyrogallol to purpurogallin in the presence of H_2O_2 at 425 nm. The reaction mixture contained 0.3 ml 0.05 M pyrogallol, 0.5 ml of 0.1 M sodium phosphate buffer solution (pH 7.0), 0.1 ml of 1.0 % H_2O_2 and 0.3 ml sample extract, then completed with distilled water up to 3 ml. The activity was expressed as absorbance change per minute (Abs/min.) (Aina et al., 2012).

Polyphenol oxidase: Polyphenol oxidase enzyme activity was determined colorimetrically by the method of (Quiles et al., 2005). The reaction mixture contained 1.0 ml of 10^3 N catechol, 1.0 ml of 0.2 M sodium phosphate buffer (pH 7.0) and 1.0 ml sample extract, then completed to a final volume of 6.0 ml with distilled water. The polyphenol oxidase activity was expressed as the change in absorbance of the reaction mixture per min. at 495 nm.

Statistical analysis: The plan of this trial as designed factorial experiment in a total randomized design with three replicates (Snedecor & Cochran, 1980). This statistical analysis was performed by using the computer program MSTAT-C statistical package version (4) using Least Significant Difference (L.S.D) test at 0.05.

Results

Frequency of the isolated fungi: Seven fungi (*Fusarium oxysporum* Schlecht., *F. roseum* Lk. Emend. Snyd. & Hans., *F. semitectum* Berk. & Rav., *F. solani* (Mart) Sacc., *Macrophomina phaseolina* (Tassi) Goid., *Pythium splendens* Braun and *Rhizoctonia solani* Kuehn) were isolated from *C. forskohlii* diseased plants collected from Giza governorate, Egypt (Table 1 and Figure 1). Data in Table (1) indicated that *R. solani* was the most frequently isolated fungus (27.01%) followed by *F. oxysporum* (21.33%), *F. semitectum* (14.69%) and *Pythium splendens* (14.22%), while *F. roseum* (4.74%), *M. phaseolina*, (7.58%) and *F. solani* (10.43%) showed the lowest isolation frequency.

Table 1: Frequency of fungi isolated from *C. forskohlii* diseased plants, collected from gardens and nurseries of Giza governorate, Egypt.

Isolated fungi	Number of isolates	Frequency (%)
Fusarium oxysporum	45	21.33
F. roseum	10	4.74
F. semitectum	31	14.69
F. solani	22	10.43
Macrophomina phaseolina	16	7.58
Pythium sp.	30	14.22
Rhizoctonia solani	57	27.01
Total	211	100

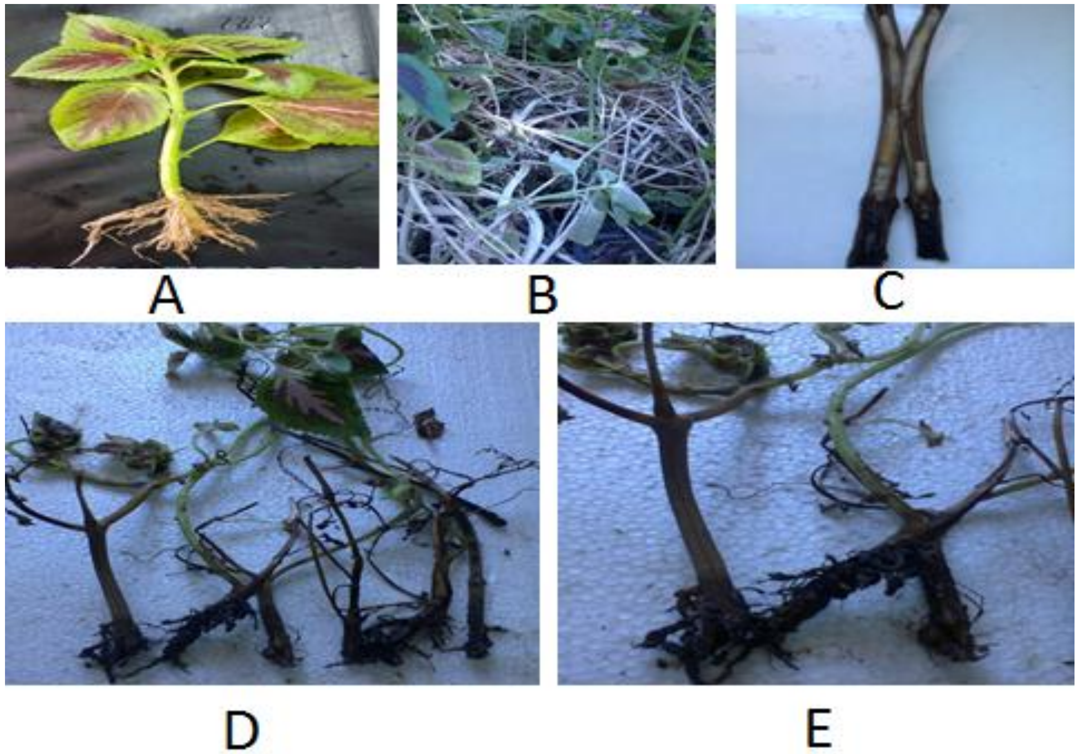

Figure 1: Coleus plants naturally infected by root and stem rot disease: A (Healthy control) and B, C, D, E (Diseased plants).

Pathogenicity tests: All fungi (Table 2) were able to infect the roots and stems of *C. forskohlii* plants, grown in artificially infested soil. The infected plant organs usually rotted, therefore stunting and/or complete death appeared on these plants. However, percentages of infection were positively correlated with the inoculum rate (0.5% and 1%) and by the time elapse from 30 to 60 days. *R. solani* was the most virulent fungi resulted in (75.0% and 91.7%), followed by *F. oxysporum* (66.7% and 83.3%), *F. solani* (66.7% and 83.3%) and *F. semitectum* (50.0% and 75.0%), at the inoculum rates (0.5% and 1%), respectively. In contrast, *Pythium splendens*, *F. roseum* and *M. phaseolina* were the least pathogenic fungi, causing (41.7%) at the inoculum rate (0.5%), while, *M. phaseolina* and *F. roseum* were the least pathogenic fungi, causing (50.0%) at the inoculum rate (1%). Symptoms of artificially infection by the pathogenic fungi on *C. forskohlii* plants, however, appear in Figure (2).

Effect of three organic acids as dipping unrooted cuttings on dead plants: The efficacy of organic acids AA, OA and SA at two concentrations (250, 500 ppm) was determined as dipping unrooted cuttings treatment, on percentages of dead plants after 30 and 60 days of planting in soil artificially infested with *R. solani*, *F. semitectum*, *F. oxysporum* and *F. solani*. Dipping unrooted cuttings with organic acids solutions and carbendazim 50%WP resulted in an increase of coleus plants resistance against infection with the tested fungi

(Table 3). Data in Table (3) indicate that SA was superior treatment than OA and AA in decreasing dead plants. SA gave the highest decrease percentage (100%) for *F. oxysporum*, *F. semitectum* and *F. solani* at concentration (500 ppm). However, OA and AA gave the highest decrease percentages (100%) for (*F. semitectum* and *F. solani*) and (*F. oxysporum*, *F. semitectum*), respectively. Carbendazim 50%WP gave the highest decrease percentages (100%) for (*F. semitectum* and *R. solani)* after 30 and 60 days from planting. Dual combinations of the three organic acids gave the highest decreasing disease incidence with all tested fungi. The highest reduction was obtained by using the combined treatments between AA+OA, AA+SA and OA+SA at a concentration of 500 ppm, which reduced the disease incidence by 100% after 30 and 60 days from planting. However, the decrease in disease incidence with all tested fungi was not significant with any treatment after 60 days from planting.

Table 2: Percentages of dead coleus plants, 30 and 60 days after planting with unrooted stem tip cuttings in infested soil under greenhouse conditions.

Fungi	Inoculum rate (0.5%)			Inoculum rate (1%)		
	Dead plants (%) after		Survivals (%)	Dead plants (%) after		Survivals (%)
	30 days	60 days		30 days	60 days	
Fusarium oxysporum	41.7	66.7	33.3	58.3	83.3	16.7
F. roseum	25.0	41.7	58.3	33.3	50.0	50.0
F. semitectum	33.3	50.0	50.0	50.0	75.0	25.0
F. solani	41.7	66.7	33.3	50.0	83.3	16.7
Macrophomina phaseolina	25.0	41.7	58.3	33.3	50.0	50.0
Pythium splendens	25.0	41.7	58.3	41.7	66.7	33.3
Rhizoctonia solani	25.0	75.0	25.0	25.0	91.7	8.3
Control (without fungus)	0.00	0.00	100.00	0.00	0.00	100.00
L.S.D. at 5%	0.4	0.5	-	2.0	0.7	-

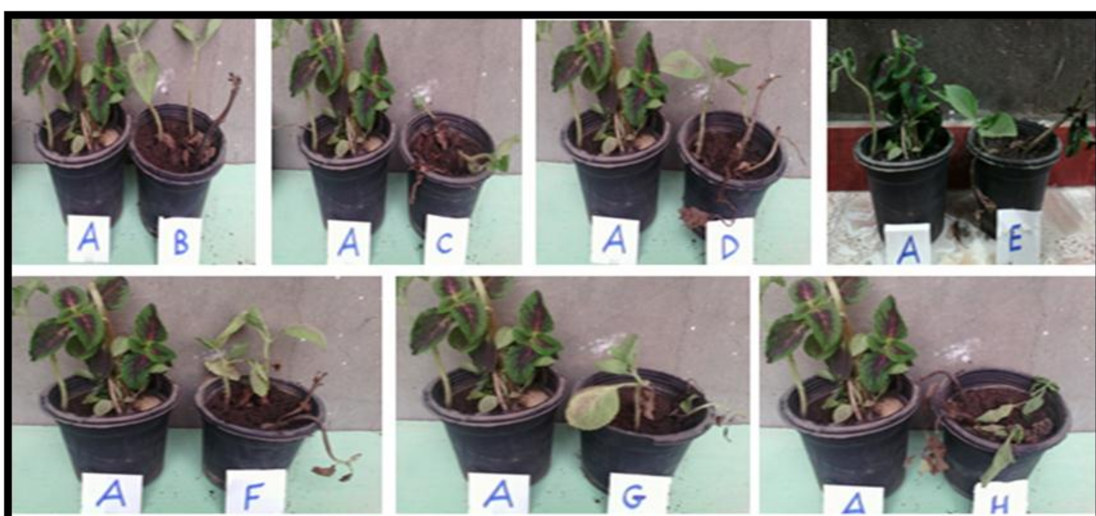

Figure 2: Coleus plants grown in soil infested with: *F. oxysporum* (B), *F. solani*, (C), *F. semitectum* (D), *P. splendens* (E) *F. roseum* (F) *M. phaseolina* (G) *R. solani* (H) showing root and stem rot disease symptoms on plant growth compared with the healthy plants (A).

Table 3: Effect of three organic acids as dipping unrooted cuttings treatment on incidence (%) of root and stem rot disease of coleus plants under greenhouse conditions..

Treatments	Conc.	*F. oxysporum*		*F. semitectum*		*F. solani*		*R. solani*	
		Disease incidence (%)		Disease incidence (%)		Disease incidence (%)		Disease incidence (%)	
		30 days	60 days	30 days	60 Days	30 days	60 days	30 days	60 days
AA	250 ppm	8.3	8.3	0.0	8.3	8.3	25.0	16.7	25.0
	500 ppm	0.0	0.0	0.0	0.0	0.0	16.7	8.3	16.7
OA	250 ppm	33.3	33.3	16.7	25.0	0.0	16.7	16.7	25.0
	500 ppm	8.3	8.3	0.0	0.0	0.0	0.0	8.3	16.7
SA	250 ppm	16.7	33.3	16.7	25.0	0.0	8.3	41.7	50.0
	500 ppm	0.0	0.0	0.0	0.0	0.0	0.0	25.0	33.3
AA + OA	250 ppm	0.0	8.3	0.0	0.0	0.0	8.3	8.3	8.3
	500 ppm	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
AA + SA	250 ppm	8.3	8.3	8.3	8.3	0.0	0.0	16.7	16.7
	500 ppm	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
OA + SA	250 ppm	8.3	8.3	8.3	8.3	0.0	0.0	16.7	16.7
	500 ppm	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carbendazim 50% WP	2 g/l	0.0	8.3	0.0	0.0	8.3	8.3	0.0	0.0
Control	-	66.7	83.3	58.3	75.0	50.0	75.0	50.0	50.0
L.S.D. at 5%	-	0.5	0.3	1.3	0.2	0.8	0.2	1.0	0.4

On the other hand, dipping unrooted cuttings in organic acids increased plant height and no. of branch/plant compared to untreated plants under infection with tested fungi. Plant height and branch number increase by increasing of organic acid concentration. Carbendazim 50%WP and dual combination between OA+SA at 500 ppm recorded superiority in increasing plant height and No. of branches per plant, followed by OA at 500 ppm compared to any of the other samples which underwent treatment with all tested fungi (Table 4).

Table 4: Effect of three organic acids as dipping unrooted cuttings treatment on some plant growth parameters of coleus plants in soil infested with each of four pathogenic fungi under greenhouse conditions.

Treatments	Conc.	*F. oxysporum*		*F. semitectum*		*F. solani*		*R. solani*	
		Plant Height (cm)	No. of branches/ plant	Plant Height (cm)	No. of branches/ plant	Plant Height (cm)	No. of branches/ plant	Plant Height (cm)	No. of branches/ plant
AA	250 ppm	10.0	4.1	11.8	4.2	10.4	5.0	11.3	4.1
	500 ppm	10.8	4.2	12.1	4.9	11.6	5.4	12.0	4.8
OA	250 ppm	12.7	5.4	13.3	5.4	15.0	6.3	14.6	6.3
	500 ppm	13.3	5.9	14.4	6.3	16.4	6.7	15.3	7.0
SA	250 ppm	10.9	4.8	12.0	5.4	12.1	5.4	12.9	5.0
	500 ppm	11.8	5.0	13.1	5.9	12.7	5.9	13.1	5.7
AA + OA	250 ppm	12.0	4.1	12.0	4.7	10.9	5.3	13.3	4.7
	500 ppm	13.0	5.2	14.4	6.7	16.1	6.3	15.1	6.7
AA + SA	250 ppm	10.9	4.7	12.7	5.4	10.8	4.2	13.1	5.7
	500 ppm	12.0	5.4	13.3	5.9	11.8	5.0	15.3	6.7
OA + SA	250 ppm	12.4	5.0	12.9	4.7	12.0	6.3	13.3	5.4
	500 ppm	13.4	5.7	14.6	6.7	16.4	7.0	16.1	7.0
Carbendazim 50% WP	2 g/l	13.5	5.9	14.7	7.0	17.8	7.3	18.1	7.3
Control	-	8.7	2.7	10.7	2.9	10.2	2.6	10.1	2.9
L.S.D. at 5%	-	0.6	0.1	0.4	0.1	0.7	0.1	0.3	0.5

Effect of three organic acids as dipping unrooted cuttings and spraying plants 30 days after planting date on the disease incidence: Dipping unrooted cuttings and spraying plants with organic acids solutions and carbendazim 50% WP fungicide resulted in an increase of *C. forskohlii* resistance against to infection with the tested fungi (Table 5). The resistance of coleus was enhanced by increasing organic acid concentration. Carbendazim 50% WP and OA gave significant decreases in disease incidence than the controls with all fungi under study, followed by SA at the highest concentration. However, AA was the least effective treatment. On the other hand, dual combination of organic acids gave the highest decreasing disease incidence with all tested fungi. The highest reduction was obtained by using the combined treatments between OA + SA at concentrations of 250 and 500 ppm and AA + SA at concentration 500 ppm, which reduced the disease incidence by 100% after 30 and 60 days from planting. . However, the decrease in disease incidence with all tested fungi was not significant with any treatment after 60 days from planting. Dipping unrooted cuttings and spraying plants 30 days after planting with organic acids and carbendazim 50% WP fungicide significantly increase all parameters compared to control under infection with tested fungi. Plant height and branch number increase by increasing of organic acid concentration. Dual combination between AA + OA at 500 ppm recorded superiority in increasing plant height and No. of branches per plant, followed by OA + SA at the same concentration compared to any of the other samples which underwent treatment with all tested fungi (Table 6).

Table 5: Effect of three organic acids as dipping unrooted cuttings before planting then spraying with them 30 days after planting on incidence (%) of root and stem rot disease of coleus plants under greenhouse conditions.

Treatments	Conc.	*F. oxysporum*		*F. semitectum*		*F. solani*		*R. solani*	
		Disease incidence (%) after		Disease incidence (%) after		Disease incidence (%) after		Disease incidence (%) after	
		30 days	60 days	30 days	60 Days	30 days	60 days	30 days	60 days
AA	250 ppm	33.3	33.3	33.3	41.7	33.3	41.7	41.7	41.7
	500 ppm	25.0	25.0	8.3	16.7	8.3	16.7	16.7	25.0
OA	250 ppm	8.3	16.7	0.0	16.7	16.7	25.0	8.3	16.7
	500 ppm	0.0	0.0	0.0	8.3	0.0	8.3	0.0	8.3
SA	250 ppm	25.0	33.3	33.3	41.7	8.3	16.7	33.3	33.3
	500 ppm	8.3	16.7	16.7	25.0	0.0	0.0	8.3	8.3
AA + OA	250 ppm	8.3	16.7	16.7	25.0	16.7	25.0	8.3	8.3
	500 ppm	8.3	8.3	8.3	8.3	8.3	8.3	0.0	0.0
AA + SA	250 ppm	16.7	16.7	8.3	8.3	8.3	8.3	8.3	16.7
	500 ppm	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
OA + SA	250 ppm	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	500 ppm	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carbendazim 50% WP	2 g/l	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Control	-	66.7	83.3	58.3	60.0	50.0	75.0	50.0	66.7
L.S.D. at 5%	-	0.8	0.1	0.5	0.3	0.8	0.6	0.5	0.6

Table 6: Effect of three organic acids as dipping unrooted cuttings before planting then spraying with them 30 days after planting on some plant growth parameters of coleus plants in soil infested with each of four pathogenic fungi under greenhouse conditions.

Treatments	Conc.	*F. oxysporum*		*F. semitectum*		*F. solani*		*R. solani*	
		Plant Height (cm)	No. of branches/ plant	Plant Height (cm)	No. of branches/ plant	Plant Height (cm)	No. of branches/ plant	Plant Height (cm)	No. of branches/ plant
AA	250 ppm	12.1	4.8	14.6	5.0	11.0	5.1	14.0	4.2
	500 ppm	13.4	5.2	15.2	5.2	13.4	5.5	15.7	5.0
OA	250 ppm	15.0	7.3	16.1	7.6	13.9	7.4	16.2	7.6
	500 ppm	16.2	8.0	17.0	8.3	16.6	8.2	18.3	8.2
SA	250 ppm	14.4	7.0	14.4	5.4	13.1	6.3	13.3	6.0
	500 ppm	14.8	7.6	15.4	6.0	15.0	6.8	16.8	6.9
AA + OA	250 ppm	18.8	8.3	17.9	9.4	19.5	9.3	19.8	8.7
	500 ppm	22.7	10.3	21.4	11.0	21.7	12.3	22.9	12.7
AA + SA	250 ppm	16.3	7.7	16.4	7.3	18.0	7.7	17.9	7.3
	500 ppm	17.9	9.4	18.0	7.3	19.8	8.7	20.4	11.6
OA + SA	250 ppm	19.3	8.8	18.0	7.0	18.0	7.3	20.0	11.0
	500 ppm	21.5	9.7	20.7	10.0	20.0	9.4	21.8	8.8
Carbendazim 50% WP	2 g/l	17.7	9.3	18.9	9.7	17.8	10.3	18.1	10.0
Control	-	8.4	2.7	10.3	2.4	10.9	3.0	10.2	3.1
L.S.D. at 5%	-	1.7	1.1	0.5	1.3	0.3	0.9	0.1	1.2

Effect of three organic acids applied as soil drenching treatment on the disease incidence: Drenching the soil with organic acid solutions resulted in an increase of coleus resistance against to infection with the tested fungi (Table 7). The results revealed that all organic acids reduced disease incidence by increasing concentration. OA gave significant decreases in disease incidence than the control with all fungi under study, followed by SA at the highest concentration. In contrast, AA was the least effective treatments in decreasing disease incidence (%), except for *F. solani*, since it gave the highest decreases percentage at concentration (500 ppm).

Table 7: Effect of three organic acids as soil drenching treatment on incidence (%) of root and stem rot disease of coleus plants, under greenhouse conditions.

Treatments	Conc.	*F. oxysporum*		*F. semitectum*		*F. solani*		*R. solani*	
		Disease incidence (%)		Disease incidence (%)		Disease incidence (%)		Disease incidence (%)	
		30 days	60 days	30 days	60 Days	30 days	60 days	30 days	60 days
AA	250 ppm	33.3	41.7	41.7	41.7	8.3	16.7	41.7	41.7
	500 ppm	25.0	25.0	25.0	33.3	0.0	0.0	25.0	33.3
OA	250 ppm	33.3	41.7	0.0	0.0	8.3	16.7	8.3	16.7
	500 ppm	8.3	16.7	0.0	0.0	0.0	0.0	0.0	8.3
SA	250 ppm	33.3	41.7	41.7	41.7	25.0	25.0	41.7	41.7
	500 ppm	16.7	25.0	33.3	33.3	8.3	16.7	16.7	25.0
AA + OA	250 ppm	16.7	16.7	8.3	16.7	8.3	16.7	16.7	16.7
	500 ppm	8.3	8.3	0.0	0.0	0.0	0.0	0.0	0.0
AA + SA	250 ppm	8.3	16.7	16.7	25.0	8.3	8.3	16.7	25.0
	500 ppm	8.3	8.3	8.3	8.3	0.0	0.0	8.3	8.3
OA + SA	250 ppm	8.3	8.3	8.3	16.7	8.3	8.3	16.7	16.7
	500 ppm	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carbendazim 50% WP	2 g/l	16.0	16.7	8.3	8.3	8.3	8.3	8.3	16.7
Control	-	66.7	83.3	58.3	75.0	50.0	75.0	50.0	66.7
L.S.D. at 5%	-	1.0	0.9	1.2	0.4	0.8	0.2	1.0	0.1

On the other hand, dual combinations of organic acids gave the highest decreasing disease incidence with all tested fungi. The highest reduction was obtained by using the combined treatments between OA+SA at concentration of 500 ppm which reduced the disease incidence by 100% after 30 and 60 days from planting. Drenching the soil with organic acids significantly increased all parameters compared to control. Soil drenching with OA single gave the highest results in all parameters (plant height and branch number) with all fungi compared to untreated plants, while, using the combined treatments between OA+SA was superiorly compared to any other treatment with all tested fungi (Table 8). On the other hand, dual combinations of the three organic acids was superiorly in both applied methods (dipping unrooted cuttings or dipping unrooted cuttings + spraying plants 30 days after planting or soil drenching).

Table 8: Effect of three organic acids as dipping unrooted cuttings treatment on some plant growth parameters of coleus plants in soil infested with each of four pathogenic fungi under greenhouse conditions.

Treatments	Conc.	*F. oxysporum*		*F. semitectum*		*F. solani*		*R. solani*	
		Plant Height (cm)	No. of branches/ plant	Plant Height (cm)	No. of branches/ plant	Plant Height (cm)	No. of branches/ plant	Plant Height (cm)	No. of branches/ plant
AA	250 ppm	14.6	5.2	14.3	6.0	15.0	6.3	15.3	7.3
	500 ppm	15.3	6.3	15.6	7.3	15.8	7.2	16.0	8.1
OA	250 ppm	20.0	8.0	20.3	8.7	20.1	7.0	19.8	8.3
	500 ppm	21.5	9.4	21.1	10.0	21.8	8.8	21.7	10.0
SA	250 ppm	15.3	6.8	16.0	7.3	16.2	7.0	17.1	7.0
	500 ppm	16.9	7.8	16.9	8.8	17.1	7.6	18.0	8.1
AA + OA	250 ppm	14.6	5.0	14.0	4.2	15.7	5.0	16.8	6.9
	500 ppm	16.2	8.3	16.1	7.6	17.0	8.3	18.3	8.2
AA + SA	250 ppm	15.0	7.3	16.1	7.6	15.0	6.8	16.2	7.6
	500 ppm	16.2	8.0	17.0	8.3	16.6	8.2	18.3	8.2
OA + SA	250 ppm	18.8	8.3	17.9	9.3	19.5	9.3	19.8	8.7
	500 ppm	22.7	10.3	20.4	11.0	21.7	12.3	22.9	12.7
Carbendazim 50% WP	2 g/l	17.7	9.3	18.9	9.7	17.8	10.3	18.1	10.0
Control	-	8.8	2.8	10.2	2.3	10.2	2.3	10.7	2.1
L.S.D. at 5%	-	3.2	1.9	2.3	2.1	1.1	0.2	0.7	0.8

Effect of three organic acids and the fungicide Carbendazim 50%WP as dipping unrooted cuttings of coleus on defense-related enzymes: Data in table (9) showed the oxidative enzyme activities (peroxidase and polyphenol oxidase) due to treating coleus cuttings unrooted with three organic acids (alone or dual combinations) and carbendazim. Dual combination between OA+SA recorded superiority in peroxidase and polyphenol oxidase activities with *F. oxysporum*, *F. semitectum* and *R. solani.* While, AA+SA recorded superiority in peroxidase and polyphenol oxidase activities with *F. solani*. In contrast, OA exhibited lower peroxidase and polyphenol oxidase activities.

Table 9: Activity of enzymes in coleus plants treated with different treatments as dipping unrooted cuttings.

Treatments	Concentrations	Enzymes	*F. oxysporum*	*F. semitectum*	*F. solani*	*R. solani*
AA	500 ppm	Peroxidase	1.81	1.38	1.90	1.90
		Polyphenol oxidase	0.70	0.48	0.76	0.74
OA		Peroxidase	1.31	1.20	1.38	1.48
		Polyphenol oxidase	0.23	0.20	0.25	0.46
SA		Peroxidase	1.38	1.80	1.75	1.49
		Polyphenol oxidase	0.25	0.71	0.76	0.53
AA + OA		Peroxidase	1.90	1.50	1.92	1.62
		Polyphenol oxidase	0.67	0.29	0.77	0.50
AA + SA		Peroxidase	1.83	1.49	1.93	1.91
		Polyphenol oxidase	0.70	0.31	0.78	0.78
OA + SA		Peroxidase	1.92	1.81	1.80	1.95
		Polyphenol oxidase	0.78	0.75	0.75	0.79
Carbendazim 50% WP	2 g/l	Peroxidase	1.83	1.77	1.80	1.56
		Polyphenol oxidase	0.71	0.50	0.76	0.59
Control	-	Peroxidase	0.76	0.65	0.55	0.68
		Polyphenol oxidase	0.13	0.12	0.14	0.12

Correlation between disease incidence and each of peroxidase and polyphenol oxidase: Table (10) showed that there was a significant or highly significant correlation between the activities of peroxidase and polyphenol oxidase in tissues infected with all fungi. The correlation between disease incidence and each of peroxidase and polyphenol oxidase was negative for *F. oxysporum* and *R. solani*, however the correlation was nonsignificant in both cases.

Table 10: Correlation between disease incidence and each of peroxidase and polyphenol oxidase.

Fungi	Variable	Variable: PO (X1)	Variable: PPO (X2)
F. oxysporum	Peroxidase (X1)		
	Polyphenol oxidase (X2)	0.986[a] (0.000)[b]	
	(%) Disease incidence after 30 days(X3)	- 0.686[a] (0.132)[b]	- 0.645[a] (0.167)[b]
F. semitectum	Peroxidase (X1)		
	Polyphenol oxidase (X2)	0.881[a] (0.020)[b]	
	(%) Disease incidence after 30 days (X3)	.[c]	.[c]
F. solani	Peroxidase (X1)		
	Polyphenol oxidase (X2)	0.950[a] (0.004)[b]	
	(%) Disease incidence after 30 days (X3)	.[c]	.[c]
R. solani	Peroxidase (X1)		
	Polyphenol oxidase (X2)	0.966[a] (0.002)[b]	
	(%) Disease incidence after 30 days (X3)	- 0.603[a] (0.205)[b]	- 0.423[a] (0.403)[b]

[a] Linear correlation coefficient, n= 6, [b] Probability level, [c] correlation cannot be computed because DI was constant.

Discussion

Coleus (*Coleus forskohlii* Briq.) is grown for use as a bedding plant for public gardens. Many soil-borne fungi are capable of causing stem and root rot of plants. Species of *Fusarium*, *Macrophomina*, *Pythium* and *Rhizoctonia* were among the most dominant fungi in isolation trials from diseased plants. The causative agents of stem and root rot as a result of isolation trials were somewhat similar to those reported by many authors (Pulla et al., 2013; Boby & Bagyaraj, 2003; Srivastava et al., 2001; Mihail, 1992). Results of the present study showed that isolated fungi were able to infect roots and stems of *C. forskohlii* plants, grown in artificially infested soil. *R. solani* was the most virulent fungi, followed by *F. oxysporum*, *F. solani* and *F. semitectum*, respectively. Similar results were reported by Shivkumar et al. (2006), Singh et al. (2009) and more recently by Pulla et al. (2013). Testing three organic acids (ascorbic acid, oxalic acid and salicylic acid) against stem and root rot diseases gave satisfactory effectiveness in most cases. Dipping unrooted cuttings with organic acids testes was of great value in decreasing disease incidence and plant growth parameters such as plant height and no. of branch/plant were greatly improved. SA was superior treatment than OA and AA in decreasing disease incidence. However, OA was significantly superior in plant growth parameters. On the other hand, dual combination of the three organic acids was superior to single treatment. The highest reduction was obtained by using the combined treatments of AA+OA, AA+SA and OA+SA. Organic acids have the properties of antioxidants which are considered to be an important mechanism of membrane deterioration during ageing of tissues (Droillard et al., 1987; Paulin et al., 1986; Mayak et al., 1983; Dhindsa et al., 198; Kellog & Fridovich, 1975). The two applications (Dipping + spraying) of plant with organic acids were of great value in decreasing dead plants and plant growth parameters. Carbendazim and OA gave significant decreases in dead plants and plant growth parameters than the controls with all fungi under study. On the other hand, dual combination of the three organic acids was superior to the single treatment. Variability in the effect of organic acids (Antioxidants) can be attributed to differences in their activity or variation in the responses dictated by the host due to the phytotoxicity of the compounds or to the ability of the compounds to penetrate their tissues (Edlich et al., 1989). Also, some studies have been shown that oxidants may enhance host resistance to fungal infection (Prusky et al., 1995; Elad, 1992; Prusky, 1988). Drenching the soil with organic acids was of great value in decreasing disease incidence and plant growth parameters. OA gave significant decreases in disease incidence and plant growth parameters than the control with all fungi under study. On the other hand, dual combination of the three organic acids was superior on single treatment. These results were in agreement with the results of Galal and Abdou (1996) who found that soil drench application of salicylic or ascorbic acid was better than foliar application to control fusarial diseases of cowpea. In the same sense, Mostafa (2006) reported that soaking cumin seeds or soil drenching with antioxidant solutions (salicylic, ascorbic, coumaric and benzoic acids) before planting resulted in resistant cumin seedlings against infection with *F. oxysporum cumini* and *Acremonium egypticum*. Treating coleus cuttings unrooted with three organic acids and the fungicide carbendazim increased in the oxidative enzyme activities (peroxidase and polyphenol oxidase). Combination between OA+SA recorded superiority in peroxidase and polyphenol oxidase activities with *F. oxysporum*, *F. semitectum* and *R. solani*, while, AA+SA recorded superiority in peroxidase and polyphenol oxidase activities with *F. solani*. The antioxidant mode of action was reported in many interactions of host-pathogen i.e. many oxidative enzymes such as peroxidase, catalase, ascorbate oxidase and polyphenol oxidase were detected as a result of infection with many pathogens (Clark *et al.*, 2002) or as a result of treatments with various antioxidants (Abdel-Monaim et al., 2011; Ragab et al., 2009; El-Khallal, 2007; Takahama & Oniki, 1994). There was a significant or highly significant correlation between the activities of peroxidase and polyphenol oxidase in tissues infected with all fungi. This correlation indicated that it is not necessary to determine the activity of the two enzymes, because any of them is indicator for the other one. The correlation between disease incidence and each of peroxidase and polyphenol oxidase was negative for *F. oxysporum* and *R. solani*, however the correlation was nonsignificant in both cases. This nonsignificant could be due to the limited sample size (n=6). In conclusion, the present study indicated that each of peroxidase or polyphenol oxidase is not correlated with disease incidence.

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