

COMPARISION OF ANTAGONISTIC EFFECTS OF THE ENDOPHYTIC FUNGI AND *TRICHODERMA* SPECIES AGAINST SOYBEAN CHARCOAL ROT DISEASE UNDER GREENHOUSE CONDITIONS

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ABSTRACT

The charcoal rot diseases of soybean caused by *Macrophomina phaseolina* consequently reduces the quantity and quality of yield, especially in drought condition which yield losses in sever epidemic years exceeded from 23-100 percent. Antagonistic ability of the entophytic fungi the main groups of symbiotic fungi associated with soybean roots (*Piriformospora indica* and *Sebacina vermifera*) related to Sebacinals from Basidiomycota and two species of *Trichoderma* including *T. harzianum* (T-100) and *T. viride* investigated by orthogonal comparisions by using SPSS software. The experiments were carried out in a completely randomized design with 47 treatments and 3 replicates. Various indices are recorded at the end of 3th month of experimentation and data analyzed. Results indicated a significant difference (P= 0.01) among various treatments in root and foliar wet and dry weights. Results of orthogonal comparisions between *P. indica* and *S. vermifera* indicated that antagonistic effects of *S. vermifera* were higher than *P. indica* fungus. Also, the study of orthogonal comparisions between *T. viride* and *T. harzianum* (T-100) revealed that the maximum antagonistic effects was related to *T. viride* fungus. Other results demonstrated that root and foliar wet and dry weights of soybean increased when antagonistic fungi inoculated earlier from pathogen in greenhouse experiments. Also, we found that the entophytic fungi not only good symbiotic relation, but also could be very effective in biological control of soybean charcoal rot disease of soybean.

KEYWORDS: Biological Control, *Piriformospora indica*, *Sebacina vermifera*, *Trichoderma harzianum*, *Trichoderma viride* and *Macrophomina phaseolina*

INTRODUCTION

Charcoal rot in soybean caused by the soil borne fungus *Macrophomina phaseolina* (Tassi) Goidanich is a serious disease of many crops, especially in soybean. The fungus can infect the root and lower stem of over 500 plant species (Wyllie, 1989). The lack of genetic resistance and absence of effective chemical control impose constraints on charcoal rot management strategy. Considerable emphasis has been given to develop biological control agents as potential means of disease control and to improve plant health (Aly *et al.*, 2007). The nuclear rDNA was used for phylogenetic studies of ectomycorrhizal Sebacinale fungi (Verma *et al.*, 1998; Glen *et al.*, 2002; Urban *et al.*, 2003 and Weiss *et al.*, 2004). Among those mycorrhizal species, *Piriformospora indica*, which was first isolated from the rhizosphere of *Prosopis juliflora* and *Zizyphus nummularia*, India (Verma *et al.*, 1998), has been shown to colonize roots and increase the biomass of both roots and shoots of numerous plant species, including cultured *Glycinemax* (Sahay and Varma, 1999; Varma *et al.*, 1999; Rai *et al.*, 2001; Kumari *et al.*, 2003 and Peskan-Berghofer *et al.*, 2004). Also *Sebacina vermifera*, an endophytic fungus has been isolated from a desert in Germany (Warcup and Talbot, 1967).

These fungi are members of Sebacinaceae family, Sebaciniales order of the Basidiomycota (Weiss *et al.*, 2004). In contrast to the obligate biotrophic AMF, *P. indica* and *S. vermifera* could be cultivated easily on synthetic media (Varma *et al.*, 2001; Peskan-Berghofer *et al.*, 2004). Beyond the stimulating effect on biomass production, *P. indica* apparently supports its host by protecting it from pathogenic fungi (Waller *et al.*, 2005). It was suggested that *P. indica* may target an as yet unidentified signaling pathways to induce systemic resistance (Serfling *et al.*, 2006). Also, the interaction between the plant and *P. indica* had been established in growth chambers, followed by incubation outdoors. Under these conditions, *P. indica* acted as both a biofertilizer and a biocontrol agent (Serfling *et al.*, 2006). The application of *Trichoderma* to the soil as biocontrol agent in the greenhouse or under field conditions, not only resulted in reduced disease severity but also enhanced plant growth (Ousley *et al.*, 1994; Harman and Bjorkman, 1998; Vazquez *et al.*, 2000; Yedidia *et al.*, 2001 and Harman *et al.*, 2004). Solubilization, increased uptake and translocation of physiologically less available minerals, production of growth hormones and vitamins are also suggested as part of the mechanism of growth promotion (Baker, 1989; Kleifeld and Chet, 1992; Inbar *et al.*, 1994 and Harman *et al.*, 2004). During early stage of root colonization by *Trichoderma* defense response was demonstrated as one of the mechanisms of biocontrol (Yedidia *et al.*, 1999, 2000; Howell *et al.*, 2000 and Howell, 2003). In the present work, because of high antagonistic effects of the endophytic fungi (*Piriformospora indica* and *Sebacinaverimifera*) and *Trichoderma* species (*Trichoderma harzianum* (T-100) and *T. viride*) for biocontrol of *M. phaseolina* *in vitro* (Abbaszadeh *et al.*, 2011), therefore, biocontrol ability of these fungi were studied under greenhouse experiments by using orthogonal contrasts.

MATERIALS AND METHODS

M. phaseolina Culture

M. phaseolina strain ML1 obtained from mycology collection of Department of Biological Sciences Rani Durgawati University Jabalpur Madhya Pradesh India. This fungus cultured on PDA medium and then five plugs of 5 mm disks of fresh PDA cultures of *M. phaseolina* were grown on sterilized rice grains into per bottle and incubated at $35 \pm 2^\circ\text{C}$ for 10 days.

Fungal Solid Culture of *P. indica* and *S. vermifera*

Piriformospora indica and *S. vermifera* were maintained on Kaefer's medium (Kaefer, 1977). *P. indica* was cultured as described previously (Verma *et al.*, 1998; Peskan-Berghofer *et al.*, 2004) in Petri dishes on a modified Kaefer's medium (NaNO₃, 7.0mM; KCl, 7.0mM; MgSO₄, 2.1mM; KH₂PO₄, 9.2mM; ZnSO₄, 0.77mM; H₃BO₄, 0.18mM; MnSO₄, 0.02mM; CoCl₂, 0.007mM; CuSO₄, 0.0065mM; FeSO₄, 0.02mM; EDTA, 0.02mM; ammonium molybdate, 0.001mM; thiamine, 0.003mM; glycine, 0.005mM; nicotinic acid, 0.002mM; pyridoxine, 0.0004mM; glucose, 110mM; peptone, 2g/l; yeast extract, 1g/l; casein hydrolysate, 1g/l, pH 6.5) with 1% (w/v) agar. The plates were inoculated with actively growing fungi and then incubated at $30 \pm 2^\circ\text{C}$ for a week.

Fungal Liquid Culture of *P. Indica* and *S. Vermifera*

Mycelium liquid culture were started in 500 ml flasks containing 200 ml of autoclaved KM liquid medium and inoculated with four mycelia disks cut from 10 days old solid culture of *P. indica* and *S. vermifera*. Flask culture were kept on a shaker (140 rpm) and incubated for 15 day at the room temperature ($30 \pm 2^\circ\text{C}$) till a dense mycelia suspension was generated. Then stored at 4°C for pot culture experiments.

Trichoderma Species Culture

Trichoderma harzianum (T-100) and *T. viride* obtained from mycology Department of Biological Sciences Rani Durgawati University Jabalpur Madhya Pradesh India. These fungi cultured on PDA medium and were grown on sterilized wheat grains into bottles and incubated at 27 ± 2 °C for 10 days.

Pot Culture Experiments

Piriformospora indica, *S. vermifera*, *T. harzianum* (T100) and *T. viride* with great inhibition zone *In vitro* (Abbaszadeh *et al.*, 2011), were investigated for their ability to reduce the incidence of charcoal rot in soybean by greenhouse experiments 2 times. Pot culture experiments were conducted in greenhouse during 2007 using a completely randomized design with 47 treatments and 3 replicates. Seeds of soybean (*Glycine max*) were surface-sterilized by soaking in 0.5% sodium hypochlorite for 2 min then rinsed three times in sterile distilled water and placed in sterilled perlite for germination. After 7 days, when the plantlets were in 3 leaflets stage, transferred to pots and were grown under greenhouse conditions. Soil had been disinfected with a 10% formaldehyde solution. Before of translation of the plantlets to pots. Pots inoculated with pathogen in two times. i.e first time; 10 days before sowing, and second time; 10 days after sowing. Antagonistic fungi inoculated concordant sowing. To produce inoculums for pathogen and antagonistic fungi, 10g/kg mixture of rice grains infected with pathogen, 10 g/kg mixture of wheat seeds distilled water infected with *Trichoderma* species (10^6 CFU/g). For inoculation with *P. indica* or *S. vermifera*, 3g/kg of crushed mycelium was added to pots. After of inoculation of soil into pots with pathogen and antagonistic fungi, 3 the plantlets were translated to per pot and were grown in a 1:1:1 mixture of soil: peat: perlite in greenhouse at 28 ± 2 °C, with a photoperiod of 16 h light/8h dark with fluorescent light intensity 1000 lux and relative humidity 10%. The control treatments was also maintained without inoculation with antagonistic fungi. Root and foliar wet and dry weights evaluated for each treatment were assessed in end of 3th month.

Treatments

T1= control (pathogen)

T2= pathogen + *P. indica*

T3= pathogen + *P. indica* + *S. vermifera*

T4= pathogen + *P. indica* + *S. vermifera* + *T.harzianum*

T5= pathogen + *P. indica* + *S. vermifera* + *T. viride*

T6= pathogen + *P. indica* + *S. vermifera* + *T. viride* + *T. harzianum*

T7= pathogen + *P.indica* + *T. harzianum*

T8= pathogen + *P. indica* + *T. viride*

T9= pathogen + *P. indica* + *T. viride* + *T. harzianum*

T10= pathogen + *S. vermifera*

T11= pathogen + *S. vermifera* + *T. harzianum*

T12= pathogen + *S. vermifera* + *T.viride*

T13= pathogen + *S. vermifera* + *T. viride* + *T. harzianum*

- T14= pathogen + *T. harzianum*
- T15= pathogen + *T. viride*
- T16= pathogen + *T. viride* + *T. harzianum*
- T17=*P. indica*
- T18= *P. indica* + pathogen
- T19= *P. indica* + *S. vermifera*
- T20= *P. indica* + *S. vermifera* + pathogen
- T21= *P. indica* + *S. vermifera* + *T. harzianum*
- T22= *P. indica* + *S. vermifera* + *T. harzianum* + pathogen
- T23= *P. indica* + *S. vermifera* + *T. viride*
- T24= *P. indica* + *S. vermifera* + *T. viride* + pathogen
- T25= *P. indica* + *S. vermifera* + *T. viride* + *T. harzianum*
- T26= *P. indica* + *S. vermifera* + *T. viride* + *T. harzianum* + pathogen
- T27= *P. indica* + *T. harzianum*
- T28= *P. indica* + *T. harzianum* + pathogen
- T29= *P. indica* + *T. viride*
- T30= *P. indica* + *T. viride* + pathogen
- T31= *P. indica* + *T. viride* + *T. harzianum*
- T32= *P. indica* + *T. viride* + *T. harzianum* + pathogen
- T33= control (plant)
- T34= *S. vermifera*
- T35= *S. vermifera* + pathogen
- T36= *S. vermifera* + *T. harzianum*
- T37= *S. vermifera* + *T. harzianum* + pathogen
- T38= *S. vermifera* + *T. viride*
- T39= *S. vermifera* + *T. viride* + pathogen
- T40= *S. vermifera* + *T. viride* + *T. harzianum*
- T41= *S. vermifera* + *T. viride* + *T. harzianum* + pathogen
- T42= *T. harzianum*
- T43= *T. harzianum* + pathogen
- T44= *T. viride*

T45= *T. viride*+ pathogen

T46= *T. viride* + *T. harzianum*

T47= *T. viride* + *T. harzianum* + pathogen

Statistical Analysis

The collected data were statistically computed using SPSS software for orthogonal contrasts. Data were subjected to analyses of variance and treatment means were compared by an approximate Duncan's multiple tests and main effectors interaction was found significant at $p < 0.01$ and $p < 0.05$.

RESULTS

Pot Culture Experiments

We determined the potential of the entophytic fungi and *Trichoderma* species to colonize soybean var. L83-570 growing in pot cultures by orthogonal comparisons by using SPSS software in 4 levels.

Comparison Bio Control Ability between the Endophytic Fungi and *Trichoderma* Species against *M. phaseolina*

In greenhouse experiments (both two times), results indicated a significant difference ($P=0.01$) among various treatments on root and foliar wet and dry weights (Table 1,2). Results of orthogonal comparisons revealed that root and foliar wet and dry weights in plants inoculated with the entophytic fungi alone or combination with *M. phaseolina* were significantly greater than in plants inoculated with *Trichoderma* species alone or combination with *M. phaseolina* and or *M. phaseolina* alone (Table 3,4 and Figure 1-10).

Comparison Bio Control Ability between *Trichoderma* Species against *M. phaseolina*

In greenhouse experiments (both two times), results indicated a significant difference ($P=0.01$) among various treatments on root and foliar wet and dry weights. Results of orthogonal comparisons between *Trichoderma* species showed that antagonistic effects of *T. viride* against pathogen was higher than *T. harzianum* (T-100) (Table 5, 6 and Figure 1, 2).

Comparison Bio Control Ability between the Endophytic Fungi against *M. phaseolina*

Root and shoot weights in greenhouse experiments, in plants inoculated with the endophytic fungi and pathogen were significantly greater than in control plants inoculated with pathogen alone. However, similar growth responses were also obtained when plants were inoculated with the endophytic fungi. But, results demonstrated that *S. vermifera* could be more effective than *P. indica* in biological control of *M. phaseolina* *In vivo* (Table 7, 8 and Figure 1, 2).

Comparison Biocontrol Ability of Antagonistic Fungi in Attention to Time of Inoculation Pathogen 10 before or after Inoculation of Antagonistic Fungi in Pot Cultures

Biocontrol ability of antagonistic fungi in attention to time of inoculation of pathogen evaluated in two times with orthogonal comparisons. In first time, pathogen inoculated 10 days before of antagonistic fungi in pot cultures and second time; pathogen inoculated 10 days after of antagonist's fungi. Results indicated a significant differences ($P=0.01$) among various treatments in root and foliar wet and dry weights. Maximum of root and foliar wet and dry weights observed in second time, which pathogen inoculated 10 days after of the entophytic fungi and *Trichoderma* species (Table 9-12).

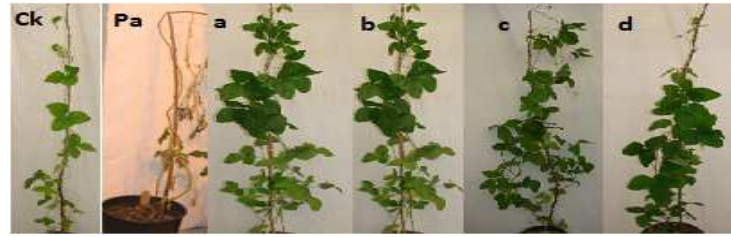


Figure 1: The Effect of the Endophytic Fungi and *Trichoderma* Species on Foliar Wet and Dry Weights
 Ck: Plant, Pa: Pathogen, a: *S. vermifera*, b: *P. indica*, c: *T. harzianum* (T-100) d: *T. viride*



Figure 2: The Effect of the Endophytic Fungi and *Trichoderma* Species on Root Wet and Dry Weights
 Pa: Pathogen, Ck: Plant, a: *S. vermifera*, b: *P. indica*, c: *T. harzianum* (T-100) d: *T. viride*

Table 1: Analysis of Variance Influence of Soybean Root and Foliar Wet and Dry Weights under Greenhouse Condition in the First Time

Variation Source	Freedom Degree	Mean Square			
		P1	P2	P3	P4
Antagonist	46	210.124**	45.206**	65.085**	3.398**
Error	94	19.369	3.386	0.845	0.321
Total	140	-	-	-	-
Coefficient of variation (cv)		12.69%	10.62%	6.02	10.71%

P1: foliar wet weight P2: foliar dry weight P3: root wet weight P4: root dry weight
 *: Significant at p<0/05; **: Significant at p<0/01 and ns: Not significant

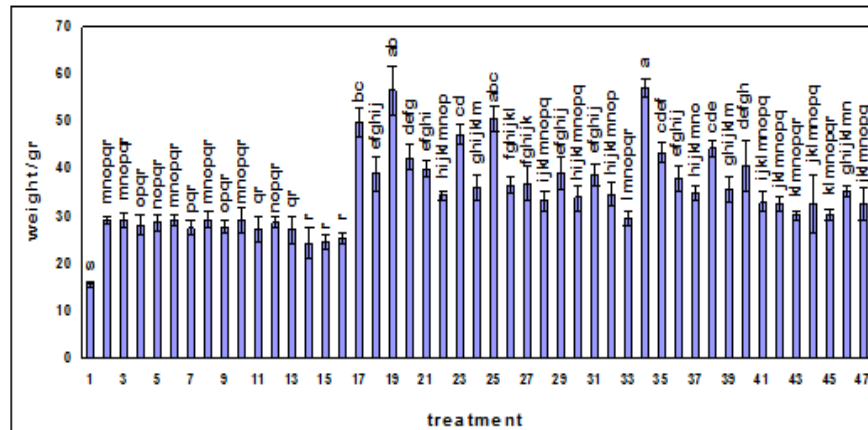


Figure 3: Mean Comparison of Influence of Antagonistic Fungi on Soybean Foliar Wet Weight under Greenhouse Conditions in the First Time by Using LSD Assay. It is Significant at P=0.01

Treatments: 1: Pa, 2: Pa+Pi, 3: Pa+Pi+S, 4: Pa+Pi+S+T100, 5: Pa+Pi+S+TV, 6: Pa+Pi+S+TV+T100, 7: Pa+Pi+T100, 8: Pa+Pi+TV, 9: Pa+Pi+TV+T100, 10: Pa+S, 11: Pa+S+T100, 12: Pa+S+TV, 13: Pa+S+TV+T100, 14: Pa+T100, 15: Pa+TV, 16: Pa+TV+T100, 17: Pi, 18: Pi+Pa, 19: Pi+S, 20: Pi+S+Pa, 21: Pi+S+T100, 22: Pi+S+T100+Pa, 23: Pi+S+TV, 24: Pi+S+TV+Pa, 25: Pi+S+TV+T100, 26: Pi+S+TV+T100+Pa, 27: Pi+T100, 28: Pi+T100+Pa, 29: Pi+TV, 30: Pi+TV+Pa, 31: Pi+TV+T100, 32: Pi+TV+T100+Pa, 33: plant, 34: S, 35: S+Pa, 36: S+T100, 37: S+T100+Pa, 38: S+TV, 39: S+TV+Pa, 40: S+TV+T100, 41: S+TV+T100+Pa, 42: T100, 43: T100+Pa, 44: TV, 45: TV+Pa, 46: TV+T100, 47: TV+T100+Pa Pa: pathogen, Pi: *p. indica*, S: *S. vermifera*, T100: *T. harzianum* (T100) and TV: *T. viride*

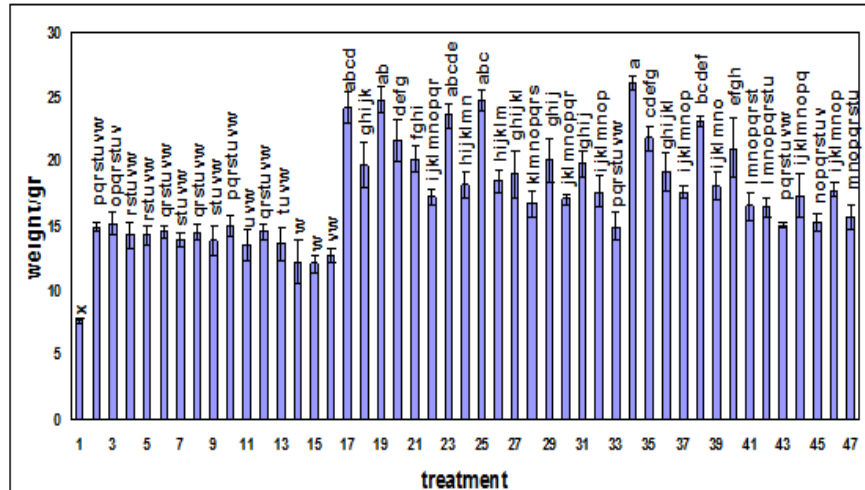


Figure 4: Mean Comparison of Influence of Antagonistic Fungi on Soybean Foliar Dry Weight under Greenhouse Conditions in the First Time by using LSD Assay. It is Significant at P=0.01

Treatments: 1: Pa, 2: Pa+Pi, 3: Pa+Pi+S, 4: Pa+Pi+S+T100, 5: Pa+Pi+S+TV, 6: Pa+Pi+S+TV+T100, 7: Pa+Pi+T100, 8: Pa+Pi+TV, 9: Pa+Pi+TV+T100, 10: Pa+S, 11: Pa+S+T100, 12: Pa+S+TV, 13: Pa+S+TV+T100, 14: Pa+T100, 15: Pa+TV, 16: Pa+TV+T100, 17: Pi, 18: Pi+Pa, 19: Pi+S, 20: Pi+S+Pa, 21: Pi+S+T100, 22: Pi+S+T100+Pa, 23: Pi+S+TV, 24: Pi+S+TV+Pa, 25: Pi+S+TV+T100, 26: Pi+S+TV+T100+Pa, 27: Pi+T100, 28: Pi+T100+Pa, 29: Pi+TV, 30: Pi+TV+Pa, 31: Pi+TV+T100, 32: Pi+TV+T100+Pa, 33: plant, 34: S, 35: S+Pa, 36: S+T100, 37: S+T100+Pa, 38: S+TV, 39: S+TV+Pa, 40: S+TV+T100, 41: S+TV+T100+Pa, 42: T100, 43: T100+Pa, 44: TV, 45: TV+Pa, 46: TV+T100, 47: TV+T100+Pa. Pa: pathogen, Pi: *p. indica*, S: *S. vermifera*, T100: *T. harzianum* (T100) and TV: *T. viride*

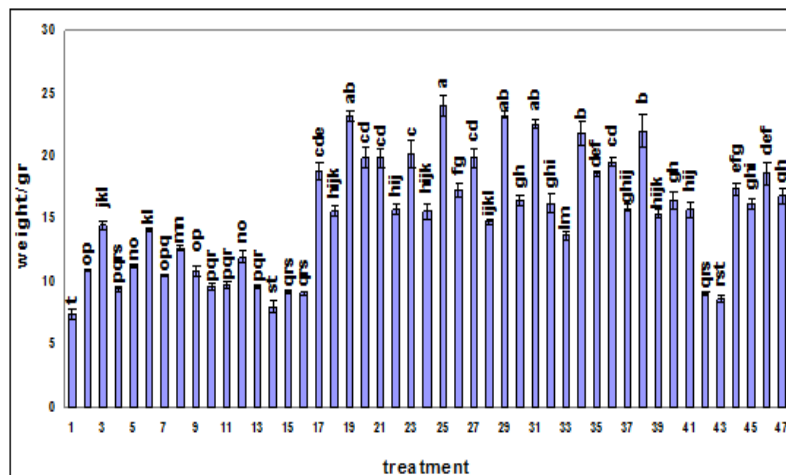


Figure 5: Mean Comparison of Influence of Antagonistic Fungi on Soybean Root Wet Weight under Greenhouse Conditions in the First Time by Using LSD Assay. It is Significant at P=0.01

Treatments: 1: Pa, 2: Pa+Pi, 3: Pa+Pi+S, 4: Pa+Pi+S+T100, 5: Pa+Pi+S+TV, 6: Pa+Pi+S+TV+T100, 7: Pa+Pi+T100, 8: Pa+Pi+TV, 9: Pa+Pi+TV+T100, 10: Pa+S, 11: Pa+S+T100, 12: Pa+S+TV, 13: Pa+S+TV+T100, 14: Pa+T100, 15: Pa+TV, 16: Pa+TV+T100, 17: Pi, 18: Pi+Pa, 19: Pi+S, 20: Pi+S+Pa, 21: Pi+S+T100, 22: Pi+S+T100+Pa, 23: Pi+S+TV, 24: Pi+S+TV+Pa, 25: Pi+S+TV+T100, 26: Pi+S+TV+T100+Pa, 27: Pi+T100, 28: Pi+T100+Pa, 29: Pi+TV, 30: Pi+TV+Pa, 31: Pi+TV+T100, 32: Pi+TV+T100+Pa, 33: plant, 34: S, 35: S+Pa, 36: S+T100, 37: S+T100+Pa, 38: S+TV, 39: S+TV+Pa, 40: S+TV+T100, 41: S+TV+T100+Pa, 42: T100, 43: T100+Pa, 44: TV, 45: TV+Pa, 46: TV+T100, 47: TV+T100+Pa. Pa: pathogen, Pi: *p. indica*, S: *S. vermifera*, T100: *T. harzianum* (T100) and TV: *T. viride*

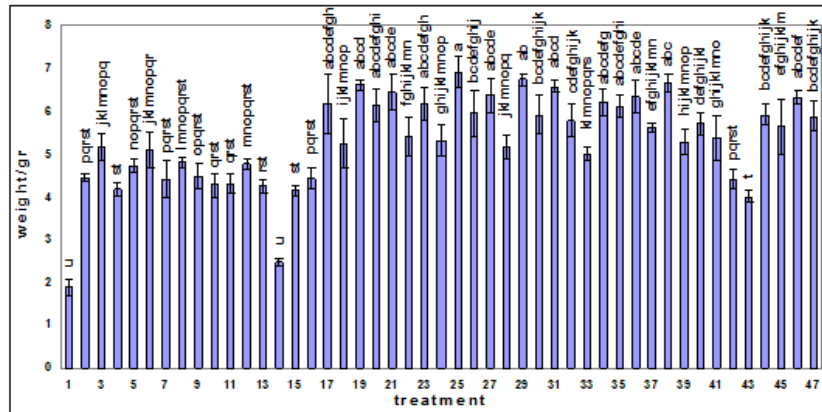


Figure 6: Mean Comparison of Influence of Antagonistic Fungi on Soybean Root Dry Weight under Greenhouse Conditions in the First Time by Using LSD Assay. It is significant at P=0.01

Treatments: 1: Pa, 2: Pa+Pi, 3: Pa+Pi+S, 4: Pa+Pi+S+T100, 5: Pa+Pi+S+TV, 6: Pa+Pi+S+TV+T100, 7: Pa+Pi+T100, 8: Pa+Pi+TV, 9: Pa+Pi+TV+T100, 10: Pa+S, 11: Pa+S+T100, 12: Pa+S+TV, 13: Pa+S+TV+T100, 14: Pa+T100, 15: Pa+TV, 16: Pa+TV+T100, 17: Pi, 18: Pi+Pa, 19: Pi+S, 20: Pi+S+Pa, 21: Pi+S+T100, 22: Pi+S+T100+Pa, 23: Pi+S+TV, 24: Pi+S+TV+Pa, 25: Pi+S+TV+T100, 26: Pi+S+TV+T100+Pa, 27; Pi+T100, 28; Pi+T100+Pa, 29:Pi+TV,30:Pi+TV+Pa, 31: Pi+TV+T100, 32: Pi+TV+T100+Pa, 33:plant, 34: S, 35: S+Pa, 36:S+T100,37:S+T100+Pa,38:S+TV,39:S+TV+Pa,40: S+TV+T100, 41: S+TV+T100+Pa, 42: T100, 43:T100+Pa, 44:TV, 45:TV+Pa, 46:TV+T100, 47:TV+T100+Pa. Pa: pathogen, Pi:*p. indica*, S:*S. vermifera*, T100:*T. harzianum* (T100) and TV:*T. viride*

Table 2: Analysis of Variance Influence of Soybean Root and Foliar Wet and Dry Weights under Greenhouse Condition in the Second Time

Variation Source	Degree Freedom	Mean Square			
		P1	P2	P3	P4
Antagonist	46	334.575**	85.623**	20.109**	1.259**
Error	94	3.729	0.879	0.569	0.518
Total	140	-	-	-	-
coefficient of variation) cv(5.93%	5.81%	3.09	26.01%

P1: foliar wet weight P2: foliar dry weight P3: root wet weight P4: root dry weight
*: Significant at p<0/05; **: Significant at p<0/01 and ns: Not significant

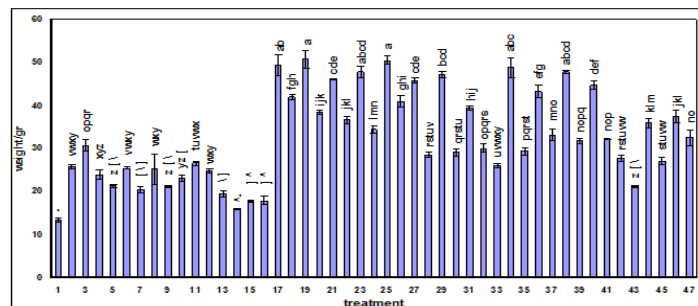


Figure 7: Mean Comparison of Influence of Antagonistic Fungi on Soybean Foliar Wet Weight under Greenhouse Conditions in the Second Time by Using LSD Assay. It is Significant at P=0.01

Treatments: 1: Pa, 2: Pa+Pi, 3: Pa+Pi+S, 4: Pa+Pi+S+T100, 5: Pa+Pi+S+TV, 6: Pa+Pi+S+TV+T100,7:Pa+Pi+T100, 8: Pa+Pi+TV, 9: Pa+Pi+TV+T100, 10: Pa+S, 11: Pa+S+T100, 12: Pa+S+TV, 13: Pa+S+TV+T100, 14: Pa+T100, 15: Pa+TV, 16: Pa+TV+T100, 17: Pi, 18: Pi+Pa, 19: Pi+S, 20: Pi+S+Pa, 21: Pi+S+T100, 22: Pi+S+T100+Pa, 23: Pi+S+TV, 24: Pi+S+TV+Pa, 25: Pi+S+TV+T100, 26: Pi+S+TV+T100+Pa, 27; Pi+T100, 28;

Pi+T100+Pa, 29:Pi+TV,30:Pi+TV+Pa, 31: Pi+TV+T100, 32: Pi+TV+T100+Pa, 33:plant, 34: S, 35: S+Pa, 36:S+T100, 37:S+T100+Pa, 38:S+TV, 39:S+TV+Pa, 40: S+TV+T100, 41: S+TV+T100+Pa, 42: T100, 43: T100+Pa, 44: TV, 45: TV+Pa, 46: TV+T100, 47: TV+T100+Pa Pa: pathogen, Pi:*p. indica*, S:*S. vermifera*, T100:*T. harzianum* (T100) and TV:*T. viride*

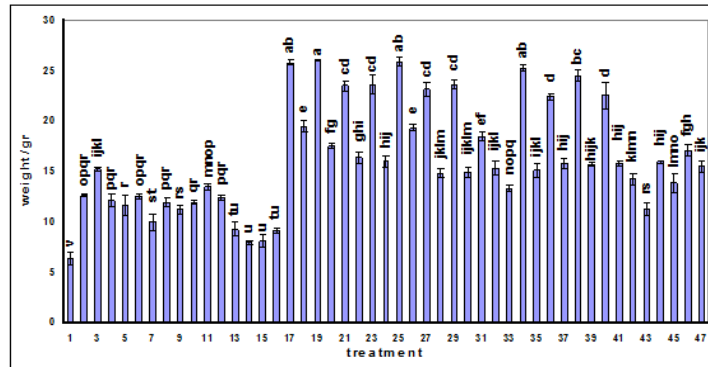


Figure 8: Mean Comparison of Influence of Antagonistic Fungi on Soybean Foliar Dry Weight under Greenhouse Conditions in the Second Time by Using LSD Assay. It is Significant at P=0.01

Treatments: 1: Pa, 2: Pa+Pi, 3: Pa+Pi+S, 4: Pa+Pi+S+T100, 5: Pa+Pi+S+TV, 6: Pa+Pi+S+TV+T100,7:Pa+Pi+T100, 8: Pa+Pi+TV, 9: Pa+Pi+TV+T100, 10: Pa+S, 11: Pa+S+T100, 12: Pa+S+TV, 13: Pa+S+TV+T100, 14: Pa+T100, 15: Pa+TV, 16: Pa+TV+T100, 17: Pi, 18: Pi+Pa, 19: Pi+S, 20: Pi+S+Pa, 21: Pi+S+T100, 22: Pi+S+T100+Pa, 23: Pi+S+TV, 24: Pi+S+TV+Pa, 25: Pi+S+TV+T100, 26: Pi+S+TV+T100+Pa, 27; Pi+T100, 28; Pi+T100+Pa, 29:Pi+TV,30:Pi+TV+Pa, 31: Pi+TV+T100, 32: Pi+TV+T100+Pa, 33:plant, 34: S, 35: S+Pa, 36:S+T100,37:S+T100+Pa,38:S+TV,39:S+TV+Pa, 40: S+TV+T100, 41: S+TV+T100+Pa, 42: T100, 43: T100+Pa, 44: TV, 45: TV+Pa, 46: TV+T100, 47: TV+T100+Pa Pa: pathogen, Pi:*p. indica*, S:*S. vermifera*, T100:*T. harzianum* (T100) and TV:*T. viride*

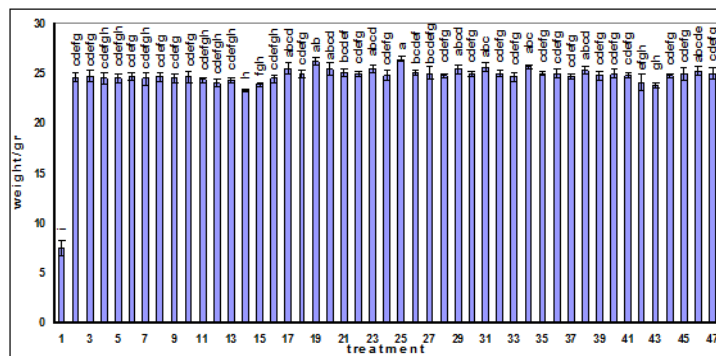


Figure 9: Mean Comparison of Influence of Antagonistic Fungi on Soybean Root Wet Weight under Greenhouse Conditions in the Second Time by Using LSD Assay. It is Significant at P=0.01

Treatments: 1: Pa, 2: Pa+Pi, 3: Pa+Pi+S, 4: Pa+Pi+S+T100, 5: Pa+Pi+S+TV, 6: Pa+Pi+S+TV+T100,7:Pa+Pi+T100, 8: Pa+Pi+TV, 9: Pa+Pi+TV+T100, 10: Pa+S, 11: Pa+S+T100, 12: Pa+S+TV, 13: Pa+S+TV+T100, 14: Pa+T100, 15: Pa+TV, 16: Pa+TV+T100, 17: Pi, 18: Pi+Pa, 19: Pi+S, 20: Pi+S+Pa, 21: Pi+S+T100, 22: Pi+S+T100+Pa, 23: Pi+S+TV, 24: Pi+S+TV+Pa, 25: Pi+S+TV+T100, 26: Pi+S+TV+T100+Pa, 27; Pi+T100, 28; Pi+T100+Pa, 29:Pi+TV,30:Pi+TV+Pa, 31: Pi+TV+T100, 32: Pi+TV+T100+Pa, 33:plant, 34: S, 35: S+Pa, 36:S+T100,37:S+T100+Pa,38:S+TV,39:S+TV+Pa, 40: S+TV+T100, 41: S+TV+T100+Pa, 42: T100, 43: T100+Pa, 44: TV, 45: TV+Pa, 46: TV+T100, 47: TV+T100+Pa Pa: pathogen, Pi:*p. indica*, S:*S. vermifera*, T100:*T. harzianum* (T100) and TV:*T. viride*

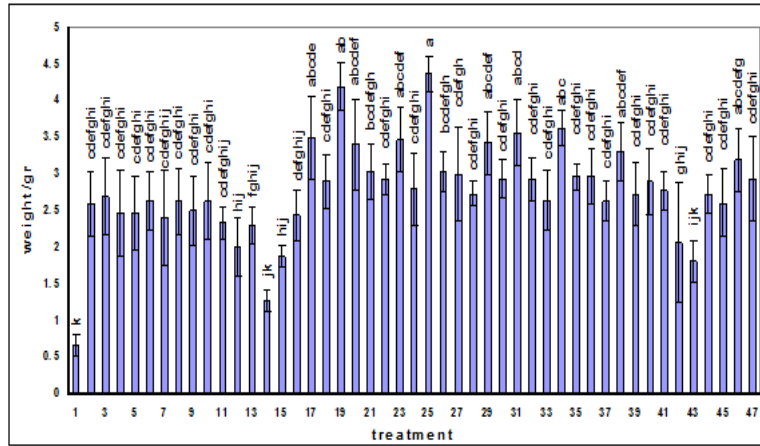


Figure 10: Mean Comparison of Influence of Antagonistic Fungi on Soybean Root Dry Weight under Greenhouse Conditions in the Second Time by Using LSD Assay. It is Significant at P=0.01

Treatments: **1:** Pa, **2:** Pa+Pi, **3:** Pa+Pi+S, **4:** Pa+Pi+S+T100, **5:** Pa+Pi+S+TV, **6:** Pa+Pi+S+TV+T100,**7:**Pa+Pi+T100, **8:** Pa+Pi+TV, **9:** Pa+Pi+TV+T100, **10:** Pa+S, **11:** Pa+S+T100, **12:** Pa+S+TV, **13:** Pa+S+TV+T100, **14:** Pa+T100, **15:** Pa+TV, **16:** Pa+TV+T100, **17:** Pi, **18:** Pi+Pa, **19:** Pi+S, **20:** Pi+S+Pa, **21:** Pi+S+T100, **22:** Pi+S+T100+Pa, **23:** Pi+S+TV, **24:** Pi+S+TV+Pa, **25:** Pi+S+TV+T100, **26:** Pi+S+TV+T100+Pa, **27:** Pi+T100, **28:** Pi+T100+Pa, **29:**Pi+TV,**30:**Pi+TV+Pa, **31:** Pi+TV+T100, **32:** Pi+TV+T100+Pa, **33:**plant, **34:** S, **35:** S+Pa, **36:**S+T100,**37:**S+T100+Pa,**38:**S+TV,**39:**S+TV+Pa, **40:** S+TV+T100, **41:** S+TV+T100+Pa, **42:** T100, **43:** T100+Pa, **44:** TV, **45:** TV+Pa, **46:** TV+T100, **47:** TV+T100+Pa **Pa:** pathogen, **Pi:***p. indica*, **S:***S. vermifera*, **T100:***T. harzianum* (T100) and **TV:***T. viride*

Table 3: Analysis of Variance Orthogonal Comparisons Antagonistic Ability between the Endophytic Fungi and Trichoderma Species in Related to Root and Foliar Wet and Dry Weights under Greenhouse Conditions (The First Time)

Evaluated Index	Mean Traits		V.c.	df	t
	The Endophytes	Trichoderma Species			
AW	41.76	29.79	107.67-	94	**9.99-
AD	20.34	14.9	48.93-	94	**10.86-
RW	16.97	12.56	39.73-	94	**17.65-
RD	5.59	4.79	7.17-	94	**5.16-

AW: foliar wet weight, **AD:** foliar dry weight, **RW:** root wet weight, **RD:** root dry weight, **V.c.:** contrast value, **df:** degree freedom and **t:** treatment. *: Significant at p<0/05; **: Significant at p<0/01 and **ns:** Not significant

Table 4: Analysis of Variance Orthogonal Comparisons Antagonistic Ability between the Endophytic Fungi andTrichoderma Species in Related to Root and Foliar Wet and Dry Weights under Greenhouse Conditions (The Second time)

Evaluated Index	Mean Traits		V.c.	df	t
	The Endophytes	Trichoderma Species			
AW	37.61	25.94	105.00-	94	**22.20-
AD	18.79	12.58	55.90-	94	**24.34-
RW	25.17	24.36	7.30-	94	**3.95-
RD	3.17	2.32	7.63-	94	**4.33-

AW: foliar wet weight, **AD:** foliar dry weight, **RW:** root wet weight, **RD:** root dry weight, **V.c.:** contrast value, **df:** degree freedom and **t:** treatment. *: Significant at p<0/05; **: Significant at p<0/01 and **ns:** Not significant

Table 5: Analysis of Variance Orthogonal Comparisons Antagonistic Ability between *T. viride* and *T. harzianum* (T100) in Related to Root and Foliar Wet and Dry Weights under Greenhouse Conditions (The First Time)

Evaluated Index	Mean Traits		V.c.	df	t
	T.v	T.h			
AW	29.15	29.09	0.20	94	0.03 ns
AD	14.84	14.52	0.97	94	0.37 ns
RW	14.24	8.56	17.03	94	13.10**
RD	5.22	3.62	4.80	94	5.99**

AW: foliar wet weight, AD: foliar dry weight, RW: root wet weight, RD: root dry weight, V.c.: contrast value, df: degree freedom, t: treatment, T.v.:*T. viride* and T.h.:*T. harzianum* (T100) *: Significant at p<0/05; **: Significant at p<0/01 and ns: Not significant

Table 6: Analysis of Variance Orthogonal Comparisons Antagonistic Ability between *T. viride* and *T. harzianum* (T100) in Related to Root and Foliar Wet and Dry Weights under Greenhouse Conditions (The Second Time)

Evaluated Index	Mean Traits		V.c.	df	t
	T.v	T.h			
AW	26.93	21.63	15.90	94	5.82**
AD	12.68	11.16	4.57	94	3.44**
RW	24.51	23.71	2.40	94	2.25*
RD	2.4	1.71	2.07	94	2.03*

AW: foliar wet weight, AD: foliar dry weight, RW: root wet weight, RD: root dry weight, V.c.: contrast value, df: degree freedom, t: treatment, T.v.:*T. viride* and T.h.:*T. harzianum* (T100) *: Significant at p<0/05; **: Significant at p<0/01 and ns: Not significant

Table 7: Analysis of Variance Orthogonal Comparisons Antagonistic Ability between *P. indica* and *S. vermifera* in Related to Root and Foliar Wet and Dry Weights under Greenhouse Conditions (The First Time)

Evaluated Index	Mean Traits		V.c.	df	t
	P.i	S.v			
AW	39.31	43.26	-11.87	94	-1.91 ^{ns}
AD	19.56	20.94	-4.13	94	-1.59 ^{ns}
RW	15.09	16.68	-4.80	94	-3.69**
RD	5.27	5.52	-0.73	94	-0.92 ^{ns}

AW: foliar wet weight, AD: foliar dry weight, RW: root wet weight, RD: root dry weight, V.c.: contrast value, df: degree freedom, t: treatment, P.i:*P. indica* and S.v:*S. vermifera* *: Significant at p<0/05; **: Significant at p<0/01 and ns: Not significant

Table 8: Analysis of Variance Orthogonal Comparisons Antagonistic Ability between *P. indica* and *S. vermifera* in Related to Root and Foliar Wet and Dry Weights under Greenhouse Conditions (The Second Time)

Evaluated Index	Mean Traits		V.c.	df	t
	P.i	S.v			
AW	39.04	33.80	15.73	94	5.76**
AD	19.30	17.44	5.57	94	4.20**
RW	25	25.07	0.23	94	-0.22 ^{ns}
RD	3	3.07	-0.23	94	-0.23 ^{ns}

AW: foliar wet weight, AD: foliar dry weight, RW: root wet weight, RD: root dry weight, V.c.: contrast value, df: degree freedom, t: treatment, P.i:*P. indica* and S.v:*S. vermifera* *: Significant at p<0/05; **: Significant at p<0/01 and ns: Not significant

Table 9: Analysis of Variance Orthogonal Comparisons between Biocontrol Ability of *Trichoderma* Species and Time of Inoculation Pathogen 10 before or after Inoculation of *Trichoderma* Species under Greenhouse Conditions (The First Time)

Evaluated Index	Mean Traits		V.c.	df	t
	Be.	Af.			
AW	24.73	31.09	-19.00	94	-3.05**
AD	12.27	15.3	9.07	94	-3.48**
RW	8.77	13.88	-15.33	94	-11.79**
RD	3.67	5.16	-4.47	94	-5.57**

AW: foliar wet weight, AD: foliar dry weight, RW: root wet weight, RD: root dry weight, V.c.: contrast value, df: degree freedom, t: treatment, Be.: inoculation pathogen 10 before inoculation of *Trichoderma* species and Af.: inoculation pathogen 10 after inoculation of *Trichoderma* species *: Significant at $p < 0/05$; **: Significant at $p < 0/01$ and ns: Not significant

Table 10: Analysis of Variance Orthogonal Comparisons between Biocontrol Ability of *Trichoderma* Species and Time of Inoculation Pathogen 10 before or after Inoculation of *Trichoderma* Species under Greenhouse Conditions (The Second Time)

Evaluated Index	Mean Traits		V.c.	df	t
	Be.	Af.			
AW	17.22	26.94	-29.17	94	-10.68**
AD	8.41	13.57	-15.50	94	-11.69**
RW	23.85	24.55	-2.10	94	-1.97*
RD	1.85	2.44	-1.77	94	-1.74 ^{ns}

AW: foliar wet weight, AD: foliar dry weight, RW: root wet weight, RD: root dry weight, V.c.: contrast value, df: degree freedom, t: treatment, Be.: inoculation pathogen 10 before inoculation of *Trichoderma* species and Af.: inoculation pathogen 10 after inoculation of *Trichoderma* species *: Significant at $p < 0/05$; **: Significant at $p < 0/01$ and ns: Not significant

Table 11: Analysis of Variance Orthogonal Comparisons between Biocontrol Ability of the Endophytic Fungi and Time of Inoculation Pathogen 10 before or after Inoculation of the Endophytic Fungi under Greenhouse Conditions (The First Time)

Evaluated Index	Mean Traits		V.c.	df	t
	Be.	Af.			
AW	29.09	41.66	-37.73	94	-6.06**
AD	15.01	21.01	-18.00	94	-6.92**
RW	11.66	18	-19.00	94	-14.61**
RD	4.62	5.82	-3.60	94	-4.49**

AW: foliar wet weight, AD: foliar dry weight, RW: root wet weight, RD: root dry weight, V.c.: contrast value, df: degree freedom, t: treatment, Be.: inoculation pathogen 10 before inoculation of the endophytic fungi and Af.: inoculation pathogen 10 after inoculation of the endophytic fungi *: Significant at $p < 0/05$; **: Significant at $p < 0/01$ and ns: Not significant

Table 12: Analysis of Variance Orthogonal Comparisons between Biocontrol Ability of the Endophytic fungi and Time of Inoculation Pathogen 10 before or after Inoculation of the Endophytic Fungi under Greenhouse Conditions (The Second Time)

Evaluated Index	Mean Traits		V.c.	df	t
	Be.	Af.			
AW	26.59	36.59	30.00-	94	**10.99-
AD	13.29	17.40	12.33-	94	**9.30-
RW	24.64	25.09	1.33-	94	1.25 ns-
RD	2.64	3.09	1.33-	94	1.31 ns-

AW: foliar wet weight, AD: foliar dry weight, RW: root wet weight, RD: root dry weight, V.c.: contrast value, df: degree freedom, t: treatment, Be.: inoculation pathogen 10 before inoculation of the endophytic fungi and Af.: inoculation pathogen 10 after inoculation of the endophytic fungi *: Significant at $p < 0/05$; **: Significant at $p < 0/01$ and ns: Not significant

DISCUSSIONS

We concluded that interaction between pathogen and antagonists led to increase root biomass and overall growth of plants. We demonstrated the potential of *P. indica* and especially *S. vermifera* to colonize soybean growing in pot cultures *In vivo*. Our study results were in agreement with findings of Barazani *et al.* (2005), which demonstrated that *Nicotiana attenuata* plants inoculated with *S. vermifera* flowered earlier, produced more flowers and matured more seed capsules than did non-inoculated plants. In this study, plants inoculated with *S. vermifera* started to flower 45 days after germination, 2 days earlier than plants inoculated with *P. indica*. Several reports have shown the ability of *P. indica* to colonize roots of different plants and demonstrated its growth-promoting effects (Sahay and Varma, 1999; Varma *et al.*, 1999; Rai *et al.*, 2001; Kumari *et al.*, 2003 and Peskan-Berghofer *et al.*, 2004). Other work revealed that inoculation of plants with *P. indica* caused a significant reduction in disease symptoms for the stem-base pathogen *Pseudocercospora herpotrichoides* on wheat under greenhouse and the field (Serfling *et al.*, 2006). In another similar study, Waller *et al.* (2005) showed that barley plants inoculated with *P. indica* have resistance to a vascular (*Fusarium culmorum*) and a leaf pathogen (*Blumeria graminis*), in addition to an increase in yield and salt stress tolerance. In addition, we also observed the reported increase in root and foliar wet and dry weights in plants inoculated with *Trichoderma* species especially *T. viride* fungus were significantly greater than plants inoculated with pathogen alone.

Trichoderma species are free-living fungi that are common in soil and root ecosystems (Sivasithamparan and Ghisalberti, 1998). Following application of *Trichoderma* species in Lettuce bean, cucumber and pepper has been showed increased growth response under greenhouse and field conditions (Baker, 1989; Kleifeld and Chet, 1992; Inbar *et al.*, 1994; Ousley *et al.*, 1994; Vazquez *et al.*, 2000 and Yedidia *et al.*, 2001). Recently, Jyotsna *et al.*, (2008), demonstrated a significant increase in growth of chickpea plants inoculated with *T. harzianum* for each of the parameters including plant height, dry weight, chlorophyll components and control of charcoal rot in chickpea plants caused by *M. phaseolina* in greenhouse conditions. Our findings indicate that *Trichoderma* species can control *M. phaseolina* and increase growth and the yield of economically important crops.

Therefore, These antagonistic fungi can use for commercial application. In addition, we in present work, demonstrated that root and foliar wet and dry weights of soybean increased when antagonistic fungi inoculated earlier from pathogen in pot cultures under greenhouse experiments. Our finding was in agreement with previous studies about that *Chaetomium* and *Phoma* endophytes of wheat, when these fungi were previously inoculated in plants, reduced severity of foliar disease caused by *Puccinia* and *Pyrenophora* spp. was observed and, the same protective effect was observed when only endophytic culture filtrates were applied to the plants (Dingle and McGee, 2003 and Istifadah and McGee, 2006). Experiments where plant protection against pathogenic fungi is observed after the inoculation of plants with endophytes, as well as after the application of endophytic culture, suggest that the endophytes may produce an antifungal compound or a substance that induces plant defense mechanisms in the plant (Liu *et al.*, 2001; Park *et al.*, 2005; Inacio *et al.*, 2006; Kim *et al.*, 2007 and Zabalgoitia, 2008).

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