Egypt. J. Phytopathol., Vol. **40**, No. 1, pp. 41-57 (2012)

Suppressive Effect of Certain Composts on the Infection by Root-Rot and Wilt Diseases of Faba Bean under Greenhouse Conditions

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Supplemented composts Rich and Planta Rich, (CMs) to infested soil with the pathogens (*Rhizoctonia solani* and *Fusarium subglutinans* the causal of root-rot and *F. oxysporum* ; the causal of wilt) at the rate of 10 gm/kg soil (Feddan = 1000 000 kg soil) caused high reduction in severity of both diseases compared with control (un-composted soil). Population density of *Bacillus subtilis Pseudomonas fluorescens,* Actinomycetes and certain fungi was increased by supplemented soil with Rich and Planta Rich CMs. The highest increase was detected in Actinomycetes population density followed by the other soil microflora. However population of fungi was the lowest. *B. subtilis Pseudomonas fluorescens,* Actinomycetes and certain fungi were isolated from both composts and proved to be antagonistic to the pathogenic fungi *in vitro*. Electrophoretic analysis of extracts of healthy and diseased faba bean plants grown in composted infested with the tested pathogens indicated that anew proteins (MW 23.3 KDa), two chitinase (Chit-1 and Chit-6), one peroxidase (PRX-5) and one estrase (ESt-4) isoforms which were induced by this treatment.

Keywords: Antagonistic microbes, compost, faba bean, isozymes, PR- proteins, root rot and wilt.

Faba bean (*Vicia faba* L.) is the most important seed legume and is a very common food in Egypt. One of the important benefits of faba bean is improved of soil fertility via providing a substantial input of N_2 fixation. Faba bean, in Egypt, is susceptible to attack by a number of soil borne pathogenic fungi that cause serious root- rot and wilt diseases, which decrease crop productivity and quality (Abou-Zeid *et al*., 1997; Abdel-Hafez, 1988; Mazen *et al*., 2008 and Elwakil *et al*., 2009). Many traditional strategies of disease control, *i.e*. biological and chemical control, resistant cultivars and agriculture practices were investigated and applied (Agrios, 2005).

Soil amendment with compost is an agronomically increasing practice as well as an attractive waste management strategy. The addition of mature compost to soil favours plant development and improves soil quality, as well as having a suppressive effect on many soil borne plant pathogens (Erhart *et al*., 1999; Cotxarrera *et al*., 2002 and Abdelhamid *et al*., 2004). Compost was found to be suppressive to certain diseases incited by soil fungi such as *Sclerotium* sp., *Pythium* sp., *Phytophthora* sp., *Aphanomyces* sp.; *Fusarium* spp. and *Rhizoctonia solani* in fields and potting mixtures in greenhouse (Tuitert *et al*., 1998; Gorodecki and Hadar, 1990; Craft and Nelson, 1996; Erhart *et al*., 1999; Reuveni *et al*., 2002; Bailley and Lazarovits, 2003; Diab and Benson, 2003 and Steinberg *et al*., 2004).

Disease suppression by compost on soil borne diseases was often related to biotic rather than abiotic factors (Reuveni *et al*., 2002 and Garbeva *et al*., 2004). Compost amendment to soil can modify the microbial community composition and as a result, enhance the competition for nutrients or antagonism and or mycoparasitism among microbes (Hoitink and Boehm, 1999; McKellar and Nelson, 2003; Steinberg *et al*., 2004 and Pérez-Piqueres *et al*., 2006). Several genera of antagonistic microorganisms (*Trichoderma, Gliocladium, Streptomyces, Pseudomonas* and *Bacillus*) have been identified as biocontrol agents in composted amended substrates. They inhibit several plant pathogenic fungi through antibioses and or mycoparasitism the common mode of action (Aryantha and Guest, 2006). Composts may also trigger indirect defence mechanisms by sensitizing the plant to create an increased state of resistance, similar to systemic acquired resistance (SAR), (Zhang *et al*., 1998 and Vallad *et al*., 2003).

Such compost mediated induced resistance appears to correlate with the activation of plant-defence related genes and subsequent accumulation of several proteins such as peroxidase, B-1-3-glucanases and pathogenesis-related 1(PR-1), (Zhang *et al*., 1998 and Kavroulakis *et al*., 2005).

The aim of this study was to evaluate the suppressive effect of Planta Rich and Rich composts at rate 10 ton/feddan. (10g/kg soil) The mechanisms by which the tested composts suppress both diseases and their incitants were also investigated.

Materials and Methods

Source of fungi and pathogenicity tests:

Fusarium subglutinans, *Rhizoctonia solani* and *F. oxysporum* were isolated from faba bean plants showing typical symptoms of root-rot and wilt diseases. Fungal isolates were purified and identified by using their morphological features and the description of Booth (1971) and Domsch *et al.* (1980). Identification was confirmed by Mycological Centre (AUMC), Assiut Univ., Assiut. The pathogenic ability of these isolates were tested and proved using faba bean cv. Masr-1 in pot experiment under greenhouse conditions.

Preparation of the fungal inocula:

Inocula of the tested isolates were prepared by inoculating sterilized conical flasks (1000 ml) containing barley medium (150 g barley seeds, 50 g clean sand, 4 g glucose, 0.2 g yeast extract, and 200 ml water) with equal discs (0.5 cm) taken from 7 days old cultures of the tested isolates grown on PDA medium at 27°C. The inoculated flasks were incubated at 27°C for two weeks then mixed with autoclaved clay soil at the rate of 3% W/W.

Compost materials:

The two compost products, Rich and Planta Rich manufactured by Agro-Egypt Company from animal manures and agricultural plant wastes, respectively, were selected for this study. They were commercially available and recommended for use as organic manures to improve productivity of wide numbers of crops include faba bean. Chemical analysis (Table 1) of the tested CM_S was kindly carried out at the Central Lab. of Soil and Water Dept., Fac. Agric., Assiut Univ.

Table 1. Chemical analysis of Rich and Planta Rich CMs

Measurement	CMs	
	Rich	Planta Rich
pH (1:10)	8.77	4.80
EC (1:5) (dS/m)	10.85	9.65
Moisture %	39.0	32.0
N %	3.10	2.15
P %	2.11	1.97
K %	2.34	1.69
Organic matter	45%	39%

Effect of two types of composts on root rot and wilt diseases under greenhouse:

This experiment was carried out in greenhouse 2010/2011, formalin disinfested pots (25cm in diameter) filled with autoclaved clay soil were infested by mixing inocula of each fungus individually with the soil at the rate of 3% W/W. Infested pots were irrigated (just after infestation) and sown 7 days later by cv. Masr 1 faba bean. Un-infested pots were served as control.

The tested two CMs were separately applied and mixed with infested and un-infested soil in pots at rate of 10 gm/kg soil. Un-composted pots were served as control. Five replicates were used for each test.

Disease severity was recorded after 30 days from planting date for root rot. The arbitrary (0-5) disease index scale described by Grunwald *et al*. (2003) was used to measure the disease severity of Fusarium root rot, in which; 0= No visible symptoms, 1= slight hypocotyls lesions, 2= lesions coalescing around epicotyls and hypocotyls, 3= lesions starting to spread into the root system with root tips starting to be infected, 4= epicotyl, hypocotyls and root system almost completely infected and only slight amount of white, uninfected tissue was left, and 5= completely infected root. However, an arbitrary (0-5) disease index scale based on disease progress, developed by Abd El-Razik (2011), was used to measure the disease severity of *Rhizoctonia* root rot, in which: 0= No visible symptoms; 1= A few small soft lesions on a part of the root system and hypocotyls; 2= Elongated, discoloured lesions spread on the entire root system and hypocotyls; 3= Deep brown necrosis grind the stem, partial root disintegration, yellowing of leaves; 4= Stem canker, root disintegration, yellowing of leaves, stunting; and 5= Collapse and death of plants.

To measure the disease severity of Fusarium wilt after 60 days, the arbitrary (0-5) disease index scale described herein based on disease progress and developed by the author was used, in which 0= No visible symptoms; 1= Light vein-clearing and chlorosis of the leaves; 2= yellowing and wilting of lower leaves and extend to upper leaves; 3= Brown (discoloration) of the vascular systems of tap rot and stem; 4= necrotic streaks on the stem base spread towards the stem apex; and 5= Premature plant death. The following equation was used to calculate percentage of disease severity for each tested isolate.

$$\text{Disease index} = \frac{\sum (\text{Rating No.} \times \text{No. of plants in the rating})}{\text{Total No. of plants} \times \text{highest rating}} \times 100$$

Effect of two types of composts on population density of soil microflora:

To determine total count of soil microflora or activity of soil microflora, core soil samples were taken from potted soil at time of planting (for comparison) and at 30 and 60 days later. They were taken from the top 2 inches of the soil. The weight of each sample was 20 g soil to represent each pot (treatment). The collected soil samples of each replicate were mixed together to form one compost sample which tested for microbial total count in different media, The densities of growing fungi, endospore forming bacteria (*Bacillus*), fluorescent *Pseudomonas* and Actinomycetes were estimated by the standard serial dilution and plotting technique. The following selective media: Martin's medium, (Nitta, 1992); ATCC medium 455, Ronald, 1993 and Starch-nitrate Agar medium ;Waksman, 1962 were used for total counts of fungi, *Bacillus subtilis*, fluorescent *Pseudomonas* and Actinomycetes , respectively, The total count of the microbial colonies developed on the selective media (using dilution plate method according to Dhingra and Sinclair, 1995) at 27-30°C was recorded after 24 hr for bacteria and 4 days for fungi and Actinomycetes. Number of microbes was calculated as CFU/g soil using the following equation:

$$\text{No. of microflora (cfu) per gram soil} = \frac{\text{Average No. of appeared colonies}}{\text{Plate} \times \text{Dilution}^{-1}}$$

Identification of isolated compost microorganisms:

Identification of fungal and Actinomycetes isolates were based on their morphological characters (conidia, conidiophores, hyphae) observed after staining with lactophenol cotton blue (Rifai, 1969; Booth, 1971; Carmichael *et al*., 1980; Domsch *et al*., 1980 and Rehner and Samules, 1994). However, bacterial isolates were identified on the base of colony features on the tested selective media and the physiological tests. (Robert *et al*., 1957).

Antagonism of soil microflora on growth of the tested pathogen in vitro:

In vitro, antagonism of each group of microbes (Fungi, bacteria and Actinomycetes) was tested against growth of the tested pathogenic fungi. Two agar discs, taken from 3 days old cultures of the isolated fungi, or two streaks of a bacterium or Actinomycetes, were placed on the surface of a Petri-dish containing PDA medium, arranged at the two sides of the central disc of the tested pathogenic fungus. Inoculated PDA medium with discs of the tested pathogenic fungi alone served as controls. The diameter of growth of the pathogenic fungi was measured when fungal growth in the control covered completely the surface of the medium at 27-30°C in the. Inhibition percentage for pathogenic fungal growth was calculated, for each antagonistic test (Ferreira *et al*., 1991).

Total microbial activity assessment:

Total microbial activity of CMs samples was estimated by measuring the rate of enzymatic hydrolysis of fluorescein diacetate (FDA) by CMs microorganisms as described by (Adam and Duncan, 2001).Two CMs samples (2 g fresh weight each) from each CMs were incubated in 15 ml of 60 mM potassium phosphate buffer pH 7.6 for exactly 20 minutes at 30°C. Enzymes present in the CMs cleave FDA to produce a yellow-green compound. The colour intensity over the incubation period was compared to known concentrations of cleaved FDA to provide an estimate of the rate of microbial enzymatic activity. Results were represented as micrograms of FDA hydrolyzed per minute per gram dry weight of soil (µg-min-$1g^{-1}$). Higher rates of microbial activity resulted in higher FDA hydrolysis values.

Determination of pathogenesis related-proteins (PR-Proteins) in faba bean plants associated with the suppressive effect of compost to root rot and wilt diseases:

Plant Samples:

Soluble proteins, esterase, peroxidase and chitinase isozymes were determined in extracts of healthy and diseased faba bean plants of Masr 1 cv. (30 or 60 day old) grown in soil artificially infested soil with the incident of root rot and wilt diseases of faba bean, and supplemented with Planta Rich or Rich composts at the rate of 10 gm/kg soil under greenhouse conditions. Plant samples were kept in deep freezer at -80°C until extracted.

Polyacrylamide gel electrophoresis:

Gel preparation:

The electrophoresis was carried out in vertical polyacrylamide gels, using the slab gel apparatus "SE 600, vertical slab gel". Polyacrylamide gel electrophoresis was carried out according to Laemmli (1970) with 7.5% acrylamide for isozymes analysis and 12% acrylamide + 1.0% SDS for protein analysis.

Preparation of samples:

Enzymes and proteins were extracted by crushing 1.0 g of sample tissue (leaves and roots) in 1.0 ml extraction buffer (0.1 M Tris-HCl + 2 mM EDTA, pH 7.8). To avoid denaturation of the enzymes by overheating, the samples were cooled with ice during crushing and preparation. The samples centrifuged for 25 minutes at 10.000 rpm under cooling 4°C. One hundred micro litre of the supernatant was mixed with one hundred micro litre of sample application buffer which prepared as follow: (2.5 mM Tris-HCl pH 6.8 +10.0% glycerol +10.0% glycerol +0.02% bromophenol blue +10% mercaptoethanol). Then, the samples were loaded directly in the electrophoresis for isozymes analysis. Meanwhile, protein samples were incubated in water path at 100°C for five minutes for protein denaturation, then loaded in the electrophoresis.

Sample loading:

100 µl per sample were then loaded directly into the wells with a Micropipette. Due to the presence of glycerol in the sample application buffer, it is higher density than the surrounding upper buffer. Hence, the sample equilibrates at the bottom of the well.

Electrophoresis:

After sample loading, the moulded gel was clamped with the upper buffer tank. The electrode buffer used in the present study consists of 0.025 M Tris, 0.192 M Glycine, 0.1% SDS pH 8.3 and added to the upper and lower tanks. Then, the electric power is turned "ON". A voltage of 100 V is used at the beginning and increased to 200 V when the tracking dye has passed the stacking gel. After 3-4 hours, or until the tracking dye migrated to the bottom of the gel, electrophoresis is stopped. The gel is then carefully removed from the mould and stained for isozymes activity or protein profile.

Enzyme activity staining:

a) Peroxidase (E.C.1.11.1.7):

Reaction: Donor + $H_2O_2 \rightarrow$ Oxidized Donor + $2H_2O$.

Stain: Redox dye, 3, 35 ,5 - tetramethylbenzidine (TMBZ).

Staining solution:

A- Na-acetate (1.0 M)	50 ml (pH 7.4)
Methanol	50 ml
TMBZ	50 mg
B- H_2O_2 (30%)	3%

The gel was incubated in solution "A" in the dark at 30°C for 30 minutes. Then the solution "B" was added and shacked the tray to ensure good staining. The chromatic band appeared in a few minutes (Guikema and Sherman, 1980).

b- Esterase (E.C.3.1.1.2):

Reaction: Aryl acetate+ $H_2O \rightarrow$ Aryl alcohol + Acetate.

Stain: Diazonium system.

Staining solution:

Na-phosphate (0.1M) 100 ml (pH 6.8)α-naphthyl acetate (1.0%) 3 ml (in acetone)
Fast Blue RR salt 100 mg

Incubate the gel under light at 25°C for 30-60 min or until the chromatic bands appear (Tanksley and Orton, 1986).

c- Chitinase (E.C.3.2.1.14):

Chitinase activities on gels were revealed by fluorescent staining using 0.01% (w/v) calcofluor white M2R in 50 mM Tris-HCl (pH 8.9) for 5 min, then washing with distilled water several times and distaining in distilled water for 2 h at room temperature. Gels were visualized under UV light;' bands with lytic activity appeared as dark zones under UV light.

Protein staining and identification:

Proteins were stained 4 hours with Comassie Brilliant Blue (0.025% Comassie Brilliant Blue R-250, 40% methanol and 7% acetic acid). Then, gels were distained for one hour in a mixture of 50% methanol and 10% acetic acid. Thereafter, the gels transferred to a destainer filled with 7% acetic acid and 5% methanol until the background is clear. Data were obtained by scanning densitometer GS 300 (Hoffer) of protein profiles. The molecular weight of protein bands were determined against protein marker (166, 66.2, 45, 35, 25, 18.4 and 14.4 KDa), using g S 365 electrophoresis data system program version 3.01 (Microsoft Windows @ version).

Statistical analysis:

Data were subjected to statistical analysis using analysis of variance and means were compared using L.S.D. test as described by Gomez and Gomez (1984).

Results and Discussion

Effect of soil amendment with CMs on severity of root rot and wilt diseases as well as population densities of soil microflora:

Data in Table (2) indicate that supplemented artificially infested soil with the tested CMs at the rate of 10 gm/kg soil before sowing with Masr1 faba bean cultivar, suppressed significantly severity of Rhizoctonia root rot, Fusarium root rot and Fusarium wilt compared with control (uncomposted soil). In general, Planta Rich CMs caused the highest suppressive effect than Rich. Data also indicate that in general CMs treatments increased population of *Bacillus* spp., Fluorescent Pseudomonas, Actinomycetes and fungi in the soil compared with non-composted soil. Total counts of soil microflora were different. Actinomycetes followed by fungi showed the lowest microbial density in composted and non-composted soil samples compared with bacterial counts. However, in infested and uninfested soil with the pathogen, population densities of Actinomycetes in composted and uncomposted soil was higher than the other tested soil microflora. Also total microbial counts 30 days after planting in soil infested with the incidents of root rot diseases or after 60 days in the soil infested with the causal of wilt disease were high compared with microbial counts in soil at the time of planting. Increase in population densities of soil microflora in soil amended with CMs which reported herein (Table 2) may explain reasons of reduction in disease severity percentages.

Table 2. Effect of soil amendment with peat-moss compost at the rate10 gm/kg soil on severity of root rot and wilt diseases as well as population density of soil microflora under greenhouse conditions

Disease	CMS type	Disease severity	Total count (C.F.U. x 10^5 /g)							
			Bacillus		Pseudomonas		Actinomycetes		Fungi	
			A	B	A	B	A	B	A	B
Rhizoctonia root rot	Planta rich	17.33*	42.00**	87.00	33.67	58.33	140.00	247.67	0.27	2.0
	Rich	28.00	22.00	57.00	24.67	46.33	86.00	224.33	0.27	2.0
	Control	61.33	16.67	38.00	18.33	28.33	58.00	100.67	0.17	1.0
Fusarium root rot	Planta rich	36.00	13.00	32.00	23.67	27.67	35.33	193.67	46.67	54.00
	Rich	40.00	46.00	30.67	11.00	26.33	36.67	83.00	47.67	27.33
	Control	61.33	12.00	10.00	7.67	10.67	19.33	56.00	27.33	17.00
Fusarium wilt	Planta rich	25.67	34.00	88.00	25.00	41.33	39.67	158.33	4.67	5.00
	Rich	32.00	25.67	70.00	15.00	29.67	41.33	122.00	2.00	3.67
	Control	76.00	17.00	42.00	11.00	21.00	22.67	53.69	1.67	2.67
L.S.D. 0.05		12.59	10.91	26.90	3.13	12.40	23.80	45.64	4.45	15.78

* Disease severity % estimated 30 in case of root rot and 60 days after sowing in case of wilt.
** Population density of microflora.
A: At time of sowing.
B: 30 and 60 days after sowing.

This may be due to changes in the overall population of the antagonistic resident soil bacteria and fungi which compete with the pathogens as well as changes in the amount and availability of soil nutrients to plants associated with CMs application as mentioned by Marcos *et al*., 1995; Serra-Wittling *et al*., 1996; Grantstein, 1997; Kim *et al*., 1992; Bess, 1999 and Aryantha and Guest, 2006. Hoitink and Boehm (1999) reported that the beneficial effects induced by composts are due to increase the activities of soil microbes in the plant rhizosphere. Some of them produce plant growth hormones and stimulate plant growth directly; others produce natural chelators called siderophores that keep iron at a high level in available form to plant in soil.

Enumeration of population and activity of compost microbes and testing their antagonistic effect against root rot and wilt causal fungi in vitro:

a- Population and activity of microbes:

Table (3) shows that population of Bacillus, fluorescent Pseudomonas, Actinomycetes and fungi were differed in Planta Rich and Rich CMs. Planta Rich CM has the highest population of such microbes compared with Rich CM, except in case of Bacillus. Planta Rich CM showed also higher microbial activity (determined by FDA hydrolyses method) than Rich CMs.

Table 3. Population density and activity of microbes in Planta Rich and Rich compost

Compost type	Microbial population ($x10^6$ cfu/g dry W)				Microbial activity FDA Hydrolyzed (µg/20 min/g fresh weight)
	Bacillus	Fluorescent Pseudomonas	Actinomycetes	Fungi	
Planta Rich	50	14	49	7	105.0*
Rich	159	4	3	2	65.0

* Average of 3 replications.

b- Evaluation of antagonistic capability of the recovered microbes from Planta Rich and Rich CMS against growth of the pathogenic fungi, in vitro:

Results presented in Tables (4 and 5) reveal that out of 39 isolates of Bacillus, fluorescent Pseudomonas, Actinomycetes and certain fungi belong to the genera *Aspergillus*, *Penicillium*, *Gliocladium*, *Rhizopus*, *Fusarium* and *Acrophiolphora*, 29 isolates proved to be antagonistic to the growth of root rot and wilt fungi, *in vitro*. The antagonistic effect of these isolates to the growth of the pathogens was differed and varied from weak to strong. Suppression of root rot and wilt diseases obtained after soil amendment with Planta Rich and Rich CMs could be due the beneficial effect of such microbes (in single way or in combination with others) on the pathogen and/or the susceptibility or on the host-parasite interaction lead to control of root rot and wilt diseases of faba bean. Such results are supported by previous reports of Craft and Nelson (1996); Serra-Wittling *et al*. (1996); Zhang *et al*. (1998); Bess (1999); Aryantha and Guest (2006); Nyoman and David (2006); Kerkeni *et al*. (2007) and Malandraki *et al*. (2008) who attribute the suppressiveness of different CMs to one or more of the microbial agents of CMs. Disease control obtained with these microbes may be attributed to completion with the pathogen for nutrients, production of antimicrobial substances against pathogens and induction of systemic resistance in plants.

Table 4. *In vitro* evaluation of antagonism of Planta Rich compost (PR) microbes against growth of *R. solani*, *F. subglutinans* and *F. oxysporum* the causal fungi of root rot and wilt diseases of faba bean

Type of microbe	Code	Inhibition (%) of colony diameter		
		R. solani	*F. subglutinans*	*F. oxysporum*
Bacillus spp.	BPR 1	15.6*	40.2	50.0
	BPR 2	21.0	3.9	13.9
	BPR 3	0	0	0
	BPR 4	9.0	6.95	37.4
	BPR 5	38.0	29.8	34.0
	BPR 6	32.0	33.6	37.5
Fluorescent *Pseudomonas*	FPR 1	9.8	11.4	19.4
	FPR 2	0	0	0
	FPR 3	11.9	16.7	29.4
Actinomycetes	APR 1	41.9	52.5	25.5
	APR 2	6.4	21.8	21.8
	APR 3	22.8	18.2	27.3
	APR 4	0	0	0
	APR 5	17.8	3.7	34.5
Fungi:				
Aspergillus flavus	As PR 1	48.7	52.8	75.0
Aspergillus niger	As PR 2	60.0	84.3	83.9
Aspergillus sydowii	As PR 3	46.0	31.6	60.7
Penicillium sp.	Pen PR 1	23.0	75.0	58.0
Rhizopus nigricans	R PR 1	17.6	13.4	14.0

* Average of 3 replications compared with control.

Table 5. *In vitro* evaluation of antagonism of Rich compost (R) microbes against growth of *R. solani*, *F. subglutinans* and *F. oxysporum*

Type of microbe	Code	Inhibition (%) of colony diameter		
		R. solani	*F. subglutinans*	*F. oxysporum*
Bacillus spp.	BR 1	0*	0	0
	BR 2	39.9	14.5	37.0
	BR 3	24.0	12.5	17.7
	BR 4	0	0	0
	BR 5	17.5	36.7	27.0
	BR 6	11.9	11.2	33.1
	BR 7	11.9	14.4	19.2
Fluorescent *Pseudomonas*	FR 1	32.9	13.3	14.7
	FR 2	11.0	15.3	5.0
	FR 3	14.6	12.8	12.0
	FR 4	0	0	0
Actinomycetes	AR 1	6.1	14.5	20.0
	AR 2	0	0	0
	AR 3	0	0	0
	AR 4	12.8	12.7	9.1
	AR 5	0	0	0
	AR 6	37.8	16.4	21.8
Fungi:				
Fusarium spp.	Fu.R 1	24.0	36.0	38.0
Gliocladium sp.	GR 1	23.7	35.3	51.0
Acrophialophora	AcR 1	0	0	0

* Average of 3 replications compared with control.

Effect of suppressive CMs on pathogenesis-related proteins, and activity of chitinase, peroxidase and estrase in faba bean plants grown in composted and non-composted soil infested with the causal fungi:

Electrophoretic analysis of extracts of healthy and infected faba bean plants of Masr 1 cv. by root rot and wilt fungi grown in infested soil with the tested pathogens and supplemented with the suppressive CMs (Planta Rich or Rich) at the rate of 10 gm/kg soil was done to determine pathogenesis-related proteins associated with the suppressive effect of CMs. Results (Figs.1-4) show that nineteen protein bands were detected with molecular weight (MW) ranged from 16.3 to 67.5 KDa. Protein bands No. 5 and 7 with MW 44 and 42.3 KDa, respectively, were induced by Rich CM treatment, however, Planta Rich CM treatment induced band No. 7 only. Induction of protein band No. 13 (MW 23.3 KDa) was only associated with fungal infection in composted soil. Also, six chitinase bands (Chit-2, Chit-3, Chit-4, Chit-5, Chit-7 and Chit-8) were detected in healthy and diseased plants grown in composted soil. However, Chit-1 and Chit-6 isoforms were induced in composted soil infested with the tested fungi. Six peroxidase bands (PRX-1, PRX-2, PRX-3, PRX-4, PRX-6 and PRX-7) were detected in extracts of healthy and diseased faba bean plants grown in soil supplemented or not supplemented with suppressive CMS. However, PRX-5 isoform was induced only in diseased plants grown in composted soil. Seven esterase bands (ESt-1, ESt-2, ESt-3, ESt-5, ESt-6, ESt-7 and ESt-8) were detected in healthy and diseased plants grown in composted soil. The ESt-4 isoform was detected only in diseased plants grown in soil treated with Rich CM.

Production of PRs including oxidative and hydrolytic enzymes during attack by fungal pathogens is generally considered to be part of a non-specific defence response initiated in plants after pathogen attack, but also a consequence of various physical, chemical and environmental stresses. However, the induction of new proteins and enzymes isoforms associated with infection or suppressive CMS treatments as reported herein appears to be a specific response which confirm previous reports (Deising *et al*., 1991; Bull *et al*., 1992; Kim *et al*., 1992; Niderman *et al*., 1995; Zhang *et al*., 1998; Huang and Bakhouse, 2006; Kavroulakis *et al*., 2006; and Radhajeyalakshmi *et al*., 2009). The results suggests that soluble proteins with molecular weight 44, 42.3 and 23.3 KDa, chitinase isozyme-1 and 6, peroxidase isozyme-5 and esterase isozyme-4 in faba bean plant extracts might be responsible for a defence against root rot and wilt pathogens in faba bean plant. Therefore, induction of such proteins in faba bean plants could possibly be a factor in the mechanism of CMs suppressiveness to the tested diseases and could be used as a protein marker to identify the tolerant lines of faba bean to root rot and wilt diseases.

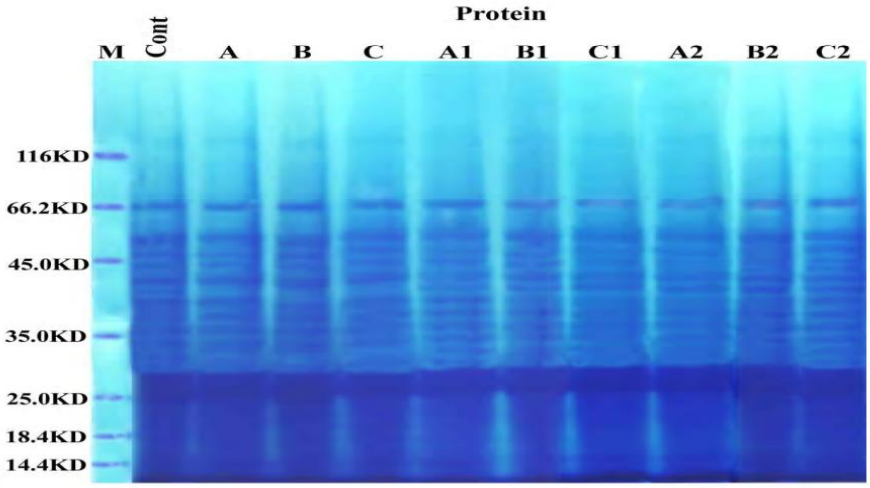

Fig. 1. Electrophoretic pattern of soluble proteins detected in extracts of healthy (control) and diseased faba bean plants grown in soil infested with the pathogens of Fusarium root rot (A), Rhizoctonia root rot (B) and Fusarium wilt (C) or in infested soil with Planta Rich CM (A1, B1 and C1) or Rich CM (A2, B2 and C2).

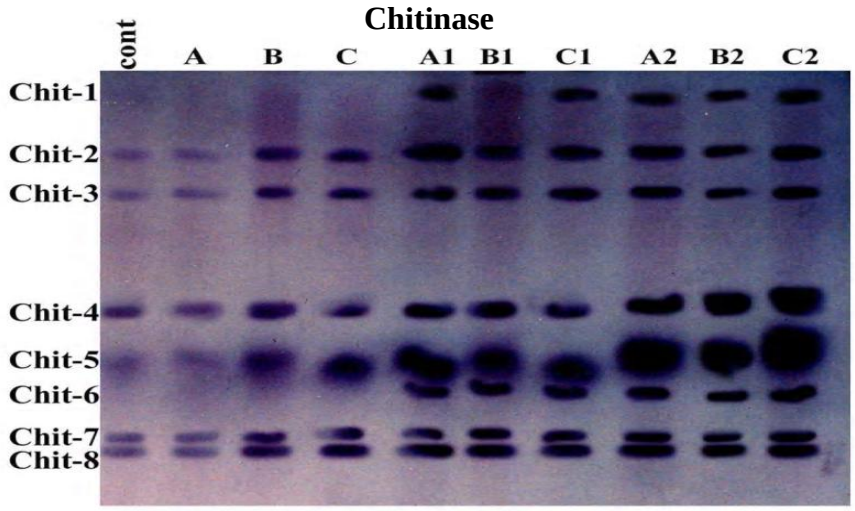

Fig. 2. Electrophoretic pattern of Chitinase isozymes detected in extracts of healthy (control) and diseased faba bean plants grown in soil infested with the pathogens of Fusarium root rot (A), Rhizoctonia root rot (B) and Fusarium wilt (C) or in infested soil composted with Planta Rich CM (A1, B1 and C1) or Rich CM (A2, B2 and C2).

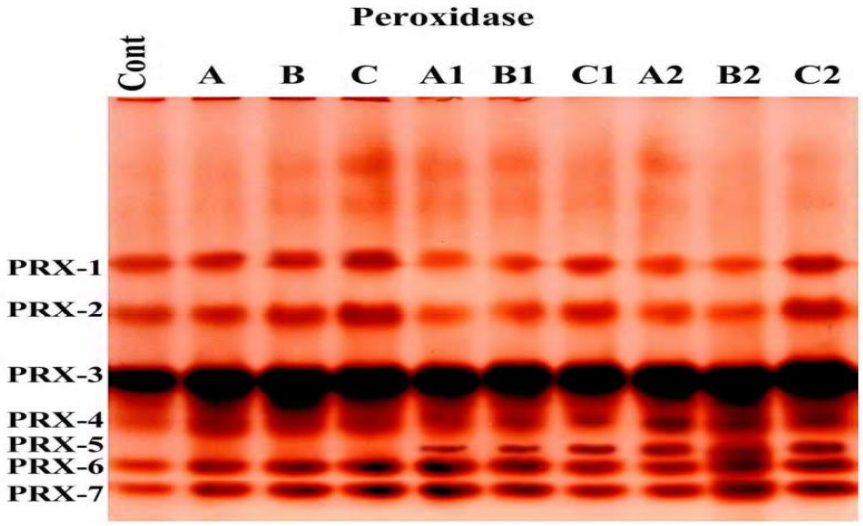

Fig. 3. Electrophoretic pattern of Peroxidase isozymes detected in extracts of healthy (control) and diseased faba bean plants grown in soil infested with the pathogens of Fusarium root rot (A), Rhizoctonia root rot (B) and Fusarium wilt (C) or in infested soil composted with Planta Rich CM (A1, B1 and C1) or Rich CM (A2, B2 and C2).

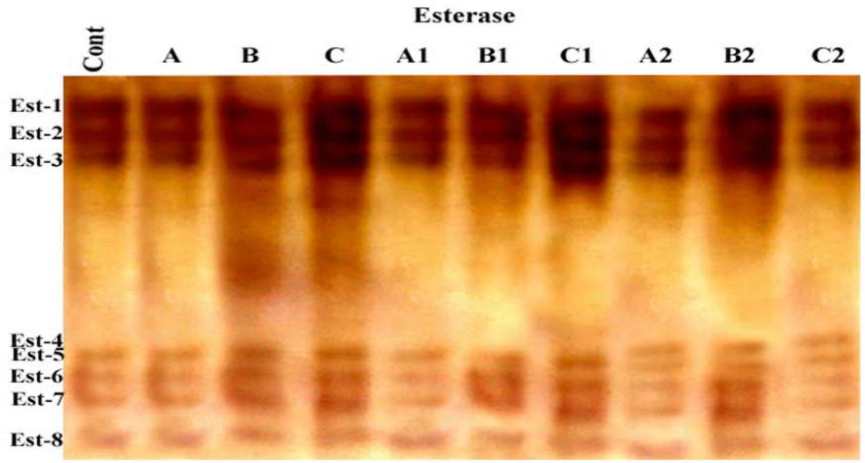

Fig. 4. Electrophoretic pattern of Esterase isozymes detected in extracts of healthy (control) and diseased faba bean plants grown in soil infested with the pathogens of Fusarium root rot (A), Rhizoctonia root rot (B) and Fusarium wilt (C) or in infested soil composted with Planta Rich CM (A1, B1 and C1) or Rich CM (A2, B2 and C2).

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(Received 12/02/2012;
in revised form 19/03/2012)

التأثير المثبط لبعض أنواع الكومبوست على مرضى عفن الجذور والذبول فى الفول

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أجرى هذا البحث بهدف دراسة التأثير المثبط لبعض أنواع الكومبوست (بلانتا ريتش وريتش) على مرضى عفن الجذور والذبول فى الفول وكذلك دراسة ميكانيكية هذا التأثير تحت ظروف الصوبة.

تمت دراسة تأثير معاملة التربة بمعدل ١٠ جم/ كجم تربه من نوعى الكومبوست بلانتا ريتش وريتش على حدوث مرض عفن الجذور المتسبب عن *Rhizoctonia solani* و *Fusarium. subglutinans* و مرض الذبول المتسبب عن *Fusarium oxysporum* وقد أدت هذه المعاملة الى تقليل كبير فى شدة المرض بالمقارنة بالنباتات التى لم تعامل التربة المنزرعة بها (كونترول) ولقد وجد أن هناك زيادة كبيرة فى أعداد بعض الكائنات الدقيقة مثل *Bacillus subtilis* و *Pseudomonas fluorescens* و Actinomycetes وبعض الفطريات فى التربة المعاملة بنوعى الكمبوست وكان أعلى معدل زيادة للأكتينوميستيات ثم البكتريا الاخرى وكانت الفطريات اقلها عدداً . وعند عزل هذه الكائنات واختبار تضادها مع الفطريات الممرضة وجد أنها تضاد الفطريات الممرضة .

أجرى تحليل الفرد الكهربائى لمستخلصات النباتات المصابة والنباتات السليمة النامية فى تربة معاملة بالكومبوست لتقدير البروتينات التى لها علاقة بالمرض وكذلك الايزوزيم والايستريز وتبين وجود بروتين جديد وزنه الجزيئى ٢٣,٣ كيلو دالتون واثنان من الشيتينيز (Chit l, Chit 6) وبيروكسيديز واحد PRX-5 وكذلك استيريز واحد (Est-4) ايزوفورم ومن هذه النتائج فان التأثير المثبط للكومبوست قد يرجع الى التأثير المضاد للكائنات التى يحتوى عليها الكومبوست أو أنه يرجع الى استحثاث المقاومة بإنتاج بروتينات جديدة أو إنزيمات .