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Biological control of soybean charcoal root rot disease using bacterial and fungal antagonists *In Vitro* and greenhouse condition

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Abstract: Soybean, *Glycine max*, is susceptible to a large number of disease agents such as seedling and root pathogens that cause serious damages to this crop plant. One of these soil-borne pathogens is *Macrophomina phaseolina*, the causal agent of charcoal root rot. In this study, two bacteria, *Pantoea agglomerans* and *Bacillus* sp. BIN, and a fungus, *Trichoderma harzianum* T100, as potential biocontrol agents, and maneb fungicide, were evaluated against soybean charcoal rot disease in *In Vitro* and greenhouse conditions. All antagonists inhibited growth of the pathogen in dual culture test by 73.8, 63.3 and 55.3%, respectively. Data from greenhouse experiments showed that in the presence of pathogen all antagonists increased the growth indices of soybean in both pasteurized and non-pasteurized soil. Reductions of microsclerotia coverage on soybean root and stem by *P. agglomerans*, *Bacillus* sp. and *T. harzianum* were up to 62.5, 87.6 and 62.5%, respectively and for maneb fungicide was 87.6% in pasteurized soil. The overall results of this study show high capability of used antagonists in reduction of initial inoculums for next season of this monocyclic disease.

Keywords: *Bacillus* sp. BIN, Biological control, *Pantoea agglomerans*, *Macrophomina phaseolina*, maneb fungicide, *Trichoderma harzianum* T100.

Introduction

Soybean, *Glycine max* (L.) Merrill, is susceptible to a large number of disease agents such as seedling and root pathogens that cause serious damages to this crop. One of these soil-borne pathogens is *Macrophomina phaseolina* (Tassi) Goid, the causal agent of charcoal root rot. This polyphagous pathogen attacks and infects more than 500 plant species in 100 families of monocots and dicots (Dhingra and Sinclair, 1977; Jana *et al.,* 2003). Fungal microsclerotia are primary inoculum source for infection which survive 2-15 years in soil and plant residues depending on environmental conditions (Cook *et al.,* 1973; Papavizas, 1977; Campbell and van der Gaag, 1993). In heavy infestation, the host plants are destroyed by fungal toxins, such as phaseolinone and vascular obstruction by mycelium (Bhattacharya *et al.,* 1994). Since the pathogen is soil-borne with high saprophytic ability, effective strategies for disease control are not available. Application of control methods are typically for reducing soil microsclerotia and minimizing inoculum contact with host roots. Use of chemicals is not recommended since effective chemicals that can protect plants from pathogen damage are not available. Use of microbial antagonists and biological control has therefore been considered and has been more or

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less successful (Gupta *et al.,* 2002; Deshwal *et al.,* 2003; Adekunle *et al.,* 2006). Thus, several studies have considered using biocontrol agents against *M. phaseolina*.

Plant Growth Promoting Rhizobacteria (PGPR) promote plant growth directly or indirectly through biological control of pathogens, production of phytohormones and by antagonistic activities such as antibiosis, hyperparasitism and competition for nutrients and site (Chet *et al.,* 1990; Whipps, 1992; Handelsman and Stab, 1996; Shoda, 2000). One of the most common and effective antagonistic fungal species used in plant disease control is *Trichoderma harzianum* Rafai species (Monte, 2001; Vinale *et al.,* 2008). The complex mechanisms of mycoparasitism, which include directed growth of *Trichoderma* toward target fungi, attachment and coiling on target fungi and production of a wide range of antifungal extracellular enzymes such as chytinolytic and gluconolytic enzymes, are fundamental aspects of antagonism in *Trichoderma* spp. against phytopathogens (Papavizas, 1985; Howell, 1998; Harman and Kubicek, 1998; Howell, 2003). The objective of this study was to evaluate plant growth promoting and antifungal activities of *Pantoea agglomerans, Bacillus sp.* BIN and *Trichoderma harzianum* T100 on soybean inoculated with charcoal rot causal agent.

Materials and Methods

Selection of Pathogenic and Antagonistic Fungal Isolates

In this study, *M*acrophomina *phaseolina* M21 isolated from soybean plants in Gorgan province of Iran was used (Vasebi, 2008). M21 isolate was evaluated in pathogenicity test as one of the most virulent isolates of this pathogenic fungus. The antagonistic fungal isolate, *Trichoderma harzianum* T100, was obtained from plant pathology department of Tarbiat Modares University, Tehran, Iran.

Selection of Bacterial Antagonists

For obtaining antagonistic bacteria, healthy soybean plants and their rhizosphere soil were collected from Gorgan province fields in 2006. Serial dilutions from washings of collected soil samples and foliar parts were prepared separately and spread on Nutrient Agar medium (NAM). The root nodules of soybeans were surface disinfested with 2% NaOCl for 20 sec, rinsed in sterile distilled water (3 min, 4 times). The nodules were then crushed and streaked on NA culture medium. Petri dishes were incubated at 26 °C for 48 h. All bacterial colonies were purified and maintained on NA at 4 °C. The isolates were characterized based on their morphological, physiological and biochemical characteristics (Schaad *et al.,* 2001).

Investigation of Antagonistic Mechanisms

Dual Culture Test

For selection of bacterial antagonists, antagonistic activity of bacterial strains was tested against *M. phaseolina* using preliminary dual culture procedure. Four bacterial strains were placed on Petri dishes at equal distances from each other, around the periphery of the central mycelial plug. In control plates only the pathogenic fungus was grown. When control plates were completely covered by the fungus, the experiment was stopped and the bacterial antagonists were selected based on their ability to produce inhibition zone against *M. phaseolina*. In complementary dual culture experiment a suspension of each antagonistic bacterial strain (10^9 cfu/ml) was smeared in a circular pattern on the inner periphery of the Petri dishes (9 cm diameter) containing fresh PDA. After 24 hours of bacterial growth, a 3-day-old PDA plug of *M. phaseolina* with mycelium and microsclerotia was placed in the center of Petri dishes. Distilled water was spread in a circular pattern on the edge of control Petri dishes instead of bacterial strain. The dishes were incubated at 28 °C. Three days after incubation, when *M. phaseolina* in controls almost completely covered the plates, radial growth of the different treatments was measured and percent inhibition of average radial growth by the antagonists was calculated relative to growth of the controls using the

formula (1) (Vasebi, 2008). The complementary dual culture experiment consisted of 40 treatments each with three replicates in a completely randomized (CRD) design.

Formula (1) $IG = ((C-T) / C) \times 100$

IG: Inhibition of Growth (%); C: Growth in Control (radial growth of fungus in control); T: radial growth of fungus in Treatment (dual culture).

For fungal antagonist, a test was performed according to Morton and Stroube (1955) as follows: A 5-mm plug of PDA containing 3-day-old colonies of *T. harzianum* T100 and a plug of PDA medium containing 3-day-old colonies of *M. phaseolina* with mycelia and microsclerotia were placed at the opposite sides of Petri dishes. Plugs of PDA medium containing *M. phaseolina* alone were used in control dishes. The percentage inhibition of pathogen radial growth relative to controls was determined after three days using the formula (1). The experiment consisted of two treatments each with three replicates in a completely randomized (CRD) design.

Colonization Test

To test the ability of *Trichoderma* in parasitizing *M. phaseolina*, a thin layer of PDA medium was prepared in Petri dishes containing sterile microscope slides. Five-mm plugs of PDA containing pathogenic and antagonistic fungal colonies were then placed on either side of microscope slide in Petri dishes. The Petri dishes were incubated in the dark at 28 °C for 24 hours. Antagonist-pathogen connection sites on slides were studied under a light microscope.

Volatile Metabolites Production Experiment

Test for antagonistic bacteria was performed following Fernando *et al.,* (2005) procedure. One hundred-microliter suspension of each antagonistic bacterial strain (10^9 cfu/ml) was smeared on fresh NAM. Sterile distilled water was used as a control. A 3-day-old PDA plug of *M. phaseolina* with mycelium and microsclerotia was placed in the center of PDA medium. Dishes containing fungal pathogen were placed inversely on NA medium dishes containing bacterial strains and were sealed with parafilm. Sealed plates were maintained in dark at 28 °C for three days. Inhibition percent of fungal mycelial growth was calculated by using the formula (1). The experiment was repeated twice and was performed in a completely randomized design with three replicates and three treatments. Production of volatile metabolites by *Trichoderma* isolate was evaluated following the methods described by Dennis and Webster (1971). A 3-day-old PDA plug each of *M. phaseolina* and *T. harzianum* was placed separately in the center of PDA medium. PDA medium without fungal antagonist was used as a control. Petri dishes containing fungal pathogen were then inverted on plates of *Trichoderma* isolate and sealed with parafilm. Petri dishes were incubated in the dark at 28 °C for three days. Radial growth of pathogen was recorded daily and inhibition percent of mycelial growth compared with control was calculated using formula (1). The experiments were performed with two treatments and three replications in a completely randomized design (CRD).

Antibiotic Production

Production of antibiotics by bacterial antagonists was determined using Kraus and Lopper (1990) method. One hundred-microliter suspension of each antagonistic bacterial strain (10^9 cfu/ml) was cultured on fresh PDA. The Petri dishes were incubated in the dark at 26 °C for 72 hours. After three days of bacterial growth, the colonies were wiped off by sterile cotton swap, the plates were then exposed to Chloroform vapor and UV light for two hours and air flow under sterile condition 30 min, in sequence. Sterile distilled water was used as a control. Then a 3-day-old PDA plug of *M. phaseolina* with mycelium and microsclerotia was placed in the center each of the above Petri dishes. The Petri dishes were incubated at 28 °C for three days. The experiment was performed with three treatments each with three replicates in a

completely randomized design (CRD). The percentage of pathogen growth inhibition was calculated by applying formula (1).

Selection of Fungicide

Two concentrations (0.5 and 1 g l^{-1}) each of maneb (WP 80%), thiram (WP 80%) and carboxin thiram (WP 75%) fungicides were added separately to PDA culture medium and a plug of 3-day-old culture of pathogen was placed at the center of Petri dishes. PDA medium without fungicide served as control. The Petri dishes were incubated in the dark at 28 °C for five days. After this time, growth of *M. phaseolina* was evaluated in comparison with control. The experiment was conducted with seven treatments and three replicates in a completely randomized design (CRD), which included; 1) control; 2) maneb 0.5 g l^{-1}; 3) maneb 1 g l^{-1}; 4) thiram 0.5 g l^{-1}; 5) thiram 1 g l^{-1}; 6) carboxin thiram 0.5 g l^{-1}; 7) carboxin thiram 1 g l^{-1}.

Pot Experiments

The effectiveness of fungal and bacterial antagonists and maneb fungicide on inhibition of pathogen and promotion of soybean growth indices was evaluated In greenhouse examinations, Plastic pots (17 × 20 × 20 cm) were filled with pasteurized and non-pasteurized sandy soils, perlite and peat moss (1: 1: 1). Inoculums of pathogen and antagonistic fungus were prepared by growing them on rice grains in 250 ml flasks as follows: The grains were soaked in distilled water, autoclaved twice (121 °C for 45 min) and inoculated with three agar discs (5-mm-diameter) of 5-day-old fungal culture. Flasks were incubated at 28 ± 1 °C in dark for 15 days. The inoculums were then mixed with soil (10 g kg^{-1} soil). Bacterial inoculum was added to the soil as suspension. Bacterial antagonists were grown in 500 ml flasks containing 250 ml NB (Nutrient Broth) at 26 °C for 48 h by shaking at 150 rpm. The cells were harvested and their concentration was adjusted to 10^9 cfu ml^{-1}. Bacterial strains and maneb fungicide (1 g/lit) were added to respective pots every 14 days after planting at the rate of 125 ml for each pot. Four soybean seedlings at three-leaf growth stage (grown earlier) were transplanted to each pot and the pots were kept at 25-33 °C and allowed to grow for100 days. There were 10 treatments in each experiment of pasteurized and non-pasteurized soils. Treatments included: 1) control (no pathogen and no biocontrol agents); 2) pathogen; 3) *T. harzianum* T100; 4) *T. harzianum* T100 + pathogen; 5) *P. agglomerans*; 6) *P. agglomerans* + pathogen; 7) *Bacillus* sp. BIN; 8) *Bacillus* sp. BIN + pathogen; 9) maneb; 10) maneb + pathogen. There were three replications for ach treatment. The following variables were measured: root and foliage fresh and dry weights and microsclerotial coverage percent on roots and stems. The experiment was conducted twice during 2007-08. In both trials, the experimental design was randomized complete block (RCB) design. For -statistical analysis data were subjected to analysis of variances (ANOVA) and the means were compared by LSD test using MSTATC statistical software (Michigan State University, version 1.42). Level of significance in different treatments was determined at 5% probability (P = 0.05).

Results

***In Vitro* Assays**

Two hundred eighty bacterial strains, isolated from samples, were purified. Eighty nine isolates in primary dual culture tests exhibited growth inhibition of the pathogen. Among them, 39 isolates showed more than 50% inhibition of which two isolates identified as *Pantoea agglomerans* and *Bacillus* sp. BIN (Vasebi *et al.,* 2009) were selected for *in vitro* and *in vivo* experiments. The results of dual culture tests showed that *P. agglomerans* and *Bacillus* sp. BIN decreased the mycelial growth of pathogen by 73.8% and 63.3%, respectively compared to control. *Trichoderma* isolate T 100 stopped the mycelial growth of pathogen, and produced spore on the mycelia of *M. phaseolina*. Inhibition of *M. phaseolina* growth in dual culture test was calculated to be 55.3%

in comparison with control (Figure 1). Examination of antagonist and pathogen mycelial connection by Light microscope showed that *T. harzeanum* T100 penetrated mycelial cells of *M. phaseolina*, moved inside and lysed them (Figure 2). Volatile metabolites produced by all antagonists including *P. agglomerans* and BIN isolate inhibited the growth of *M. phaseolina* up to 34.5% and 25%, respectively. Results also showed that the amount of volatile metabolites production and their effects on inhibition of pathogen growth and microsclerotia formation were increased with time and colonization by the antagonist T100. The amount of pathogen growth inhibition was estimated at 0, 7.4 and 12.3%, respectively in three successive days. Production of antibiotics by *Bacillus* isolate and *P. agglomerans*, inhibited the mycelial growth of pathogen by 100% and 89%, respectively. In addition, results showed that maneb fungicide at both concentrations inhibited the growth of *M. phaseolina* by 100%, but thiram and carboxin thiram didn't reduce mycelial growth of pathogen at any of the concentrations (Figure 3). Thus, maneb at concentration of 1 g l^{-1} was used in the greenhouse experiments.

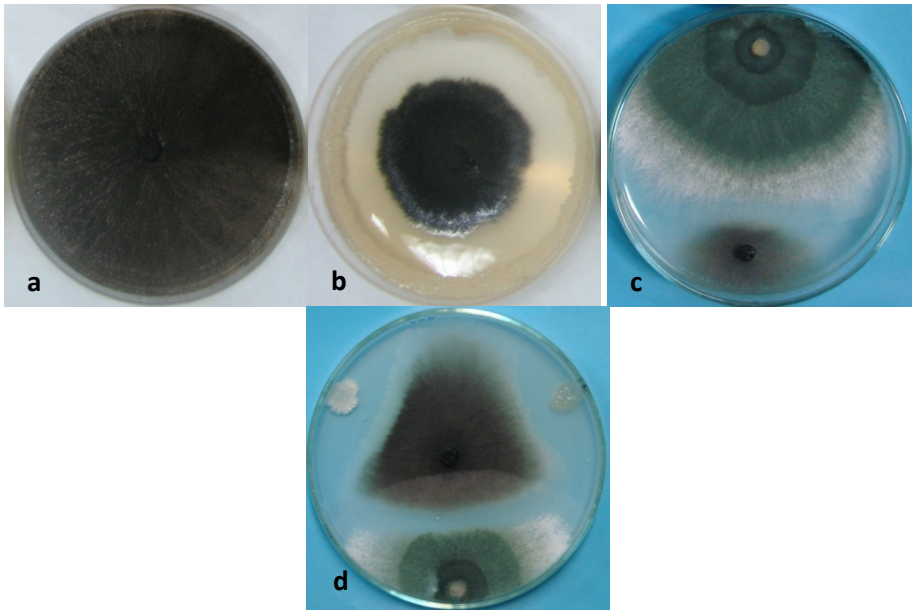

Figure 1 Dual culture test a: *Macrophomina phaseolina* (control), b: *Bacillus* sp. BIN, C: *Trichoderma harzianum* T100 and d: *Pantoea agglomerans*, *Bacillus* sp. BIN and *T. harzianum* T100.

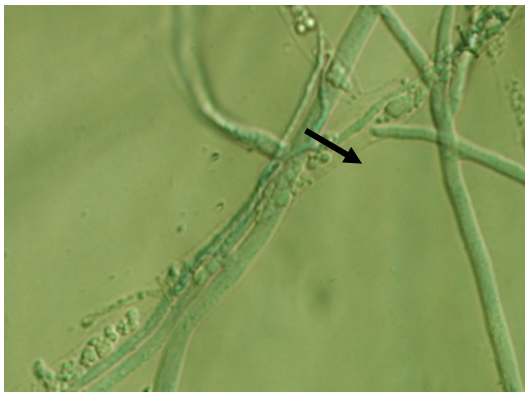

Figure 2 Mycelial interaction between *Trichoderma harzianum* T100 and *Macrophomina phaseolina,* Arrows show penetration sites of antagonist and lysis of pathogen cells.

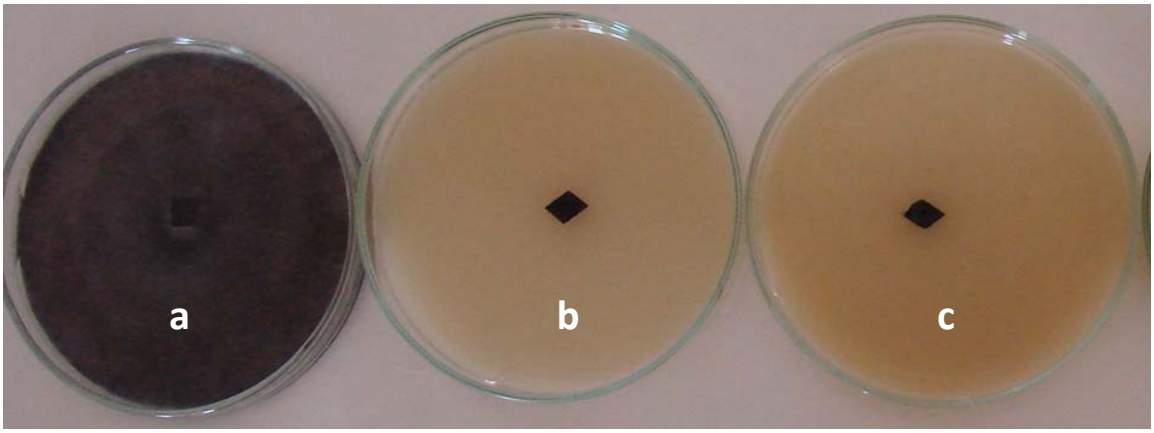

Figure 3 Mycelial growth inhibition of *Macrophomina phaseolina,* by different concentrations of maneb fungicide a: control (no fungicide); b: maneb (0.5 g l^{-1}) and c: maneb (1 g l^{-1}).

Greenhouse Studies

Analysis of greenhouse results showed that there were statistical differences among treatments in increasing soybean (Williams Cultivar) growth indices and inhibition of charcoal rot causal agent at 5% level in LSD test (Table 1). In pasteurized soil and presence of pathogen, *T. harzianum* T100 was the most effective antagonist against *M. phaseolina* and increased soybean fresh and dry root weights by 58.5% and 56.6% and fresh and dry foliage weights by 26.4% and 16.4%, respectively in comparison with control. Among bacterial antagonists, *Bacillus* sp. BIN was more effective than *P. agglomerans*. BIN isolate in pasteurized soil increased soybean fresh and dry root weights by 64.5% and 19.3% and fresh and dry foliage weights by 26.3% and 3.5%, respectively in comparison with control (Figure 4). Microsclerotial covering of root and stem was decreased by all antagonists by 72.7% in non-pasteurized and 62.5% by *P. agglomerans* and *T. harzianum* T100 and 87.6% by *Bacillus* sp. BIN in pasteurized soil (Table 1). Maneb in the presence of pathogen increased the host plant growth indexes by 87.6% and 82% in pasteurized and non-pasteurized soils, and decreased root and stem microsclerotial coverage by 87.6% and 82.3% in pasteurized and non-pasteurized soil, respectively.

Figure 4 Effects of a: *Baccillus* sp. BIN, b: *Trichoderma harzianum* T100, c: *Pantoea agglomerans* on growth of foliar part of soybean in presence of *Macrophomina phaseolina* compared with control (d: *M. phaseolina* only) in greenhouse.

Table 1 Effects of *Pantoea agglomerans, Bacillus* sp. BIN, *Trichoderma harzianum* T100 and maneb fungicide alone and in combination with *Macrophomina phaseolina* on soybean growth indices and microsclerotia coverage on soybean root and stem in pasteurized and non-pasteurized soil in greenhouse 100 days after transplanting.

No	Treatment	FRW(g)		FAW(g)		DRW(g)		DAW(g)		MC	
		S	NS	S	NS	S	NS	S	NS	S	NS
1	**C**	27.8a	21abc	128.7bcde	125bc	4.8ab	4.4b	43a	45.2ab	-	--
2	**M**	11.2b	14.7bc	105.3e	115bc	2.7c	3.7bc	34ab	38.9bcd	53.3%	73.3%
3	**P.a**	21.3ab	23.5ab	156.6ab	144.6bc	4.7abc	4.4ab	36ab	35.8bcd	-	-
4	**P.a + M**	11.4b	11c	114de	102c	2.9bc	2.6c	31.4b	29d	20%	20%
5	**B**	23.7a	26.4ab	178.7a	205.3a	4.6abc	4.3bc	41.4a	57a	-	-
6	**B + M**	18.2ab	18.6abc	133bcde	162.7ab	3.3abc	3.7bc	35.3ab	43bc	6.6%	20%
7	**T**	18.8ab	16.4abc	141bcd	157.3abc	4abc	2.8bc	34.7ab	38bcd	-	-
8	**T + M**	17.8ab	18.6abc	133bcde	137.3bc	4.3abc	3.6bc	39.7ab	31.4cd	20%	13%
9	**F**	21.4ab	27.6a	144.3bc	121.7bc	4.7abc	6.1a	39.2ab	35bcd	-	-
10	**F + M**	23.9a	23.2ab	126.3cde	137.7bc	5.1a	4.5ab	35.1ab	35.4bcd	6.6%	20%

Means with different letters in each column are significantly different in fisher′s LSD test (p = 0.05). Data are the means of three replicates. FRW: Fresh Root Weight (g); DRW: Dry Root Weight (g); FAW: Fresh Foliar parts Weight (g); DAW: Dry Foliar parts Weight (g); S: pasteurized soil; NS: non- pasteurized soil; MC: Microsclerotial Coverage; M: *M. phaseolina*; P. a: *P. agglomerans*; B: *Bacillus* sp. BIN; T: *T. harzianum* T100; F: maneb.

Discussion

Soil-borne plant pathogens such as bacteria, fungi and nematodes annually cause major economical losses to soybean plants. *Macrophomina* charcoal root rot on soybean is one of the most important diseases in Gorgan province of Iran. The lack of chemical fungicides against *M. phaseolina* and the environmental contaminations by chemicals has encouraged researchers around the world to have an increasing interest in biocontrol agents. Rhizosphere microorganisms provide primary defense line against pathogen attacks (Weller, 1988). The predominantly reported biocontrol agents for managing *M. phaseolina* include mycoparasitic fungi and bacteria such as *Trichoderma* spp., *Bacillis* spp. and *Pseudomonas* spp. (Gupta *et al.,* 2002; Hajieghrari *et al.,* 2008; Singh *et al.,* 2008). In this study, we reported antagonistic effects of three different bacterial and fungal antagonists against *Macrophomina* charcoal rot on soybean. High reduction of pathogen growth in *In Vitro* tests was observed by all antagonists. Penetration, progression, colonization and sporulation of *Trichoderma* isolate on *M. phaseolina* were also observed. Similar results with other fungi have previously been reported (Harman and Kubicek, 1998; Yedidia *et al.,* 1999; Eteberian *et al.,* 2000; Benitez *et al.,* 2004; Harman *et al.,* 2004). Volatile metabolites produced by *Bacillus* sp. BIN, *P. agglomerans* and *T. harzianum* T100 inhibited mycelial growth of the pathogen in our study. Inhibitory effects of antagonists' metabolites have been reported in various studies (Ghisalberti and Sivasithamparam, 1991; Pal *et*

al., 2001; Monte and Liobell, 2003). Production of antibiotics by bacterial antagonists against *M. phaseolina* could be considered as one of the main mechanisms of antagonism by these biocontrol agents.

In vivo assays both pasteurized and non-pasteurized soils were used. soils pasteurization, autoclaving or fumigation usually allows the pathogen to re-clonize (Burgess *et al.,* 1988). Some soils may be toxic after pasteurization (Armstrong and Armstrong, 1975) but the growth of healthy seedlings in the pasteurized soil suggests that pasteurization did not result in phytotoxicity. Our results showed that soybean growth indices were improved in pasteurized soil in comparison with non-pasteurized soil. One possible explanation is that some microorganisms in non-pasteurized soil had negative effects on applied antagonists and decreased their efficiency which is similar to field conditions. In laboratory experiments, *P. agglomerans* showed higher antagonistic effects on the pathogen than the other antagonists. However, in greenhouse experiments *T. harzianum* T100 was the more effective antagonist in inhibition of *Macrophomina* charcoal rot and increased plant growth indices next to maneb fungicide. In this study, it was determined that direct interaction of antagonist isolates with soybean increased fresh and dry weight of root and foliar parts. Similar studies have previously shown that antagonists increas seed germination and promote plant growth (Inbar *et al.,* 1994; Altomare *et al.,* 1999; Ryder *et al.,* 1999; Harris and Adkins, 1999; Valiente *et al.,* 2008). Our greenhouse experiments showed that in presence of the pathogen, all antagonists controlled soybean charcoal rot disease same as maneb did. The reduction observed in soybean root and stem microsclerotia covering (at least 62.5% and maximum 87.6% in comparison with pathogen) could be related to mycoparasitism, antibiosis, competition and promotion of plant growth ability of these antagonists that have also been reported in other studies. Decrease in root and stem microsclerotia coverage, as the primary inoculum source of the pathogen, caused by the antagonists and the fungicide, respectively was similar to the effects of antagonists and fungicide on soybean growth indices. These results are comparable with El-fiki *et al.,* (2004) and Etebarian (2006) results.

Considering that this pathogen causes a mono-cyclic disease and has a broad host range and high saprophytic power, its microsclerotia play the main role in the initiation and development of disease in all stages of host plant growth, thus reduction of primary inoculum source, is very important and should be the main goal in prevention of disease initiation. Overall results of this study showed that *T. harzianum* T100, *P. agglomerans* and *Bacillus* sp. BIN isolates each were able to control charcoal rot by restricting microsclerotia production and promoting host plant growth indices. The introduced antagonists therefore, may be effectively used in reduction of primary inoculum source by the end of growing season which can result in effective management of this devastating disease.

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کنترل بیولوژیکی بیماری پوسیدگی زغالی ریشه سویا توسط آنتاگونیست‌های باکتریایی و قارچی در آزمایشگاه و گلخانه

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چکیده: سویای زراعی ،Glycine max، به تعداد زیادی از عوامل بیماریزا ازجمله بیمارگرهای ریشه و طوقه حساس می‌باشد. یکی از این عوامل بیماریزای خاکزاد قارچ *Macrophomina phaseolina* عامل پوسیدگی زغالی ریشه می‌باشد. در این مطالعه، تأثیرات بازدارندگی دو جدایه باکتریایی، *Pantoea agglomerans*، *Bacillus* sp. BIN، و یک جدایه قارچی، *Trichoderma harzianum* T100، به‌عنوان عوامل بیوکنترل پوسیدگی زغالی سویا در مقایسه با قارچ‌کش مانب در آزمون‌های آزمایشگاهی و گلخانه‌ای بررسی شدند. عوامل آنتاگونیست در آزمون‌های کشت متقابل توانستند رشد رویشی بیمارگر را به‌ترتیب ۷۳,۸٪، ۶۳,۳٪ و ۵۵,۳٪ کاهش دهند. نتایج حاصله از آزمون‌های گلخانه‌ای نشان داد که همه آنتاگونیست‌ها در خاک‌های پاستوریزه و غیر پاستوریزه و در حضور بیمارگر شاخص‌های رشدی میزبان را افزایش دادند. کاهش پوشش میکرواسکلروتی ریشه و ساقه سویا توسط آنتاگونیست‌ها (به‌ترتیب ۶۲,۵٪، ۸۷,۶٪ و ۶۲,۵٪) و قارچ‌کش مانب (۸۷,۶٪) در خاک‌های پاستوریزه نشان‌دهنده توانایی بالای آنتاگونیست‌ها در کاهش میزان مایه تلقیح اولیه بیمارگر برای سال زراعی آتی می‌باشد.

واژگان کلیدی: *Bacillus* sp. BIN، کنترل بیولوژیکی، *Pantoea agglomerans*، *Macrophomina phaseolina*، *Trichoderma harzianum* T100 ، قارچ‌کش مانب.