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Induced Resistance in Bean Plants Against Root Rot and Alternaria Leaf Spot Diseases Using Biotic and Abiotic Inducers under Field Conditions

F. Abd-El-Kareem

Plant Pathology Dept., National Research Centre, Giza, Egypt.

Abstract: Bean seeds coating with *T. harzianum* and or seedlings spray with humic acid were evaluated against root rot and Alternaria leaf spot disease under field conditions. All tested concentrations of humic acid had no inhibitory effect on tested fungi. Meanwhile, *T. harzianum* reduced the linear growth of *R. solani, F. solani* and *A. alternata* by 84.4,91.1 and 83.3 %, respectively.In greenhouse experiments, results indicate that integrated treatments between seeds coating with *T. harzianum* followed by seedlings spray with humic acid at concentrations 6 or 8 ml / l reduced the root rot incidence more than 85.1 and 81.9 % for *F. solani* and *R. solani*, respectively, in addition to more increase in chitinase activity was observed. In field experiments results indicate that the most effective treatment was integrated treatments between seeds coating with *T. harzianum* followed by seedlings spray with humic acid at concentrations 6 or 8 ml / l which reduced the root rot incidence more than 80.5 %. Single treatment with *T. harzianum* as seed coating or humic acid at concentrations 8 ml /l as foliar spray reduced the disease incidence more than 65.7 % during two successive seasons. Alternaria leaf spot was less appeared, on treated plants with humic acid at concentrations 6 or 8 ml / l when applied as single or integration with *T. harzianum*. Marked increase in bean yield was obtained with integrated treatments between *T. harzianum* and humic acid which increased the yield more than 56.3 % as compared with untreated plants. It could be suggested that integrated treatment between seed coating with *T. harzianum* followed by seedlings spray with humic acid as safety method might be used commercially for controlling bean diseases under field conditions.

Keywords: Bean plants - Root rot - T. harzianum - humic acid- Induced resistance

INTRODUCTION

Bean plants (*Phaseolus vulgarus* L.) is one of the most important leguminous crops in Egypt. Root rot and Alternaria leaf spot diseases caused by *Fusarium solani, Rhizoctonia solani* and *Alternaria alternata* respectively are a serious and persistent diseases problem of bean plants during growing season[1,2,3,4].

There is a growing need to develop alternative approaches for controlling plant diseases. Induced resistance in some plants against plant diseases was reported by[5,6,7,8,9]. Humic acid is a suspension, based on potassium-humates, which can be applied successfully in many areas of plant production as a plant growth stimulant or soil conditioner for enhancing natural resistance against plant diseases and pests[10,11], stimulation plant growth through increased cell division, as well as optimized uptake of nutrients and water,[12,13].Moreover, humic acid stimulated the soil microorganisms[12,14]. Furthermore, Scheuerell and Mahaffee[10,11] reported that the most effective treatments for suppression damping off in many plants and gray mould in Geranium was compost tea plus kelp extract and humic acid.

Trichoderma spp. are effective biocontrol agents for a number of soilborne plant pathogens, and also known for their ability to enhance plant growth[15,16]. Recently it was suggested that *Trichoderma* affects induced systemic resistance (ISR) mechanism in plants[7,17].

Challenge of *Trichoderma* pre inoculated plants with the leaf pathogen *Pseudomonas syringae* pv. *lachrymans* resulted in higher systemic expression of the pathogenesis-related genes encoding for chitinase 1, beta-1,3-glucanase, and peroxidase relative to noninoculated, challenged plants[6]. The purpose of the present work was designed to evaluate the effect of *T. harzianum* and humic acid as single or integration treatments on the bean root rot disease as well as on Alternaria leaf spot incidence under field conditions.

MATERIALS AND METHODS

Source of Pathogenic Fungi and Bean Seeds: Pathogenic fungal isolates, *i.e. Rhizoctonia solani; Fusarium solani* and *Alternaria alternata* as the causal agents of bean root rot disease and Alternaria leaf spot, respectively were kindly obtained from Plant Pathology

Corresponding Author: F. Abd-El-Kareem, Plant Pathology Dept., National Research Centre, Giza, Egypt.

Dept., National Research Centre, Giza, Egypt. Meanwhile, Bean seeds cv. Giza 3 were obtained from Vegetable Crops Research Dept., Agricultural Research Centre, Giza, Egypt.

Testing of Humic Acid and *T. harzianum* on the Linear Growth of Pathogenic Fungi *in vitro*: The inhibitory effect of humic acid and *T. harzianum* against linear growth of bean fungi was evaluated. Humic acid at five concentrations, *i.e.* 0, 2, 4, 6 and 8 ml /l. were added to conical flasks containing sterilized PDA medium before its solidifying and rotated gently then disbanded into sterilized Petri-plates (9 cm diameter). Plates were individually inoculated at the centre with equal disks (6 mm diameter) taken from 10 days old cultures of each *F. solani, R. solani* and *A. alternata*, then incubated at 25^{-2} °C. As for *T. harzianum*, disks of *T.harzianum* and disks of each pathogenic fungi were placed on opposite sides of Petri plates containing PDA medium. Inoculated plates were incubated for 7 days at 25^{-2} °C. Linear growth of tested fungi was measured, when the control plates reached full growth and the average growth diameter was calculated. Each treatment was represented by 5 plates as replicates.

Greenhouse Experiments:

Soil Infestation with Root Rot Fungi: One pathogenic isolate of each *R. solani* or *F. solani* was grown on sand-barley medium (1:1 w:w and 40 % water) for 20 days at $25^{\circ}C^{-2}$. Plastic pots (30-cm-diameter) containing sandy loam soil were artificially infested individually with prepared fungal inoculum at the rate 3 % of soil weight. Infested soil irrigated every other day and left for 15 days.

Testing of Two Application Methods of Humic Acid and *T. harzianum* on Root Rot Disease of Bean Plants under Greenhouse Conditions: Two application methods of *T. harzianum* and humic acid *i.e.* seed treatments and foliar spray were evaluated against bean root rot disease incidence. Bean seeds cv. Giza 3 were sown in transplants foam trays containing peat-moss soil. Seed treatment was carried out as seed soaking for 2 hours in five concentrations of Humic acid *i.e.* 0, 2, 4, 6 and 8 ml /l or spore suspension (10^{6} spores / ml) of *T. harzianum*. While, foliar spray was carried out as seedling at first true leaf growth stage sprayed with humic acid at 0, 2, 4, 6 and 8 ml /l or spore suspension (10^{6} spores / ml) of *T. harzianum*. On the other treatment integration treatments was carried out as seed coating with spore suspension (10^{6} spores / ml) of *T. harzianum* followed by foliar spray with humic acid at concentrations 6 or 8 ml / l as mentioned before. Treated bean seedlings was transfer to pots previously infested with *R. solani or F. solani.* Bean transplants were then planted at the rate of 6 transplants / pot and 6 pots / treatment were used. The percentage of bean root rot incidence was recorded up to 40 days of transplant.

Determination of Chitinase Activity of Bean Plants Treated With:

***T. harzianum* as Seed Coating Followed by Humic Acid as Foliar Spray:** *T. harzianum* as seed coating followed by humic acid at concentrations 6 or 8 ml /l as foliar spray were applied to study their effect on chitinase activity.Bean seed coated with spore suspension (10^{6} spores / ml) of *T. harzianum* then sown in foam trays. Been seedlings at first true leaf growth stage were sprayed with humic acid at concentrations 6 or 8 ml /l.Treated bean seedlings were planted in previously infested soil.

Extraction of enzyme: Chitinase activity was determined after 20 days of transplants. The enzyme extracted from bean plants and the supernatant was prepared according to method of Tuzun *et al*.,[18]. The chitinase activity was determined by colourimetric method of Boller and Mauch,[19]. Colloidal chitin was used as substrate and dinitrosalicylic acid as reagent to measure reducing sugars. Chitinase activity was expressed as mM N-acetylglucose amine equivalent released/gram fresh weight tissue/60 minutes.

Root Rot Assessment under Greenhouse Conditions: The average percentage of bean root rot incidence was recorded until 40 days after transplant.

Field Experiments:

Testing of Seed Coating with *T. harzianum* and / or Seedlings Spray with Humic Acid on Bean Root Rot and Alternaria Leaf Spot Diseases: The promising treatments in greenhouse experiments was applied under field conditions. *Trichoderma harzianum* as seed coating and / or humic acid at concentrations 6 or 8 ml /l as foliar spray were applied to study their effect on bean root rot and Alternaria leaf spot diseases during tow cultivation seasons.

Bean seeds cv. Giza 3 were coated with spore suspension (10^{6} spores / ml) of *T. harzianum* then sown. Seedlings at first true leaf growth stage was sprayed with humic acid at 6 or 8 ml / l.

Booster treatments was carried out as foliar spray with humic acid at concentration 6 ml / l after 70 days of sowing.

Rhizolex-T (50% WP) at 3 g/kg seeds was used as comparison treatment

Field experiment was carried out, in El Sadat city, Behera Governorate. Field experiments were conducted under natural infection in plots (4x8 m) each comprised

of 8 rows and 32 holes / row, in a completely randomized block design with three replicates (plots) for each particular treatment. as well as untreated plants.

Disease Assessment under Field Conditions:

Root Rot Disease: The average percentage of bean root rot incidence was recorded until 30 and 90 days, of sown for pre-and post emergence respectively.

Alternaria Leaf Spot: Disease scale from 0 to 4 according to Vakalunakis[20], based on the leaf area infected was used, as follows:

- 0 = No leaf lesions.
- 1 = 25 % or less.
- 2 = 26 to 50
- 3 = 51 to 75
- 4 = 76 to 100 % infected leaf area.

Determination of Bean Yield: Obtained yield (kg per Fadden) was calculated.

Statistical Analysis: Tukey test for multiple comparisons among means was utilized[21].

RESULTS AND DISCUSSIONS

Results:

Effect of Humic Acid and *T. harzianum* on the Linear Growth of Bean Fungi: The inhibitory effect of humic acid at five concentrations, *i.e.* 0, 2, 4, 6 and 8 ml /l. and *T. harzianum* against linear growth of Bean fungi was evaluated.Results in Table (1) indicate that all tested concentrations of humic acid had no inhibitory effect on tested fungi. Meanwhile, the high reduction was obtained with *T. harzianum* which reduced linear growth of *R. solani, F. solani* and *A. alternate* by 84.4,91.1 and 83.3 %, respectively.

Greenhouse Experiments:

Effect of Two Application Methods of Humic Acid and *T. harzianum* on Root Rot Disease of Bean Plants: Two application methods of *T. harzianum* and humic acid *i.e.* seed treatments and foliar spray were evaluated against bean root rot disease. Results in Table (2) indicate that all treatments significantly reduced the root rot incidence of bean plants. The most effective treatments was *T. harzianum* when applied as seed treatment which reduced disease incidence by 72.5 and 73.0 % for *F. solani and R. solani* respectively.While, humic acid at concentrations 6 or 8 ml / l. as foliar spray reduced the root rot incidence more than 77.0 and 66.0 % for *F. solani and R. solani* respectively. It noticed that, the high reduction in root rot disease was obtained with *T. harzianum* and humic acid at concentrations 6 or 8 ml / l when applied as seed treatment and foliar spray respectively.

Effect of Seed Coating with *T. harzianum* and / or Seedlings Spray with Humic Acid on Bean Root Rot Disease of Bean Plants: *Trichoderma harzianum* as seed coating and / or humic acid at concentrations 6 or 8 ml /l as foliar spray applied to study their effect on suppression bean root rot incidence.

Results in table (3) indicate that all treatments significantly reduced the root rot incidence of bean plants. Seed coated with spore suspension of *T. harzianum* followed by foliar spray with humic acid at concentrations 6 or 8 ml / l reduced the disease incidence more than 85.1 and 81.9 % for *F. solani* and *R. solani*, respectively. Single treatment with *T. harzianum* as seed treatment reduced the disease incidence by 72.0 and 66.9 for *F. solani* and *R. solani*, respectively.While humic acid at concentrations 6 or 8 ml / l. as foliar spray reduced the root rot incidence more than 65.6 and 56.9 % for *F. solani* and *R. solani*, respectively.

Effect of Seed Coating with *T. harzianum* and or Seedlings Spray with Humic Acid on Chitinase Activity of Bean Plants: Effect of *T. harzianum* as seed coating and humic acid at concentrations 6 or 8 ml /l as foliar spray alone or in combination on chitinase activity was determined. Results in Table (4) indicate that all treatments increased the chitinase activity. The most effective treatment was combined treatments between seed coated with spore suspension of *T. harzianum* and foliar spray with humic acid at concentrations 6 or 8 which Increased the activity more than 113.3 and 118.8 % for *F. solani and R. solani* respectively. Single treatment with *T. harzianum* as seed treatments or humic acid at concentrations 6 or 8 ml / l. as foliar spray Increased the chitinase activity more than 66.7 and 65.6 % for *F. solani and R. solani* respectively.

Field Experiments:

Effect of Seed Coating with *T. harzianum* And/ or Seedlings Spray with Humic Acid on Bean Root Rot and Alternaria Leaf Spot Diseases: *Trichoderma harzianum* as seed coating and / or humic acid at concentrations 6 or 8 ml /l as foliar spray were applied to study their effect on bean root rot and Alternaria leaf spot diseases in addition to determine bean yield during two cultivation seasons.

Bean Root Rot Disease: Results in Table (5) indicate that all treatments significantly reduced the root rot incidence of bean plants. during two successive seasons. The most effective treatments was integrated

Table 1: Effect of humic acid and *T. harzianum* on linear growth of Pathogenic fungi

Treatments	Conc. (ml / l)l	Linear growth (mm)		
		Root rot fungi	Alternaria leaf spot	
		F. solani	R. solani	A. alternata
Humic acid	2	90.0 a	90.0 a	90.0 a
	4	90.0 a	90.0 a	90.0 a
	6	90.0 a	90.0 a	90.0 a
	8	90.0 a	90.0 a	90.0 a
T. harzianum	-----	b14.0	b8.0	15.0 b
Control	0	90.0 a	90.0 a	90.0 a

1-Figures with the same letter are not significantly different (P= 0.05)

Table 2: Root rot incidence of bean plants in response to different application methods of humic acid and *T. harzianum* under greenhouse conditions.

Treatments	Bean root rot incidence			
	Application methods			
Humic acid (ml /L)ll	Seed treatments		Foliar spray	
	F. solani	R . solani	F. solani	R . solani
2.0	38.5b	42.0b	25.5c	38.0c
4.0	33.0b	37.0c	20.0d	31.0d
6.0	27.5c	31.5d	17.5de	23.4e
8.0	23.0c	27.0d	14.0e	20.2e
T. harzianum	14.5d	16.0e	32.5b	41.0b
Control	53.0a	60.0a	53.0	60.0

1-Figures with the same letter are not significantly different (P= 0.05)
2- Seed treatment was carried out by coating bean seeds with individual *T. harzianum* or humic acid before sowing
3- Foliar spray was carried out by spraying the seedlings at first true leaf with
T. harzianum or humic acid

Table 3: Effect of seed coating with *T. harzianum* and / or seedlings spray with humic acid on bean roo rot disease

Application		Bea root rot incidence			
		F. solani		R . solani	
Seed treatments	Foliar spray (H.A.) ml /l	Disease	Reduction %	Disease	Reduction %
0.0	6.0	19.0b	65.5	25.0b	56.9
0.0	8.0	15.0c	72.8	22.5bc	61.2
	0.0	15.4c	72.0	18.0c	68.9
T. harzianum	6.0	8.2d	85.1	10.5d	81.9
	8.0	7.4d	86.5	8.1d	86
0.0	0.0	55.0a	-----	58.0a	----

Figures with the same letter are not significantly different (P= 0.05)

treatments between seed coated with spore suspension of *T. harzianum* followed by foliar spray with humic acid at concentrations 6 or 8 ml / l which reduced the post- emergence more than 80.5 % during two successive seasons. Single treatment as seed coating with *T. harzianum* or humic acid at concentrations 8

Table 4: Effect of seed coating with *T. harzianum* and / or seedlings spray with humic acid on chitinase activity of bean plants under greenhouse cndtions.

Application		Chitinase activity			
		F. solani		R . solani	
Seed treatment	Foliar spray (H.A) ml/l	Activity	Increase %	Activity	Increase %
0	6	c5.0	66.7	bc5.3	65.6
0	8	b5.5	83.3	b5.6	86.7
	0	d4.5	50	c5.0	56.3
T. harzianum	6	a6.4	113.3	a7.0	118.8
	8	a6.8	126.7	a7.3	128.1
0	0	e3.0	????	d3.2	------

Figures with the same letter are not significantly different (P= 0.05)

Table 5: Root rot incidence in bean plants as affected by seed coating with *T. harzianum* and / or seedlings spraying with humic acid under field conditions

Application		Bean root rot incidence			
		First season		Second season	
Seed treatment	Foliar spray (H.A) ml/l	Pre- emergence	Post- emergence	Pre- emergence	Post- emergence
0.0	6.0	-------	22.5b	------	25.2b
0.0	8.0	------	16.1c	-----	18.5c
T. harzianumm	0.0	13.5b	15.2cd	14.5b	16.0c
	6.0	12.0b	8.5e	12.5b	10.5d
	8.0	11.5b	7.0e	12.0b	10.0d
Fungicide	0.0	10.0b	11.5de	11.0b	12.0d
0.0	0.0	45.5a	47.0a	52.0a	54.0a

Figures with the same letter are not significantly different (P= 0.05)
Pooster treatment was carried out after 70 days of sowing by spraying plants with humic acid at 6 ml / l.

Table 6: Alternaia leaf spot in bean plants as affected by seed Coating with *T. harzianum* and seedlings spraying with humic acid under field conditions

Applicationl		Alternaria leaf spot			
		First season		Second season	
Seed treatment	Foliar spray (H.A) ml/l	Disease incidence	Reduction %	Disease incidence	Reduction %
0.0	6.0	0.7c	72.0	0.6c	75.0
0.0	8.0	0.5c	80.0	0.5c	79.2
T. harzianumm	0.0	1.5b	40.0	1.4b	41.7
	6.0	0.6c	76.0	0.6c	75.0
	8.0	0.4c	84.0	0.4c	83.3
0.0	0.0	2.5a		2.4a	

Figures with the same letter are not significantly different (P= 0.05)
Pooster treatment was carried out after 70 days of sowing by sparing plants with humic acid at 6 ml / l.

ml /l as foliar spraying reduced the disease incidence more than 70.3 and 65.7 %.As for pre -emergence no significant different between single or integrated treatment was observed. Fungicidal treatment reduced the pre- and post emergence more than 78.7 and 81.5 % respectively.

Table 7: Dray yield of bean plants as affected by seed coating with *T. harzianum* and / or seedlings spray with Humic acid under field conditions

Application		Bean yield (kg / Fadden)			
		First season		Second season	
Seed treatment	Foliar spray (H.A) ml/l	Yield	Increase %	Yield	Increase %
0.0	6.0	650	35.4	640	39
0.0	8.0	700	45.8	700	52.2
T. harzianum	0.0	660	37.5	670	45.7
	6.0	750	56.3	780	69.6
	8.0	800	66.7	820	78.3
Fungicide		650	35.4	670	39.6
0.00	0.00	480	460		

Alternaria Leaf Spot: Results in Table (6) indicate that all treatments significantly reduced the Aternaria leaf spot incidence of bean plants during two successive seasons. The most effective treatments was humic acid at concentrations 6 or 8 ml / l when applied as single or in combination with *T. harzianum* which reduced the disease incidence more than 72.0 % during two successive seasons. Meanwhile, *T. harzianum* as single treatment reduced the disease incidence more than 40.0 %.

Bean Yield: Results in table (7) indicate that all treatments increased the bean yield. The highest increase was obtained with integrated treatments between seed coated with spore suspension of *T. harzianum* and foliar spray with humic acid at concentrations 6 or 8 ml / l which increased bean yield more than 56.3 and 66.7 % respectively during two successive seasons.The moderate increase was achieved with single treatment of humic acid at concentrations 6 or 8 ml /l, *T. harzianum* and fungicidal treatment which increased bean yield more than 35.4 %.

Discussion: Root rot and Alternaria leaf spot diseases caused by *F. solani, R. solani* and *A. alternate* respectively are the most important diseases attack bean plants during growing season[1,2,3,4,20]. In present study results indicate that all tested concentrations of humic acid had no inhibitory effect on the growth of tested fungi. Meanwhile, the highest reduction was obtained with *T. harzianum* which reduced linear growth of *R. solani, F. solani* and *A. alternate* by 84.4,91.1 and 83.3 % respectively. In greenhouse and field experiments In present study synergistic effect between seed coating with *T. harzianum* and seedlings spray with humic acid at concentrations 6 or 8 ml / l was observed.

Humic acid can be applied successfully in many areas of plant production as a plant growth stimulant, soil conditioner,*i.e.* enhanced natural resistance against plant diseases and pests [10, 11]. Present results indicate that humic acid at concentrations 6 or 8 ml / l had no inhibitory effect against bean fungi and reduced root rot disease in greenhouse and field experiments as well as Alternaria leaf spot under field conditions. In this respect, Scheuerell and Mahaffee[10,11] reported that the most effective treatments for suppression damping off in many plants and gray mould in Geranium was compost tea plus kelp extract and humic acid. The role of humic acid for reducing root rot and Alternaria leaf spot diseases in addition to increase yield of bean plants may be due to enhanced natural resistance against plant diseases and pests[10,11], stimulated plant growth through increased cell division, as well as optimized uptake of nutrients and water,[12,13,22] and stimulated the soil microorganisms[12,13]. In present study results indicate that humic acid at concentrations 6 or 8 ml / l stimulated the chitinase activity more than 65.0 and 113.1 % when applied as single or integrated treatments with *T. harzianum* respectively. In this respect, *B*-1,3-glucanases and chitinases are able to hydrolyze *B*-1,3-glucan and chitin, respectively, the major components of fungal cell walls[24,25,26].It was suggested that *Trichoderma* spp. are effective biocontrol agents for a number of soilborne plant pathogens and it was affects induced systemic resistance (ISR) mechanism in plants[7,27].

In this study, *T. harzianum* when applied as single or integrated treatments with humic acid reduced root rot and Alternaria leaf spot diseases under field conditions in addition to increase bean yield. In this respect, Shoresh, *et al*.,[6] reported that Challenge of *Trichoderma*-preinoculated plants with the leaf pathogen *P. syringae* pv. *lachrymans* resulted in higher systemic expression of the pathogenesis-related genes encoding for chitinase 1, beta-1,3-glucanase, and peroxidase relative to noninoculated, challenged plants.

In present study results indicate that *T. harzianum* as single or combined treatment with humic acid at concentrations 6 or 8 ml / l stimulated the chitinase activity more than 50.0 and 113.1 % respectively. In this respect Xue *et al.*,[28] reported that inoculated bean plants with non pathogenic of Rhizoctonia sp. Resulted in higher increasing of peroxidases, 1,3-_-glucanases, and chitinases activities. Induced resistance against cotton root rot and Alternaria leaf spot diseases using non pathogenic isolate of *Rhizoctonia* sp. was also reported by Jabaji-Hare and Neate[29].

Trichoderma spp. are also known for their ability to enhance plant growth[15,16].

It could be suggested that combined treatment between seed coating with *T. harzianum* and seedlings spraying with humic acid as safety method might be used commercially for controlling bean diseases under field conditions.

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