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ORIGINAL PAPER

Influence of Some *Trichoderma* spp. in Combination with Compost and Resistance Inducing Chemicals Against Pea Damping-Off and Root-Rot Diseases

Attia, A.M.F.*[1]; Youssef, M.M.[1]; El-Sayed, S.A.[2] El-Fiki, I.A.I.[3]
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ABSTRACT

Trichoderma bioagents, *i.e.*, *Trichoderma hamatum*, *T. harzianum* and *T. viride* were isolated from the rhizospheric soil of the healthy pea plants. They were used in combination with the resistance inducing chemicals, Bion and compost for studying their impact on managing pea damping-off and root-rot diseases caused by *Macrophomina phaseolina* (Tassi) Goid (the pycnidial stage of *S. bataticola* Taub.) and *Rhizoctonia solani* Kuhn (the anamorph stage of *Thanatephorus cucumeris* (Frank) Donk.).
The inhibitory effect of the three bioagents as well as compost and resistance inducing chemicals (RICs), Bion, chitosan and salicylic acid was investigated to determine their inhibitory effect against each of *M. phaseolina* and *R. solani in vitro* and *in vivo*.
Data demonstrated significant inhibition to the linear growth of both *M. phaseolina* and *R. solani* due to using the tested bioagents, compost and the RICs compared to control treatment. This reduction was gradually increased by increasing the incorporated concentration to PDA medium. In addition, RICs were less efficient in this regard than *Trichoderma* spp. and compost.
The obtained data showed that soaking pea seeds in 50 mM Bion for six hours then pelleting with the inoculum of *Trichoderma* spp. and sown in soil amended with compost resulted in significant reduction to the incidence of damping-off and root-rot severity caused by each of the tested fungi with significant increase to the produced green pods yield, phenolic compounds, % nitrogen and % protein content compared with control treatment. This research provides alternative and safe compounds to management damping-off and root-rot diseases and consequently reduces environmental pollution as well as improving plant growth and seed yield of pea plants.

Keywords: Pea, *Pisum sativum*, damping-off, root-rot, bioagents, Compost, resistance inducing chemicals, phenolic compounds, nitrogen, protein content.

***Correspondence: Attia, A.M.F.**
amany.farouk@agr.cu.edu.eg
Amany M.F. Attia
https://orcid.org/0000-0002-0075-3438
Maryan M. Youssef,
https://orcid.org/0000-0002-1944-669X
1- Plant Pathology Department, Faculty of Agriculture, Cairo University, 12612 Giza, Egypt.
Sahar A. El-Sayed
https://orcid.org/0000-0002-2230-8087
2- Plant Pathology Research Institute, Agricultural Research Center, 12619, Giza, Egypt.
Ibrahim A.I. El-Fiki
3- Plant Pathology Department, Faculty of Agriculture, Benha University, Egypt.

INTRODUCTION

Pea (*Pisum sativum* L.) is economically an important legume crop in Egypt either for exportation or local consumption. Pea is considered as a rich source of vitamins, carbohydrates, protein and other compounds. Moreover, it enriches the soil fertility via nitrogen fixation.

Pea is subjected to different pathogens causing many fungal, bacterial, viral and nematode diseases additional the physiological disorders. Fungal diseases, damping-off and root-rot diseases, are considered the major deleterious diseases to the yield (Kraft and Pfleger, 2001; Muhanna *et al*., 2018 and Ketta *et al*., 2021).

Recently, many reports were received from pea growers for damping-off and root-rot symptoms during warm conditions each year, where most of these growers re-sown pea after pea due to the economic importance of this crop. Therefore, this work was achieved to find a practical safe method to manage damping-off and root-rot diseases of pea.

Management of plant disease represents a major concern facing the farmers in the cropping systems management. Both *Rhizoctonia solani* and *Macrophomina phaseolina* are soil-borne fungi causing pea damping off and root-rot diseases which reduce pea quality and the yields.

Taking into account, the growing population demands, the production of food must rise each year to provide enough food for the seasonal increase in the people. Agricultural sector is a key in which commerce, beverages and food are considered it as raw materials main supplier.

Increasing outputs with limited resources is the main challenge. Fungal diseases considerable major causes of decreasing agricultural crops yield worldwide (Makovitzki *et al.* 2007). Plant pathogenic fungi management is mainly achieved using synthetic fungicides. Even so, inappropriate massive use of the synthetic fungicides on plants resulted in severe detrimental effects on multi-levels. On contrary, plant pathogens have evolved resistance to the fungicides (Rossall, 2012). Thus, new formulations for controlling plant diseases perform a real demand nowadays context of sustainable development in both agriculture and ecology area. Several secondary metabolites *i.e*., terpenoids, flavonoids, tannins and alkaloids are produced by plants (Butu *et al.*, 2014) and serve as important sources of biologically active molecules showing antifungal, antibacterial (Negi, 2012) and antioxidant properties (Butu *et al.*, 2014). In recent times, traditional fungicide, chemical treatments with the integrated disease management used for controlling *Macrophomina phaseolina* and *R. solani* assume to be not completely effective. Hence the diseases remain a persistent concern for the farmers to deal with (Muhanna *et al*., 2018 and Ketta *et al*., 2021).

Systemic resistance induction is a new approach for plant protection that is much less harmful to the environment and the plant yield compared to deadly agrochemicals used to control plant diseases (Yan *et al.*, 2003; Carrion *et al.*, 2019 and Ketta *et al*., 2021).

This research aimed to assess the efficacy of some Trichoderma strains, compost, and resistance inducing chemicals on both *R. solani* and *M. phaseolina in vitro* and *in vivo*. Also, estimation of total phenolic compounds in pea roots as well as determination the percentages of nitrogen and the protein content in the dry pea seeds were estimated in Trichoderma, compost, RICs treatments and pathogen-treated pea plants.

MATERIALS AND METHODS

2.1. Source of the Causal Fungi:

Virulent isolates of *Macrophomina phaseolina* (Tassi) Goid (the pycnidal stage of *Sclerotium bataticola* Taub.) and *Rhizoctonia solani* Kuhn. (The anamorph of *Thanatephorus cucumeris* (Frank) Donk to pea roots were kindly provided by Prof. Dr. Abada, K.A. Prof. of Plant Pathol., Plant Pathol. Dept., Fac. Agric., Cairo Univ., Egypt.

2.2. Isolation, Purification, and Identification of the Trichoderma Antagonists:

Soil samples collected from the rhizosphere of pea plants with vigorous plant growth were used to isolate bioagents belonging to *Trichoderma* spp. A serial dilution plate technique was used to isolate *Trichoderma* spp. on the soil extract medium (Oedjijono and Dragar, 1993).

The isolated *Trichoderma* spp. fungal cultures purified and identified based on their cultural and microscopic morphological characteristics (Rifia, 1969 and Bissett, 1991).

2.3. Effect of the Isolated Bioagents and the Resistance Inducing Chemicals (RICs) and Compost on the Linear Growth of the Two Pathogenic Fungi *in Vitro*:

We have grown *Trichoderma* spp. in gliotoxin fermentation medium (GFM) as represented by Brain and Hemming (1945) for 14 days, we used 0.25µm syringe filter to sterilize the filtrate.

The calculated amounts of the culture filtrate of each Trichoderma bioagent were mixed each alone with PDA medium after sterilization, just before solidification, to obtain final concentrations of 40, 60 and 80 %. The medium was then poured into the Petri-dishes (20 ml/plate).

The concentrations, 30, 40 and 50 mM of the RICs, *i.e.,* Bion (benzothiadiazole; BTH), salicylic acid (monohydroxy benzoic acid) and chitosan (cellulose with the hydroxyl at position C_2 substituted with an acetamido group) were ready based on their molecular weight. The calculated sterilized amounts of the RICS were mixed each alone with sterilized PDA medium, just before solidification. The medium was then poured into the Petri-dishes (20 ml/plate).

Two kg plant compost were soaked overnight in five liters water in a plastic container then filtrated through two layers of cheesecloth and collected in a plastic container. Compost tea filtrate was sterilized using a 0.25µm syringe filter. The sterilized filtrated compost tea was mixed with sterilized PDA medium just before solidification in different proportions, *i.e.*, 40, 60 and 80 %. The medium plus compost tea filtrate was poured into sterilized Petri-dishes (20 ml/plate).

After solidification the aforementioned treatments, Petri-dishes were inoculated with 5 mm. discs of any of the two tested pathogens cut from five days old culture under aseptic conditions. Plates were then incubated in an incubator at 28±2°C. Five replications were prepared for each treatment. The growth of the tested fungi was measured, and the average was recorded.

2.4. Effect of *T. viride*, Bion and Compost, Each Alone and in Different Combinations on Damping-Off and Root-Rot Severity and Some Crop Parameters:

This experiment was carried out in the greenhouse at Fac. Agric., Cairo Univ. Pea seeds (cv. Hendi) were surface sterilized with 1.0% solution of sodium hypochlorite for 1 min and then thoroughly washed with sterilized water. The growth of *T. viride* in liquid gliotoxin fermentation medium (GFM) (Brain and Hemming, 1945) was filtered through Whatman filter paper (No.1), mixed with sterilized talc powder at the rate of 1:1 (v/w), then dried at 25±1ºC and kept in a refrigerator at 10 ºC until usage. Formalin sterilized sandy clay soil was divided into twenty treatments (Table, 3) as follows:

- Pea seeds mixed with the inoculum of the tested bioagent *T. viride* (5g/kg. seeds), planted in pots contained sterilized soil.
- Pea seeds soaked for six hours in the RIC Bion (50 mM), planted in pots contained sterilized soil.
- Pea seeds were planted in pots contained sterilized soil + 50 g. compost/ pot.
- Pea seeds mixed with the inoculum of the tested bioagent, *T. viride* (5g/kg. seeds), planted in soil infested with 2% inoculum level of *M. phaseolina*.
- Pea seeds mixed with the inoculum of the tested bioagent *T. viride* (5g/kg. seeds), planted in soil infested with 2% inoculum level of *R. solani*.
- Pea seeds soaked for six hours in the RIC Bion (50 mM), planted in soil infested with 2% inoculum level of *M. phaseolina*.
- Pea seeds were soaked for six hours in the RICs Bion and planted in soil infested with 2% inoculum level of *R. solani*.
- Pea seeds planted in soil infested with 2% inoculum level of *M. phaseolina* and amended with 50 g. compost/pot.
- Pea seeds planted in soil infested with 2% inoculum level of *R. solani* and amended with 50 g. compost/pot.
- Pea seeds soaked for six hours in the RIC Bion (50 mM) and treated with the inoculum of the tested bioagent, *T. viride* (5g/kg. seeds) planted in soil infested with 2% inoculum level of *M. phaseolina*.
- Pea seeds soaked for six hours in the IRC Bion (50 mM) and treated with the inoculums of the tested bioagent, *T. viride* (5g/kg. seeds) planted in soil infested with 2% inoculum level of *R. solani*.
- Pea seeds mixed with the inoculum of the tested bioagent *T. viride* (5g/kg. seeds), planted in soil infested with 2% inoculum level of *M. phaseolina* and amended with 50 g. compost/pot.
- Pea seeds mixed with the inoculum of the tested bioagent *T. viride* (5g/kg. seeds), planted in soil infested with 2% inoculum level of *R. solani* and amended with 50 g. compost/pot.
- Pea seeds soaked for six hours in the RIC Bion (50 mM), planted in soil infested with 2% inoculum level of *M. phaseolina* amended with 50 g. compost/pot.
- Pea seeds soaked for six hours in the RIC Bion (50 mM), planted in soil infested with 2% inoculum level of *R. solani* amended with 50 g. compost/pot.
- Pea seeds soaked for six hours in the RIC Bion (50 mM) and treated with the inoculum of the tested bioagent, *T. viride* (5g/kg. seeds) planted in soil infested with 2% inoculum level of *M. phaseolina* amended with 50 g. compost/pot.
- Pea seeds soaked for six hours in the RIC Bion (50 mM) and treated with the inoculum of the tested bioagent, *T. viride* (5g/kg. seeds) planted in soil infested with 2% inoculum level of *R. solani* amended with 50 g. compost/pot.
- Pea seeds planted in soil infested with 2% inoculum level of *M. phaseolina.*
- Pea seeds planted in soil infested with 2% inoculum level of *R. solani.*
- Pea seeds planted in un-infested soil.

Five seeds were planted in each pot and eight pots were used for each treatment, where disease severity was assessed in three randomized pots 75 days after sowing to assess the severity of the disease and the remained five pots of each treatment were left to the end of the experiment to estimate the yield of the green pod yield.

The incidence of pre-and post-emergence damping off as well as root-rot severity were calculated 10, 21 and 75 days after sowing, respectively. Also, plant height (cm), the number and weight of pods (g)/plant were recorded at the end of the experiment (100 days).

2.5. Disease assessment:

We have calculated the occurrence of pre- and post-emergence damping-off 10 and 21 days after planting. Also, root-rot severity was assessed 75 days after sowing, the plants of each pot were carefully uprooted, washed in water and the roots were measured, disease severity was estimated using the devised scale (0-5) adopted by Salt (1982) and then disease severity percentage was estimated using the following formula:

Disease severity % = ∑ (n × v) / 5N × 100

Where:

n = Number of infected roots in each category.

v = Numerical values of each category.

N = Total number of the infected roots.

Biochemical changes associated with the experimental treatments, *i.e.*, Trichoderma-bioagents- Bion- Compost and pathogen-treated pea plants:

Estimation of the content of total phenolic compounds:

One gram of pea roots representing each treatment was used, the phenolic compounds amount was expressed as mg gallic acid per g plant material according to the method described by Zieslin and Ben-Zaken, (1993).

Determination the percentages of Nitrogen and protein content of pea seeds:

A random samples of pea pods were taken from the yield of each treatment. The percentage of nitrogen in the seeds was calculated with reference to the method represented by Hafez and Mikkelsen (1981). In addition, protein percentage was calculated by multiplying nitrogen content by 6.25.

Statistical analysis:

We have used the standard technique for complete randomize block, split and split - split designs as mentioned by Snedecor and Cochran (1989) in analyzing the data. The averages were compared at 5% level using the least significant differences (L.S.D) according to Fisher (1948).

RESULTS

Isolation, purification, and identification of Trichoderma antagonists:

Isolation trials from the rhizospheric soil of healthy pea plants yielded many fungal isolates. Isolates belonging to the genus *Trichoderma* were selected, purified with hyphal tip technique, and identified as *Trichoderma hamatum* Bonord., *T. harzianum* Rifai and *T. viride* Pers.

Effect of the culture filtrates of three Trichoderma strains, the inducer resistance inducing chemicals (RICs) and compost on the linear growth of the two pathogenic fungi *in Vitro*:

Effect of culture filtrates of three Trichoderma strains on the linear growth of both pathogenic fungi:

Data shown in Table (1) indicate that culture filtrates of the three tested bioagents, *i.e.*, *T. hamatum, T. harzianum* and *T. viride* resulted in significant inhibitory effect to the linear growth of both *M. phaseolina* and *R. solani* compared with control treatment. This inhibitory effect was gradually increased by increasing the incorporated concentration to PDA medium, being 71.0, 34.8 and 13.2 mm. linear growth, on the average, at the concentrations of 40, 60 and 80%, respectively. Meanwhile, *R. solani* was, to somewhat, affected by the tested bioagents more than *M. phaseolina,* being 38.9 and 40.4 mm. linear growth, on the average, respectively. In addition, *T. viride* was the most efficient bioagent in this regard followed by *T. harzianum* then *T. hamatum,* being 30.2, 37.7 and 52.7 mm. linear growth, on the average, respectively. Furthermore, the linear growth of the two tested pathogens was completely inhibited by *T. viride* at the concentration of 80 %. Therefore, *T. viride* was chosen to test its capability to the biological control of the tested two pathogens under greenhouse conditions.

Effect of three RICs on the linear growth of both pathogenic fungi:

Tabulated data (Table 2) indicate that the resistance inducing chemicals (RICs), *i.e.,* Bion, chitosan and salicylic acid caused significant inhibitory effect on the linear growth of both pathogenic fungi compared to control treatment. This inhibitory effect was gradually increased by increasing the incorporated concentration to PDA medium, being 72.5, 46.7, and 10.3 mm. linear growth, on the average, at concentrations of 30, 40 and 50%, respectively. *R. solani* was, to somewhat, affected by the tested RICs more than *M. phaseolina,* being 42.4 and 43.8 mm. linear growth, on the average, respectively. In addition, Bion was the most efficient one in this regard followed by salicylic acid then chitosan, being 36.4, 44.3 and 48.8 mm. linear growth, on the average, respectively. Furthermore, Bion at the concentration of 50 %, completely inhibited the linear growth of the two tested pathogens therefore, Bion was chosen to test its capability to management of the tested two pathogens under greenhouse conditions.

Effect of compost tea on the linear growth of both pathogenic fungi:

Results presented in Table (3) reveal that compost tea resulted in significant inhibitory effect on the linear growth of both pathogenic fungi compared with control treatment. This inhibitory effect was gradually increased by increasing the incorporated concentration to PDA medium, being 64.4, 41.5 and 0.0 mm. linear growth, on the average, at the concentration of 40, 60 and 80%, respectively. Also, the fungus *R. solani* was significantly affected by compost tea more than the fungus *M. phaseolina*, being 34.5 and 36.0 mm. linear growth, on the average, respectively.

Table (1): Effect of culture filtrates of three *Trichoderma* strains on the linear growth of *M. phaseolina* and *R. solani*, five days after incubation at 28 ±2°C.

Trichoderma strains	The pathogenic fungi	Average linear growth (mm) at concentrations of (%)				
		40	60	80	Mean	General mean
T. hamautm	*M. phaseolina*	78.8	49.8	27.2	51.9	52.7
	R. solani	77.2	47.0	26.8	53.3	
T. harzianum	*M. phaseolina*	70.6	32.4	14.0	39.0	37.7
	R. solani	67.4	30.2	11.2	36.3	
T. viride	*M. phaseolina*	66.2	25.0	0.0	30.3	30.2
	R. solani	65.4	24.6	0.0	30.0	
Control*	*M. phaseolina*	90.0	90.0	90.0	90.0	90.0
	R. solani	90.0	90.0	90.0	90.0	
Mean	*M. phaseolina*	71.9	35.7	13.7	40.4	-
	R. solani	70.0	33.9	12.7	38.9	
General mean		71.0	34.8	13.2	-	-
L.S.D at 0.05		Trichoderma strains (T) = 3.9, Concentrations (C) = 2.8, Fungi (F) = n.s, T × C = 3.0, T × F = 2.2, C × F = 1.9, T × C × F = 3.7.				

*Not included in the mean.

Table (2): Effect of three RICs on the linear growth of *M. phaseolina* and *R. solani*, five days after incubation at 28 ±2°C.

Resistance inducing Chemicals (RICs)	The pathogenic Fungi	Average linear growth (mm) at concentrations of (mM)				
		30	40	50	Mean	General Mean
Bion	*M. phaseolina*	68.6	41.8	0.0	36.8	36.4
	R. solani	67.2	40.4	0.0	35.9	
Chitosan	*M. phaseolina*	78.6	52.0	18.0	49.5	48.8
	R. solani	76.4	51.2	16.6	48.1	
Salicylic acid	*M. phaseolina*	73.2	48.0	14.0	45.1	44.3
	R. solani	70.8	46.6	12.8	43.4	
Control*	*M. phaseolina*	90.0	90.0	90.0	90.0	90.0
	R. solani	90.0	90.0	90.0	90.0	
Mean	*M. phaseolina*	73.5	47.3	10.7	43.8	-
	R. solani	71.5	46.0	9.8	42.4	
General men		72.5	46.7	10.3	-	-
L.S.D at 0.05		RICs (I) = 3.1, Concentrations(C) = 3.0, Fungi (F) = n.s, I × C = 3.1, I × F = 2.0, C × F = 1.6, I × C × F = 3.4.				

*Not included in the mean.

Effect of *T. viride*, *Bion* and compost, each alone and in different combinations on the incidence of damping-off and root-rot severity as well as some crop parameters:

Effect of *T. viride*, Bion and compost on the infection by damping-off and root-rot severity:

Data illustrated in Table (4) and Figure (1) show the effect of *T. viride*, Bion and compost on the infection by damping-off and root-rot severity. The obtained results reveal that there was significant decrease in both damping-off and root-rot severity due to these treatments. In addition, no damping-off was recorded when the three items, *i.e.*, *T. viride*, Bion and compost were combined and used as treatments to the infested soil with any of both pathogenic fungi. Also, the lowest percentages of root-rot severity were occurred when the three items were combined and used as treatments to the infested soil with any of both pathogenic fungi, being 2.0 and 2.1% for *M. phaseolina* and *R. solani*, respectively. However, significant reduction was recorded when two items from these treatments were combined and used as treatments to the infested soil with any of both pathogenic fungi. Soil without any treatment and infested with any of *M. phaseolina* and *R. solani* recorded the highest percentages of damping-off and root-rot severity, being 44.0 and 52.0% damping-off and 46.8 and 47.8% for root-rot severity, respectively. No. apparent symptoms of damping-off or root-rot were seen in case of control plants (uninfested soil).

Effect of *T. viride*, Bion and compost on some crop parameters:

Data presented in Table (5) illustrate the effect of *T. viride*, Bion and compost on some crop parameters, plant height, number and weight of pods/ plant in the presence or absence of the tested fungi. The obtained results show that there was significant increase in the crop parameters of the plants due to treatment by the tested three treatments and sown in infested soil with any of the two pathogenic fungi. This increase was gained when the plants were raised from seeds treated with *T. viride*, Bion and compost and planted in infested soil with any of the tested fungi.

Also, considerable increase was occurred when one or two of the three items were used as treatment to the soil infested with any of both pathogenic fungi. Soil without any treatment and infested with any of *M. phaseolina* and *R. solani* recorded the shortest plants and the lowest values of pods number and weight of green pods(g)/ plant, being 30.2 cm., 6.2 pod and 20.1 g. for *M. phaseolina* and 30.0 cm., 6.0 pod and 20 g./plant for *R. solani*, respectively. Pea plants sown in un-infested soil (control) recorded the highest figures of crop parameters, being 65.0 cm., 18.8 pod and 72.8 g. for plant height, pods number /plant and pod average weight/plant, respectively.

Table (3): Effect of compost tea on the linear growth of the two pathogenic fungi, five days after incubation at 28±2°C.

Conc. (%)	Linear growth (mm)		Mean
	M. phaseolina	*R. solani*	
40	65.8	63.0	64.4
60	42.4	40.6	41.5
80	0.0	0.0	0.0
Control *	90.0	90.0	90.0
Mean	36.0	34.5	-----
L.S.D at 0.05	Concentrations (C) = 2.8, Fungi (F) = 1.1, C × F = 3.8		

*Not included in the mean.

Table (4): Effect of *T. viride*, Bion and compost and their combinations on damping-off and root-rot severity caused by each of *M. phaseolina* and *R. solani*, greenhouse experiment.

Treatments	% Damping-off		Total	% Root-rot severity
	Pre-emergence	Post-emergence		
T. viride (TV)	0.0	0.0	0.0	0.0
Bion (B)	0.0	0.0	0.0	0.0
Compost (C)	0.0	0.0	0.0	0.0
TV + *M. phaseolina*	12.0	8.0	20.0	10.8
TV + *R. solani*	12.0	8.0	20.0	11.0
B + *M. phaseolina*	20.0	4.0	24.0	13.2
B + *R. solani*	20.0	4.0	24.0	13.2
C + *M. phaseolina*	16.0	4.0	20.0	11.5
C + *R. solani*	8.0	8.0	16.0	9.9
TV + B + *M. phaseolina*	8.0	8.0	16.0	10.8
TV + B + *R. solani*	4.0	4.0	8.0	7.0
TV + C + *M. phaseolina*	4.0	4.0	8.0	7.1
TV + C + *R. solani*	4.0	4.0	8.0	7.8
B + C + *M. phaseolina*	4.0	4.0	8.0	7.0
B + C + *R. solani*	4.0	4.0	8.0	7.7
TV + B + C + *M. phaseolina*	0.0	0.0	0.0	2.0
TV + B + C + *R. solani*	0.0	0.0	0.0	2.1
M. phaseolina	24.0	20.0	44.0	46.8
R. solani	28.0	24.0	52.0	47.8
Control (Uninfested soil)	0.0	0.0	0.0	0.0
L.S.D. at 0.05	2.3	2.5	2.6	3.5

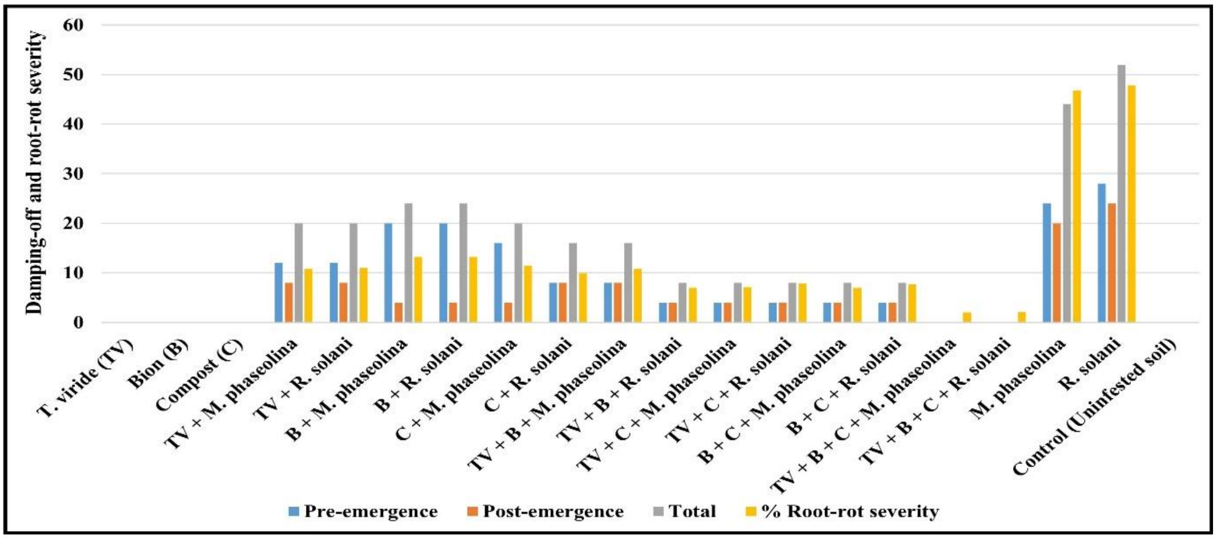

Figure (1). Effect of *T. viride*, Bion and compost and their combinations on damping-off and root-rot severity caused by each of *M. phaseolina* and *R. solani*, greenhouse experiment.

Table (5): Effect of the bioagent *T. viride* and the RIC Bion, each alone or in different combinations, on plant height and the produced green pod yield / plant, greenhouse experiment.

Treatments	Plant height (cm)	Average No. of pods /plant	Pods average weight(g)/plant
T. viride (TV)	55.8	20.2	85.6
Bion (B)	57.3	21.6	84.0
Compost (C)	59.9	24.0	86.1
TV + *M. phaseolina*	50.1	14.0	56.9
TV + *R. solani*	50.0	13.4	55.4
B + *M. phaseolina*	49.7	14.8	57.2
B + *R. solani*	50.0	15.0	58.0
C + *M. phaseolina*	48.0	14.0	56.5
C + *R. solani*	53.6	16.0	62.0
TV + B + *M. phaseolina*	53.5	16.0	62.0
TV + B + *R. solani*	55.8	16.4	62.4
TV + C + *M. phaseolina*	56.0	16.0	62.1
TV + C + *R. solani*	58.9	17.2	63.8
B + C + *M. phaseolina*	58.0	17.0	63.5
B + C + *R. solani*	60.0	17.0	63.2
TV + B +C+*M. phaseolina*	60.0	17.0	63.5
TV + B + C + *R. solani*	64.6	18.4	68.4
M. phaseolina	30.2	6.2	20.1
R. solani	30.0	6.0	20.0
Control (Uninfested soil)	65.0	18.8	72.8
L.S.D. at 5 %	2.1	0.9	2.3

Biochemical changes in pea plants raised from seeds treated with Trichoderma bioagents- Bion-Compost and infested with the Pathogen:

Estimation the content of total phenolic compounds:

Induction of defense-related biochemicals such as total phenolic compounds as well as nitrogen and protein were studied in plants raised from seeds treated with Trichoderma bioagents-compost- RICs and planted in soil infested with the tested pathogens under different combinations (Table 6). Data reveal that *Trichoderma* strains, compost and the RICs induced considerable content of phenolic compounds compared with control treatment and both fungi. However, we recorded little changes due to the pathogenic fungi whereas in the untreated control; no valuable modification was recorded in total phenolic contents. Great increase was recorded in the plants treated with the RICs then compost and Trichoderma bioagents, being 0.58, 0.58 and 0.59 mg gallic acid/g plant fresh leaves for the RICs, respectively, 0.56 mg gallic acid / g plant fresh leaves for compost and 0.53, 52 and 53 mg gallic acid / g plant fresh leaves for the bioagents, respectively.

Also, the percentages of total nitrogen and protein constitutes of pea dry seeds were, generally, greatly higher in the treatments of the RICs followed by compost then Trichoderma bioagents compared with the values of the two pathogenic fungi and the un-infested control treatment.

Table (6): Effect of *Trichoderma* bioagents- compost -RICs and pathogen-treated pea plants on the content of phenolic compounds and the percentages of nitrogen and protein content of pea seeds.

Treatments	Gallic acid in mg / g plant leaves after (days)			Mean	% Total nitrogen	% Protein
	15	30	60			
T. hamatum	0.40	0.57	0.63	0.53	4.20	26.25
T. harzianum	0.40	0.55	0.61	0.52	4.10	25.63
T. viride	0.40	0.57	0.62	0.53	4.15	25.94
Compost	0.40	0.64	0.65	0.56	4.21	26.31
Bion	0.40	0.66	0.71	0.58	4.24	26.50
Chitosan	0.40	0.65	0.68	0.58	4.23	26.44
Salicylic acid	0.40	0.68	0.70	0.59	4.26	26.63
M. phaseolina	0.40	0.50	0.60	0.50	3.24	20.25
R. solani	0.40	0.48	0.59	0.49	3.20	20.00
Control	0.32	0.33	0.36	0.34	3.95	24.69
Mean	0.41	0.56	0.62	-	-	-

DISCUSSION

Recently, farmer's main goal is to reduce the usage of chemical inputs to manage plant diseases and pests, especially in vegetable crops. Since plant disease management exemplify a huge challenge that farmers are facing in the cropping systems. *Rhizoctonia solani* and *Macrophomina phaseolina* are soil-borne fungal pathogens and cause damping-off and root-rot diseases to a wide range of crops and also effect quality of crops and decreasing yields.

Soil-borne diseases are from the destructive items in the crop production of vegetable crops, they are susceptible to many pathogenic organisms that either decrease the yield and marketability or many other sides combined. The dispose of many chemicals and increasing the resistance development, and climate change requires seeking for alternative proper management choices (Panth *et al.*, 2020).

Isolation from the rhizospheric soil around healthy pea plants yielded many fungal isolates. From these isolates, we have selected those belonging to the genus *Trichoderma*, purified with hyphal tip method and were identified as *Trichoderma hamatum, T. harzianum* and *T. viride.*

The culture filtrates of the three previously tested bio- agents, resulted in major inhibitory effect to the linear growth of tested fungi. This inhibitory effect was regularly increased by increasing the merged concentration to PDA medium. In specific, T. *viride* gave the most effective results in this regard, secondly; *T. harzianum* and thirdly *T. hamatum*. Moreover, the linear growth of the tested pathogens was inhibited by *T. viride* at 80% concentration.

The study was extended to understand the role of the inducing resistance chemicals (RICs) which significantly inhibited the linear growth of both tested fungi compared with control.

This inhibition was increased by increasing the combined concentration to PDA medium. *R. solani* was extremely affected by the RICs more than *M. phaseolina*. Also, the most efficient treatment in this regard was Bion followed by salicylic acid then chitosan. Moreover, the Bion at the concentration of 50 %, totally inhibited the growth of the two tested pathogens as a result, Bion was chosen to check its ability to the manage the tested two pathogens under greenhouse conditions.

In recent times, the effect of compost in plant disease management was taken to attention. Compost tea showed high inhibitory effect to the linear growth of both pathogenic fungi when compared to control. The inhibition was increased by increasing the concentration to PDA medium. Also, *R. solani* was highly affected by compost tea more than *M. phaseolina*. The results obtained from testing these treatments showed that there was significant reduction in both damping-off and root-rot severity. Also, damping off percentage was zero when the three tested items, *i.e.*, *T. viride*, Bion and compost were mixed and used as treatments to the soil infested with any of the tested pathogenic fungi. Moreover, the lowest percentages of root-rot severity were recorded when the three treatments were mixed and used as treatments to the soil infested with any of both pathogenic fungi.

However, significant reduction was obtained when two items from these treatments were mixed and used with soil infested with any of the tested pathogenic fungi. Soil without any treatment and infested with any of *M. phaseolina* and *R. solani* showed the highest severity percentages of damping-off and root-rot.

Trichoderma spp.; are a hopeful and effective biological agent against plant pathogenic fungi. *Trichoderma* spp. as antimicrobials controlling a wide range of microbes, have been well documented and demonstrated for more than seven decades, their usage came much later under field conditions (Ragab *et al*., 2015) and their parasitism mechanism is more complex, *i.e*., food competition, hyper parasitism, antibiotics, cell wall and space-breaking enzymes (Abd-El-khair *et al*., 2010). *Trichoderma* spp. are famous to stop the growth of pathogens either indirectly using competition for life needs, or plant parameters increasing and improve mechanisms of plant defense, or directly by mycoparasitism and enzyme production (Faheem *et al*., 2010; Zaher *et al.*,2013 and Ragab *et al*., 2015).

Non-pathogenic microorganisms can enhance defense mechanisms in the plant in a similar way to pathogenic microorganisms; as strengthening of plant cell walls, synthesis of pathogenesis-related proteins (PRs), production of antimicrobial and phytoalexins (Hammond-Kosack and Jones, 1996), also, they help in expressing these defense responses upon challenge inoculation with a pathogen, a mechanism known as "priming‟ (Conrath *et al.*, 2006). Activation of defense reactions indicates that even a beneficial microorganisms may be supposed by the plant as a possible threat, and that such observation involves production of resistance-eliciting compounds that act mechanistically like the elicitors formed by plant pathogenic bacteria and fungi.

In respect to using fungicides, it is well known that chemical management means are fast, easy and effective, but they have a dark side as they disturb the surround environment, destroy aquatic ecosystems, badly affect human health and finally reduce populations of beneficial microorganisms in the soil. For that reason, using of bioagents in the soil is an alternative way to suppress soil-borne plant pathogens by competition for nutrition, parasitism, production of antagonistic chemicals and resistance induction in plants against disease-causing pathogens. Applying bioagents in soil, such as *T. harzianum* and *T. viride*, which were used in this work, have been found to be effective against soil-borne plant pathogens causing root rot in many crops (Zaher *et al.*, 2013; Ragab *et al.*, 2015 and Shafique *et al.*, 2016).

Trichoderma spp. have been effectively applied to control damping-off and root-rot diseases (Ragab *et al*., 2015 and Sarhan *et al*, 2018 and Ketta *et al*., 2021). In this respect, these species were used to inhibit plant pathogens by one of two ways; directly by mechanisms such as mycoparasitism and enzyme production or indirectly by competing for food and space, increase plant growth and improving plant defensive mechanisms (Izquierdo-García *et at.*,2020).

Induced acquired resistance is pathogen nonspecific (Doubrava *et al.*, 1988). Larcke (1981) found that they are the main reason for localized defense, and they induced phytoalexins and lignifications and enhance activities of chitinase and P-glucanase (Dean and Kuc, 1985 and Metranx and Boller, 1986 and Dutta *et al*., 2016). Furthermore, Kessmann *et al*. (1994) reported that the mechanism of resistance inducing chemicals is in fact multifaced, resulting in stable broad spectrum disease control and they could be used as long-lasting protection.

Results of the present study showed a significant increase of crop parameters as a result of using the tested three treatments *i.e*., *T. viride*, Bion and compost and sowing the seeds in the infested soil with the tested pathogenic fungi. Also, we recorded an increase when one or two of these three items were used as treatments to the soil infested with any of tested pathogenic fungi. Soil without any treatment and infested with any of *M. phaseolina* and *R. solani* recorded the shortest plants and the lowest values of pods number and green pods weight (g)/ plant. Pea plants sown in un- infested soil (control) recorded the higher records of crop parameters.

The importance of compost has been studied by scientists, applied at any rate, herein, commercially as a natural fertilizer for many vegetables as well as to inhibit several plant diseases as (Bonanomi *et al.*,2007; Martin and Brathwaite, 2012 and Abada *et al.*, 2014 and 2018). Compost was used as organic alteration to the soil, especially for new reclaimed soils as it improves soil characteristics, crop productivity and also assist in inhibiting soil-borne pathogens.

Obtained data revealed that *Trichoderma* strains, compost and the RICs resulted in considerable induction of phenolic compounds compared with control treatment and both tested fungi, while minute changes were observed due to the presence of the pathogenic fungi, meanwhile no variable change was observed in total phenolic contents of untreated control. However, considerable increase in the total phenolic compounds was observed of plants raised from treated seeds with the RICs then compost and *Trichoderma* bioagents.

The amount of total nitrogen and protein constitutes of pea dry seeds were greatly higher in the treatments of the RICs followed by compost then Trichoderma bioagents compared with the values of seeds collected from plants grown in infested soil without any treatment and in those of the un-infested control treatment.

Bion (BTH) is an elicitor of systemic acquired resistance, which reduces plant fungal diseases by its exogenous applications (Barilli *et al.*, 2015 and Abada *et al.*, 2018 and Sarhan *et al*., 2018). It’s effect depend on the induction of the phenol pathway, but the particular metabolites involved have not been determined yet. This suggests fungal growth impairment by both direct toxic effect as well as plant cell wall strengthening.

IPM is a sustainable approach to managing pests by combining biological, cultural, physical and chemical materials in a technique reduces economic, health and environmental risks. This work evaluated the integrated use of *Trichoderma*-based bioagents with RICs and compost. It is well known that integration of many tools provides strength to the disease management programs. Integration of bioagents with other disease management tools resulted in maximum crop adaptation and both more efficacious and consistent disease management. Noble and Coventry (2003) noticed the suppressive effect of the use of composts to suppress several diseases in the field, whereas the disease suppressive effect of compost was increased with rate of application. Biological control agents BCAs have become an important element in sustainable agriculture (Umesha *et al.*, 2018). Although many beneficial microbial strains inhibit pathogens under laboratory or greenhouse conditions, successful BCA application in field-based crop production are infrequent (Mazzola and Freilich, 2017), this is mainly due to inadequate colonization of host rhizosphere connected with inefficient inhibition of soil-borne pathogen growth (Sarma *et al.*, 2015 and Mazzola; Freilich, 2017 and Ketta *et al*., 2021).

Lookwood (1988) reported that the compost supply stimulated suppressive activity for the microorganisms of the soil through four mechanisms, *i.e.*, competition, predation, hyper-parasitism, antibiosis and the induction of systemic acquired resistance in the host plant.

Lattanzio *et al.* (2006) mentioned that compounds such as phenolic and polyphenolic compounds are ubiquitous in plants and effect mainly in the non-host resistance to pathogenic fungi.

Also, if the produced antifungal phenolics are exist in healthy plants at antimicrobial levels, their levels can be raised further in response to a pathogens attack. Phenolic formation is raised when a plant attacked by a pathogen Waterman and Mole (1995). Phenolic compounds induction using bioagents was firstly reported by Van Peer *et al*. (1991). However, they alone are not dependable for suggestion of disease resistance in plant Waterman and Mole (1995). Akram *et al.* (2013) reported that there was a raise in the total phenolic contents in bacterial-treated plants. Melo *et al*. (2007) and Farkas and Kiraaly (2008) recorded that dependent upon active phenol oxidase system the endogenous provide phenol compound in the plant disease resistance.

In addition, Reddy *et al.* (2005) reported that the increase in soluble nitrogen may be approved to increase hydrolysis of proteins. Also, El-Sayed (2017) found that there was considerable increase in the percentages of total nitrogen and protein content of faba bean seeds due to treating the plants with bio-products, RICs and the fungicide Rizolex-T compared with untreated plants.

CONCLUSION

Damping-off and root-rot diseases can cause the decay of germinating seeds, kill young seedlings, and rot the roots, which represent for farmers the most important yield constraints both in nurseries and fields. To obtain effective management of damping-off and root-rot of the plants, it requires the deployment of several strategies, fungicides, bioproducts, resistant cvs., RICs, organic manure or compost, agricultural and sanitary methods, which can be used in combination to give efficacy in managing damping-off and root-rot. Thus, it requires to combine all or most of them within IPM.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

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