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Evaluation of Soil Amendment with Three Plants on *Fusarium* spp. Isolated from Carnation Plant

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Soil amendment for controlling three *Fusarium* species, *Fusarium oxysporum* (Sehlect. Emend Snyd. & Hans), *F. solani* (Mart.) (Apple and Wr. Emend. Snyd. & Hans) and *F. moniliforme* (Sheldon) were isolated from carnation. Cuttings were planted in soil artificially infested with the pathogenic fungi tested and amended singly with powder dry material of Eucalyptus, Leek or Thyme to study their effect on % infection and some growth parameters in soil infested with the pathogenic tested fungi of *fusarium* spp. under greenhouse conditions. The possible biochemical changes (phenolic compounds, oxidative enzymes and chlorophyll and carotene contents) associated soil amendment with three tested dry plants for controlling the disease in the present study was investigated. Herb of thyme was amended with soil proved to be the most effective one in the prevention of infection in case of infested with any three tested fungi while leek the least one. Also, fungicide (Topsin M-70) was tested and other commercial bioproduct Plant-Guard (original component *Trichoderma harzianum*). Mixing infested soil with tested powder materials lead to increase levels of phenolics compounds, oxidative enzymes and chlorophyll and carotenoides contents compared with those obtained from non treated cuttings in infested soil.

Key words: Soil amendment, *fusarium*, Carnation, Biochemical studies.

The carnation *(Dianthus caryophllus* L.) growers all over the world, complain from losses due to certain fungal pathogens which cause wilt and root rot diseases which attack the root system, crown or basal stem parts. Plant diseases contribute significantly to the total crop losses both at global and national level Snyder and Hansen (1945). Akhmed (1990) recorded that *fusarium oxysporum, f. moniliforme* and *f. solani* were isolated from carnation.

On the other hand, development of synthetic chemical fungicides to control plant diseases has become difficult because of strict regulations of health and environmental safety with no lenient efficacy selectivity and toxicity requirements as well as their general impact on environment (McLaren, 1986). Consequently, the need to find alternatives to these chemicals has promoted in the last two decades as higher plants are found to be a source of important natural

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extracts which may probably have fungi toxic effect on different plant pathogens (Al-Abed *et al.,* 1993). Any part of the plant as barks, stems, leaves and flowers contain many active natural compounds that can be used as an antimicrobial (Emmanual *et al.,* 2010, Nair and Chanda, 2004 and Cragg and Newman, 2001).

The application of organic soil amendment has some advantages over chemical control because the former is more useful, less hazardous, and pollution free.

Present study was carried out to examine the effect of Eucalyptus, Leek or Thyme to study their effect on % infection and some growth parameters in soil infested with *fusarium* spp. fungicide (Topsin M-70) was tested and other commercial bioproduct Plant-Guard (original component *Trichoderma harzianum*).

MATERIALS AND METHODS

Collection of samples

The samples were collected from Egypt in April, 2010 and duration of this study 9 months.

Isolation of *fusarium* spp. associated with carnation wilt plants

Infected roots and basal steam parts of carnation were cut into small fragments, washed thoroughly with tap water, then sterilized with sodium hypochlorite solution of about (1% chlorine) for one minute, rinsed several times in sterile distilled water and dried between two sterilized filter papers. Fragments were then placed on potato dextrose agar media in sterilized Petri dishes and incubated at 25 °C for 7 days. The developed fungal colonies were recorded as percentage of frequencies for each fungus and purified using either hyphal-tip or single-spore technique .The isolated fungi were picked from the edges of growing colonies or germination spores and transferred onto water agar plates as described by (Nelson *et al.,* 1983 and Booth 1971), then the purified colonies were transferred on PDA slants. All the obtained isolates were microscopically identified according to the morphological features using the description of Wterhouse (1956). Identification of the selected isolates were confirmed by the fungal Taxonomy Department, Plant Pathology Research Institute, Agricultural Research Centre, Giza.

Pathogenicity tests

Inocula of *Fusarium oxysporum* f. sp. *dianthi, F. solani* and *F. moniliforme* were the most frequently isolated from carnation wilt prepared by inoculating maize meal-sand medium in 500 ml. glass with 5mm. disk of 7-day-old. Pure culture of each fungus then incubated at 27ºC for 15 days. Throughout all the greenhouse trials, pots of 25 cm in diameter were used. Pots were always sterilized by immersing them in 5% formalin solution for 15 minutes and then air dried for 7 days. Sterilized soil infestation was carried out by adding the inoculum of the fungus at the rate of 3% of soil weight (w/w) Mazen (2004). Fungi inocula were thoroughly mixed with the soil and regularly watered every day for a week before planting to ensure even distribution and growth of each particular fungus. Soil mixed alone with the same amount of autoclaved maize meal- sand medium served as a check treatment. Each replicate pot was planted by five seeds of basil and five pots were used for each treatment. All plants were observed daily.

Effect of soil amendment with three dry plants on %infection and some growth parameter: Collection of plant

Leaves of Eucalyptus and Leek and herb of Thyme were collected and dried under shade. After drying all plant parts were separately ground and their powder was used for further studies. The three tested plants (Eucalyptus, Leek and Thyme) were tested for controlling the disease in three separated experiments. dry materials were amendment with soil at the rate of 1%w/w (Emmanual *et al.,* 2010) then planted in sterilized pots were filled with infested soil with each of the three pathogenic fungi as previously mentioned. The soil was watered daily to allow decomposition of the material. After 10 days of amendment, ten cuttings of carnation were planted in each pot and four replicates were used for each treatment. Non amended soil served as control. Data were recorded after 30 days after planting as percentage of infection and some growth parameters.

Effect of Topsin M-70 as fungicide on %infection and some growth parameter

The fungicide was tested in pots experiment under greenhouse conditions according to Abo-Zeid *et al*. (1987).

Effect of Plant-Guard as bioproduct on %infection and some growth parameter

Effectiveness of commercial bioproduct Plant-Guard (original component *Trichoderma harzianum*) was tested also in the laboratory and in pots under greenhouse conditions.The treatment was carried out in pots (20 cm.) containing unfested soil. The inoculum of the pathogenic fungi (*F. oxysporum f.sp. dianthi , F. solani* and *F. moniliforme*) was thoroughly mixed with the soil at the rate of 3% (W/W) and regularly watered every other day for a week before planting. Plant-guard was applied at the rate of 4.0 cm/L water (as a recommended dose) by complete soaking of the cuttings for 15 minutes before planting. Treated cuttings were then planted in the infested soil in the greenhouse. Ten cuttings/ pot were used in four replicates. Untreated cuttings were served as check pots. Data were recorded 30 days after planting as percentage of infection and growth parameters were measured.

Biochemical studies

The possible biochemical changes associated Soil amendments with three tested dry plants for controlling the disease in the present study were investigated. Healthy and infected plants with *Fusarium oxysporum* f. sp. *dianthi, F. solani* and *F. moniliforme* and cuttings were grown on soil infested with tested pathogens and amended singly with powder dry material of Eucalyptus, Leek or Thyme , fungicide as well as the biocide were always sampled at the age of 30 days at the end of each pot experiment. The investigated parameter included changes in phenolic compounds, oxidative enzymes, chlorophyll and carotenoides contents.

Phenolic compounds content

The phenolic compounds content was calorimetrically determined using the Follin reagent according to Snell and Snell (1953).

Estimation of peroxidase activity

Enzyme extraction from the leaves was prepared as recommended by (Maxwell and Bateman, 1967). The leaf tissues were grounded with 0.1 M sodium phosphate buffer at pH 7.1 (2 ml buffer/g of fresh leaf tissues), in a mortar. These triturated tissues were strained through four layers of cheesecloth and the filtrates were centrifuged at 3000 rpm for 20 min at 6ºC. The supernatant fluid was used for enzyme assays. Peroxidase activity was estimated according to the method of Allam and Hollis (1972).

Polyphenoloxidase assay

The activity of phenoloxidase was measured with the colourimetric method of Maxwell and Bateman, 1967). The reaction mixture contained 0.2 ml enzyme extract, 0.5-ml sodium phosphate buffer at PH 7 and 0.5 ml of catechol brought to a final volume of 3 ml with distilled water. The activity of phenoloxidase was expressed as the change in absorbance /1 ml of extract per min at 495 nm.

Estimation of Chlorophyll and carotene contents

The photosynthetic pigments were extracted from treated and untreated fresh leaves after 10, 20 days of inoculation. Ten disks (1cm) were taken from each treated leaf and pigments were extracted for 48 h in the dark in a tube containing 10 ml of 85% acetone according to the methods described by Procter, (1981). The total chlorophyll pigments were determined by measuring the optical density (O.D) at 663, 452 and 645 nm and calculated using the formula recorded by Arnon, (1949).

Total chlorophyll = 8.02 x O.D at 663 + 20.20 x O.D at 645.

Carotene = 4.75 x O.D at 452 – total chlorophyll.

Statistical analysis

The obtained data were subjected to analysis of variance following (Steel and Torrie, 1960), whereas the differences between treatments were tested by calculating Least Significant Differences (L. S. D) at 5% level.

RESULTS

Isolation and pathogenicity test of fusarium spp. were isolated from carnation wilt Carnation

Fusarium isolates were identified into three species, *Fusarium oxysporum* (Sehlect. Emend Snyd. & Hans), *F. solani* (Mart.) Apple & Wr. Emend. Snyd.& Hans, and *F. moniliforme* (Sheldon). Natural infection samples showing rot and /wilt symptoms which obtained in fig. (1).

Data in Table (1) show that all the tested isolates were pathogenic to carnation plants and percentages of infection increased up to 30 days after planting were recorded. The highest mean percentages of infected plants were observed in soil infested with *F. oxysporum* f. sp. *dianthi* (42.5%) and *F. moniliforme* (41.6%). *F. solani*

exhibited the least mean infection percentage (36.7%) as compared to the control plants (0.0% infection).

Table (2) revealed that Carnation cuttings were planted in soil artificially infested with the pathogenic fungi tested and amended singly with powder dry material of Eucalyptus, Leek or Thyme to study their effect on % infection in soil infested with the pathogenic tested fungi of *fusarium* spp. under greenhouse conditions. A significant positive against correlation between effect of all tested plants as amendment soil and control on % infection in case of three tested fungi. Herb of thyme was amended with soil proved to be the most effective one in prevent percentage of infection in case of infested with any three tested fungi followed by carnation cuttings with Plant-Guard. Amended soil with Eucalyptus leaves powder gave modulate effect on decrease %infection and equal with effective of Topsin M70, while amended soil with leek was the least one. and no significant between treatment with Eucalyptus or thyme and Topsin M-70 .

On the other side, the same table recorded that effect of soil amendment with Eucalyptus, Leek or Thyme, Topsin (M-70) and Plant-Guard on some growth parameters of carnation infested with *F. oxysporum, F.solani*, *F. moniliforme.* Topsin (M-70) was the best on plant height ,fresh weight and dry weight followed by Plant-Guard then Eculayptus in case of *F.oxysporum* and *F.solani* whereas, soil amendment with Eculayptus or Thyme were the best in case of *F. moniliforme*. with a significant positive against correlation between all treatments and control and no significant between treatment with Eucalyptus and treatment with thyme in case of plant height. No significant mention between all treatments and

Table 1. Pathogenicity tests of the isolated *fusarium spp.* from carnation after 10, 20 and 30 days from planting.

Tested isolated of fusarium spp	Days after planting			Mean
	10 days	20 days	30 days	
F. oxysporum f. sp. *dianthi*	27.5	35.0	65.0	42.5
F. solani	20.0	32.5	57.5	36.7
F. moniliforme	17.5	42.5	65.0	41.6
Control	0.0	0.0	0.0	0.0
Mean	24.4	39.4	65.0	-

LSD 5% For Fungi (F)= 5.1, Periods(P)= 5.3, and (FxP)= 8.8

Table 2. Effect of soil amendment with some dry materials of three medicinal plants , fungicide and bio product on growth parameters of carnation infested with *F. oxysporum, F. solani , F. monilinforme*

Treatment	*F. oxysporum*				*F. solani*				*F. moniliforme*			
	%I	P.H	F.W	D.W	%I	P.H	F.W	D.W	%I	P.H	F.W	D.W
Eucalyptus	7.5	13.5	3.14	0.47	5.0	13.6	3.02	0.60	5.0	13.8	5.18	0.81
Leek	40.0	12.4	2.91	0.45	15.0	12.3	3.45	0.73	12.5	12.1	3.14	0.47
Thyme	0.0	13.4	3.28	0.46	0.0	13.5	3.53	0.62	0.0	13.8	3.92	0.75
Topsin (M-70)	7.5	13.7	3.84	0.79	0.0	13.7	4.01	0.79	0.0	13.6	3.91	0.65
Plant-Guard	5.0	13.6	3.34	0.64	7.5	13.4	3.24	0.56	12.5	13.2	3.11	0.51
Control (infested)	80.0	9.6	2.78	0.34	50.0	9.6	2.55	0.31	60.0	9.5	2.89	0.40

%I=% infection, P.H=plant height (cm.), F.W=fresh weight (g.) and D.W=dry weight (g).
L.S.D at 5% for % infection Treatment(T)= 4.6 Fungi(F)=2.8 TXF=6.79
L.S.D at 5% for plant height Treatment(T)= 0.17 Fungi(F)=0.12 TXF=0.28
L.S.D at 5% for fresh weight Treatment(T)= 0.092 Fungi(F)=0.052 TXF=0.13
L.S.D at 5% for dry weight Treatment(T)= 0.34 Fungi(F)=0.032 TXF=0.078

control in case of weight (fresh and dry weight) .

Biochemical studies

Phenolic compounds

Table (3) recorded that phenolics compounds contents were evaluated in carnation cuttings planted in Soil amendment of the three medicinal plants Leek, thyme and Eucalyptus. Data indicated that mixing infested soil with tested powder materials increased levels of phenolics compounds compared with those obtained from non treated cuttings in infested soil. Among the used powder materials of the used medicinal plants, Thyme increased total phenolic compounds contents of carnation cuttings in soil infested with *F. solani* (3.21) compared with the two check treatments (0.89) and (3.12) in non treated plants in infested and non infested soil, respectively, It is worth to note that Plant Guard recorded the highest values of total phenolic compounds in carnation cuttings planted in soil infested with *F. oxysporum* (3.34).

Oxidative enzymes

Data in Table (4) did not make statistical analysis for the chemical estimation, however, it shows evaluated values of the activity of oxidative enzymes (peroxidase and polyphenoloxidase) in carnation cuttings grown in soil infested with three tested pathogen and soil amendment with plant powder materials caused an increase in peroxidase and polyphenoloxidase activity in carnation cuttings than those planted in soil infested only. However, the highest values of peroxidase and polyphenoloxidase activity were recorded in soil mixed with leave powder of Eucalyptus in soil infested with *F. oxysporum* f. sp. *dianthi* or *F. moniliforme* while soil amendment with powder herb of Thyme was the best in case of soil infected with *F. solani*. It worth to not that, powder leaves of leek mixed with soil were not effective in increasing activity of peroxidase and polyphenoloxidase.

Table 3. Effect of Soil amendment with dry three medicinal plants, Topsin M70 and Plant-Guard on phenolic compound (mg/g fresh weight) of carnation cuttings planted in soil infested with *F. oxysporum* f. sp. *dianthi*, *F. solani*, *F. moniliforme*, under greenhouse conditions

Treatment	*F. oxysporum*			*F. solani*			*F. moniliforme*		
	Free	Conjugated	Total	Free	Conjugated	Total	Free	Conjugated	Total
Eucalyptus	0.63	0.49	1.12	0.74	0.20	0.94	0.61	0.26	0.87
Leek	1.64	0.73	2.19	1.78	1.43	3.21	1.13	0.64	1.77
Thyme	1.93	0.85	2.78	1.74	1.01	2.75	1.22	0.73	1.95
Topsin (M-70)	1.48	0.98	2.46	1.32	0.87	2.19	1.61	1.53	3.14
Plant-Guard	1.70	1.64	3.34	1.40	1.12	2.61	1.50	1.43	2.93
Control (infested)	0.62	0.12	0.74	0.75	0.14	0.89	0.35	0.11	0.46

Table 4. Effect of Soil amendment with dry three medicinal plants, Topsin M70 and Plant-Guard on activity of peroxidase and polyphenoloxidase enzymes of carnation cuttings planted in soil infested with *F. oxysporum* f. sp. *dianthi*, *F. solani*, *F. moniliforme*, under greenhouse conditions

Treatment	*F. oxysporum*		*F. solani*		*F. moniliforme*	
	PO	PPO	PO	PPO	PO	PPO
Leek	1.26	0.31	1.13	0.19	0.83	0.21
Thyme	1.75	0.34	2.51	0.67	1.43	0.41
Eucalyptus	1.89	0.52	1.73	0.39	1.52	0.45
Topsin (M-70)	1.72	0.42	1.61	0.39	1.98	0.56
Plant-Guard	1.84	0.47	1.77	0.54	1.81	0.41
Control (infested)	0.61	0.23	1.22	0.29	0.72	0.28

PO= Peroxidase enzymes; PPO= Polyphenoloxidase enzymes

Table 5. Effect of Soil amendment with dry three medicinal plants, Topsin M70 and Plant Guard on chlorophyll (a and b) and carotenoides (mg./ml) of carnation cuttings planted in soil infested with *F. oxysporum* f. sp. *dianthi*, *F. solani*, *F. moniliforme*, under greenhouse conditions

Treatment	*F. oxysporum*			*F. solani*			*F. moniliforme*		
	a	b	c	a	b	c	a	b	c
Leek	0.31	1.22	0.44	0.55	1.52	0.59	0.66	1.85	0.61
Thyme	0.89	2.30	1.11	0.65	1.94	0.95	0.73	2.06	1.42
Eucalyptus	0.58	1.90	1.33	0.59	1.86	1.43	0.66	2.01	1.52
Topsin (M-70)	1.31	0.81	0.86	1.15	0.64	0.75	1.02	0.67	0.57
Plant-Guard	1.47	0.82	1.11	1.16	0.63	1.30	1.24	0.78	1.03
Control (infested)	0.24	0.32	0.32	0.30	0.25	0.27	0.35	0.17	0.24
Control (non infested)	1.40	0.88	1.22	1.40	0.88	1.22	1.40	0.88	1.22

a= chlorophyll (a) b= chlorophyll (b) c= carotenoides

Chlorophyll and carotenoides

Regarding determination of the chlorophyll and carotenoides contents, the data in (Tables 5) revealed that contents in healthy carnation leaves grown in non infested soil were l.40, 0.88 for chlorophyll a and chlorophyll b, and 1.22 (mg/ml) for carotenoides. These levels were reduced too much in carnation grown in soil infested with the 3 fusarium isolates where chlorophyll a ranged between 0.24-0.35 mg/ml, chlorophyll b ranged between 0.17-0.32 mg/ml and carotenoides ranged between 0.24-0.32 mg/ml. It is worth to notice the effect of both fungicidal and biocide treatments on the chlorophyll contents as compared to control treatment in non infested soil, where no sharp effects was observed in soil infested with F *oxysporum* but sharp decrease was noticed in soil infested with *F. solani* or *F. moniliforme,* meanwhile carotenoides were mostly decreased.

In case of soil treated with Soil amendment with three medicinal plants, the highest value of chlorophyll a &b were obtained in soil mixed with Thyme herb and infested with *F. oxysporum , F.* and *F. moniliforme*. Plant Guard occupied the second rank in this respect in most cases while Leek recorded the lowest. Carotenoides highest values were obtained in soil mixed with Eucalyptus followed by soil amendment with Thyme in any three tested fungi.

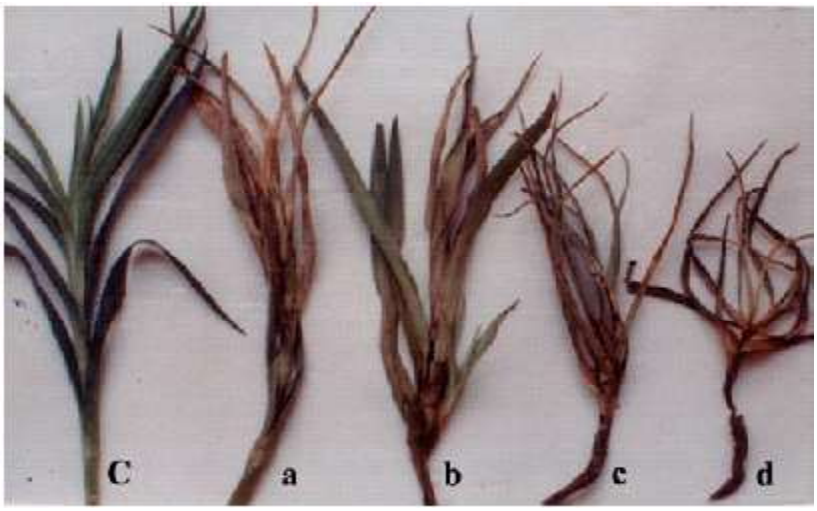

Fig. 1. Carnation plant with natural infection, (C) =control (a, b, c, d)= different stages from natural infection

DISCUSSION

The results obtained here clearly demonstrate that Thyme herb mixed with the infested soil completely prevented infection of carnation with the tested pathogenic fungi followed by those of Eucalyptus which was similar to those obtained by Plant-Guard and Topsin M-70. The used material may release substances that reduce inoculum density of the pathogenic fungi in the soil leading to disease control. These results are somewhat similar to those obtained by Salama *et al.,* 1988 and Zedan, 1993. They reported that incorporation of Eucalyptus leaves into the soil inoculated with *S. cepivorum* not only reduced the percentage of infection but also delayed disease incidence and increased onion growth parameters as well as the yield. They concluded that the pathogen suppressed in soil probably due to phenolic compounds and/or other inhibitory substances present in Eucalyptus leaves. Shaukat and Saddiqui (2002) reported that phenolic compounds or some other chemicals were exuded from dry materials lead to suppression of fungi infection.

Tariq *et al.,* (2008) reported that mangrove plant parts powder used lead to increased all growth parameters of potato plants were diseased with root rot. Plants produce Emmanual *et al.,* 2010 reported that Leaves and stem powder of *Sida pakistanica* and *Senna holosericea*s howed reduction in infection of *R. solani* and *M. phaseolina* on okra and mash bean and significantly enhanced plant weight of mash bean. The oxidative enzyme play an important role in induced resistance by the oxidation of phenols to oxidized products (quinone) which limit the fungal activity Baraka et al. (2004) and Hassan et al. (2007). However, Bonner and Varner, 1965 reported that plant extract might be stimulate photosynthesis pigments due to substances that act as activators for chlorophyll synthesis degradation and/or inhibit the effect of causal fungi chlorophyll degradations well as carotenoides.

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