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Research Article

Efficacy of peanut root nodulating symbiotic bacteria in controlling white stem rot

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Abstract: Stem rot with the causal agent *Sclerotium rolfsii* is a major disease of peanut in Guilan province, Iran. The aim of this investigation was to determine the inhibitory effect of native isolates of peanut root nodulating symbiotic bacteria on this fungus based on *in vitro* and *in vivo* studies. Several bacterial strains were isolated and purified from peanut roots collected from different fields. Eight of them were detected as the main symbiotic nodulating strains. These eight isolates were identified as *Bradyrhizobium* based on *16S rDNA* gene analysis and different biochemical tests. The inhibitory effect of these strains on the radial growth of *S. rolfsii* was studied *in vitro* using sealed plate and dual culture methods. Strains significantly inhibited radial growth of the fungus on the PDA medium. Br9, Br18 and Br16 were recognized as strong inhibitors and Br14 as weak strain in dual culture method and used in greenhouse experiments. Ability of the selected strains in controlling the stem rot disease, reducing the disease parameters and enhancing the peanut growth parameters was investigated in greenhouse conditions. The strains significantly decreased the white rot index and increased peanut dry matter ($P \leq 0.01$) in greenhouse.

Keywords: *Bradyrhizobium*, dual culture, groundnut, sealed plate, southern blight

Introduction

Groundnut or peanut (*Arachis hypogaea* L.) is an annual legume crop cultivated in tropical and subtropical zones of the world (Hammons, 1994). It is used extensively for feed and food as a major source of edible oil, vitamins and amino acids (Savage and Keenan, 1994). Groundnut is also a main crop in Guilan province of Iran with about 3500 hectares cultivation area located in Astaneh Ashrafiyeh County (Eslami *et al*., 2015). This crop has good capacity for nitrogen fixing through symbiosis by root nodulating bacteria including *Bradyrhizobium* spp. (Van Rossum, 1994).

This crop is under the pressure of different diseases and so its optimum productivity has not been reached because of yield loss. Among the diseases of groundnut; collar rot or stem rot caused by *Sclerotium rolfsii* Sacc. is considered as an economically important disease. Control of this fungus is difficult since it overwinters as sclerotia on plant debris and in soil (Punja, 1985). However, one of the disease management and yield loss reduction methods is to use biocontrol agents. Plant-growth-promoting bacteria (PGPB) stimulate the plants growth either directly or indirectly. PGPB affect the plant growth directly by phytohormones production or by facilitating the uptake of nutrients such as nitrogen, phosphorus or iron.

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Preventing the deleterious effects of phytopathogens by antibiosis, competition for space and nutrients, production of siderophores, and induction of systemic resistance in plants against a broad spectrum of root and foliar pathogens are due to the indirect positive effects of PGPB on plants (Sturz and Christie, 2003; Podile and Kishore, 2006; Lugtenberg and Kamilova, 2009; Fabra *et al*., 2010).

Rhizobia as a wide group of microorganisms among PGPB, show their biocontrol effect through the secretion of secondary metabolites such as antibiotics and HCN (Hydrogen Cyanide). They also produce siderophore in iron stress conditions which results in exclusion of pathogens due to iron starvation as an added advantage for rhizobia (Deshwal *et al*., 2003b).

Rhizobial strains were used for biocontrol of plant pathogenic fungi and also some plant parasitic nematodes in different studies (Shaban and El-Bramawy, 2011; Hemissi *et al*., 2011a, b; Al-Ani *et al*., 2012; Khalifa *et al*., 2013; Srinivasan *et al*., 2013; Figueredo *et al*., 2014). About the biocontrol of *S. rolfsii* by rhizobial strains, Balasundaram and Sarbhoy (1988) observed that six rhizobial strains inhibited only the germination of brown sclerotia of *S. rolfsii*, but the fast growing rhizobial strains were found to completely inhibit the growth of white sclerotia of *S. rolfsii*.

Integrated management of stem rot disease of groundnut using a combined application of *Rhizobium* and *Trichoderma harzianum* (ITCC -4572) was performed by Ganesan *et al*. (2007). The results indicated that the application of these native microorganisms successfully decreased the stem rot incidence and also increased the growth of groundnut plants.

Srinivasan *et al*. (2013) also demonstrated the biocontrol effect of a *Rhizobium* strain against *S. rolfsii* in rainfed groundnut. The studied strain significantly reduced the incidence of stem rot due to *S. rolfsii* in groundnut when compared to control. Also, Figueredo *et al*. (2014) reported the biocontrol effect of the peanut nodulating strain, *Bradyrhizobium* sp. SEMIA 6144 on *S. rolfsii*.

The aim of the present investigation was to determine the inhibitory effect of native isolates of peanut root nodulating symbiotic bacteria on *S. rolfsii* based on *in vitro* and *in vivo* studies.

Materials and Methods

Isolation of the fungal pathogen

S. rolfsii was isolated from infected peanut stems collected from the fields in Astaneh Ashrafiyeh County. Stems were surface sterilized with 5% sodium hypochlorite for 3-4 minutes, cultured on PDA medium and maintained at 28 °C until the fungus growth**.** Purification of the fungus was done through hyphal tip isolation.

Isolation of the bacteria

Peanut healthy root nodules were collected from the fields in Astaneh Ashrafiyeh County in July 2014. The bacteria were isolated from the nodules based on the method of Vincent (1970). The root nodules were washed with distilled water to remove the soil particles and surface sterilized in 3% sodium hypochlorite for 3-4 and 95% ethanol for 1-2 minutes, respectively and then washed in sterile water for 4 times. The nodules were crushed on watch glasses separately and a loopful of the extracts were transferred on YEMA (Yeast Extract Mannitol Agar) medium containing Congo red (Deshwal and Chaubey, 2014) in Petri dishes. Cultures were maintained at 28 °C for 5-8 days.

Biochemical characterization

Biochemical tests including gram staining (Somasegaran and Hoben, 1994), catalase (Graham and Parker, 1964), oxidase (Kovacs, 1956), bromothymol blue (Sharma *et al*., 2010), starch hydrolysis (Küçük *et al*., 2006) and urease activity (Deshmukh *et al*., 2013) were done for identification of the isolates. The ability of the isolates to grow on different carbon and nitrogen sources (Hungria *et al*., 2000; Bedi and Naglot, 2011) and the isolates tolerance to different salt concentrations and pH was also determined (El Sheikh and Wood, 1989; Graham *et al*., 1994). Intrinsic antibiotic resistance (IAR) test was done by disk diffusion method (Ladha and So, 1994). The methyl red

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test was done based on Kölbel-Boelke method (Kölbel-Boelke *et al*., 1988).

Molecular identification based on *16S rDNA* gene

A loopful of young colonies was transferred to 200 µl micro tubes containing 25µl DNAse free water. After spinning to have suitable suspension, tubes were kept in a 95 °C water bath for 10 minutes and then quickly centrifuged in 10000 rpm at 4 °C for 5 minutes. Five µl supernatant of any tube was transferred to a new tube (Jiang *et al*., 2006).

Each PCR sample contained 2 µl of each primer with 10 µM concentration [27F Unive (5´-AGTTTGATCCTGGCTCAG-3´) and 1492R Unive (5´-GGCTTACCTTGTTACGACTT-3´) (Jiang *et al*., 2006)], 2 µl DNA sample, 25 µl master mix ($MgCl_2$, Taq DNA polymerase, PCR buffer and DNTPs) by SinaClon Co., Iran, and 19 µl DNAse free water. Thermal cycler program included initial denaturation at 93 °C for 5 minutes, 35 cycles containing: 93 °C for 45 seconds, 55 °C for 45 seconds and 72 °C for 1.5 minutes and final extension at 72 °C for 7 minutes (Jiang *et al*., 2006).

Three µl of PCR product was run on 1% agarose electrophoresis gel containing DNA safe stain for 25 minutes. PCR products were sequenced (FazaPajooh Co., Iran). Sequences were edited by Mega5 ver. 5.2.2 and then blasted in gene bank (NCBI).

***In vitro* screening of the isolates for antagonistic activity**

Dual culture method

The bacterial isolates were cultured on YMA (Yeast Manitol Agar) medium. Bacterial suspensions of 10^8 CFU/ml were prepared from 3 days old cultures. Fifty µl drops of the prepared suspensions were located on the circumference of a circle with 2.5 cm distance from each other and 2.5 cm from the plate center on the 9 cm Petri plates containing PDA medium and maintained at 28 °C for 24 hours. After 24 hours, a 5 mm disc from 3 days old culture of *S. rolfsii* was transferred to center of the plates and the cultures were maintained at 24 ± 1 °C for 5 days (Sadfi *et al*., 2001). Drops of sterile water were used as control. The fungus radial growth was measured at 24 hours intervals (Fiddman and Rossall, 1993).

Sealed plate method

Bacterial suspensions of 10^8 CFU/ml were prepared from 3 days old cultures. The prepared suspensions were transferred to the 9 cm plates containing YMA medium as lawn culture. Five mm disc from 3 days old culture of *S. rolfsii* was transferred to the center of the plates containing PDA medium and these plates were inverted and placed over the bacterial cultures. The two plates were sealed together with parafilm and incubated at 25 °C. The fungus radial growth was measured at 24 hours intervals (Fiddman and Rossall, 1993). Sterile water was used as control treatment. Growth inhibition (%) of *S. rolfsii* was calculated by Whipps (1987) formula:

(R1-R2)/R1*100, where R1 and R2 are radial growth of the fungus in control and antagonist containing plates, respectively.

Antagonistic activity of the isolates in greenhouse conditions

Barley seeds were boiled in distilled water for twenty minutes and then 12 gr of seeds were added to each 100 ml Erlenmeyer flask and autoclaved twice at 121 °C and 1.5 atmospheric pressure for 30 minutes. Then a 5 mm disk of 3 days old culture of *S. rolfsii* on PDA medium was transferred to the Erlenmeyer flasks containing sterilized barley seeds and the cultures were maintained in the growth chamber (27 ± 1 °C) until the mycelium covered all the barley seeds and enough sclerotia were formed (Sennoi *et al*., 2010).

The applied soil (1:1:2 clay, compost, sand, pH = 6.7) was autoclaved at 121 °C and 1.5 atmospheric pressure for 30 minutes and added to the pots with 500 gr soil capacity (Dange, 2006).

Seeds of a local susceptible peanut germplasm were sterilized with sodium hypochlorite 1% solution for 3 minutes and rinsed with sterilized distilled water three times, then soaked in sterilized distilled water. The peanut seeds were placed in a moist chamber at 25 ± 5 °C for 72 h to germinate (Toribio *et al*., 1992).

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Based on the *in vitro* analysis, strains with high and low inhibitory effects were selected. These strains were cultured in YMB (Yeast Manitol Broth) in Erlenmeyer flasks and maintained for 3 days on shaker incubator under 120 rpm and 28 °C conditions. Before sowing peanut seeds, the germinated seeds were immersed in the bacterial suspension for 15 minutes and then cultivated in the prepared pots (one seed per pot) (Somasegaran and Hoben, 1994). Five days later, each pot was inoculated with 30 infected barley seeds and the seeds were covered with a thin soil layer (Adandonon *et al*., 2005; Dange, 2006; Flores-Moctezuma *et al*., 2006; Sennoi *et al*., 2010). The experiment was done as a complete randomized block design with three replications. Three sets of control pots also were prepared including the pots inoculated with the fungus and the bacterial strains separately and the pots without any inoculation. The pots were maintained in greenhouse conditions at 25 ± 5 °C (Erkilic *et al.*, 2006; Flores-Moctezuma *et al*., 2006; Sennoi *et al*., 2010). The pots were irrigated so as to prevent water stress.

Disease symptoms were monitored daily starting one week after fungus inoculation, when the symptoms were observed. At the plants maturity (about 6 weeks after seeding), all the plants were uprooted at the same time and the roots were washed in running tap water to remove soil particles (Yaqub and Shahzad, 2005).

Disease severity was assessed by scoring the plant wilting, yellowing or death, mycelia or sclerotia production on the soil surface or on plant stem, stem area affected (%) and lesion length (Le *et al*., 2012). Disease severity index was calculated for each treatment using these scores according to the Townsend-Heuberger formula (Erkilic *et al.*, 2006) as bellow:

$$DS(\%) = \frac{\sum(n \times v)}{N \times V} \times 100$$

Where,
n: degree of infection according to the scale (Le *et al*., 2012),
v: number of seedlings per category,
N: total number of seedlings were screened and
V: highest degree of infection.

Root and shoot wet and dry weight and plant height were also recorded at this stage (Yaqub and Shahzad, 2005; Sennoi *et al*., 2010). The data were analyzed by the one-way ANOVA followed by Tukey's multiple range test for mean comparison using SAS v. 9.0 software at $P \leq 0.01$.

Results

Identification of the isolates

Based on the biochemical tests and molecular analysis of *16S rDNA* (Fig. 1), all the bacterial isolates belonged to *Bradyrhizobium.* In this bacterial genus, colonies are hemispherical, smooth, sticky and producing a lot of exo-polysaccharide (EPS). It is a genus of gram negative bacteria, pleomorphic (x, y and t like) while fixing nitrogen and rod shape in pure culture (Oke and Long, 1999; Garrity *et al.*, 2004). The isolates were oxidase and catalase positive and were able to use urea as an energy source. Br16 was the only strain that could use starch as carbon source. All the strains were growing slowly as described by Garrity *et al.* (2004) and the bromothymol blue containing medium changed to blue. In methyl red test, Br2 and Br15cultures changed to red and orange, respectively and the rest of the strains didn't have any color change. Eight of the strains were identified as main symbiotic nodulating strains by nodulating test on peanut roots based on Raul *et al*. (2009) method.

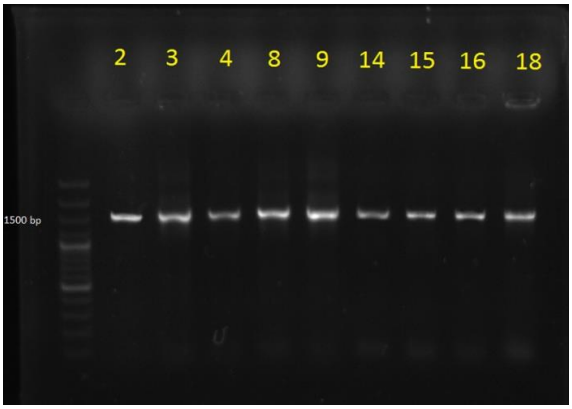

Figure 1 Amplification of *16S rDNA* gene of the studied bacterial strains by 27F Unive and 1492R Unive primers resulted in a 1500 bp band (left column is 100 bp Plus ladder, Fermentas).

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In vitro screening of the isolates antagonistic activity

The studied bacterial strains inhibited the *S. rolfsii* radial growth significantly in both dual culture and sealed plate methods. Strains Br9 and Br16 showed the highest inhibitory effect in dual culture method and Br2, Br3 and Br8 were the best ones in sealed plate method (Figs. 2 and 3).

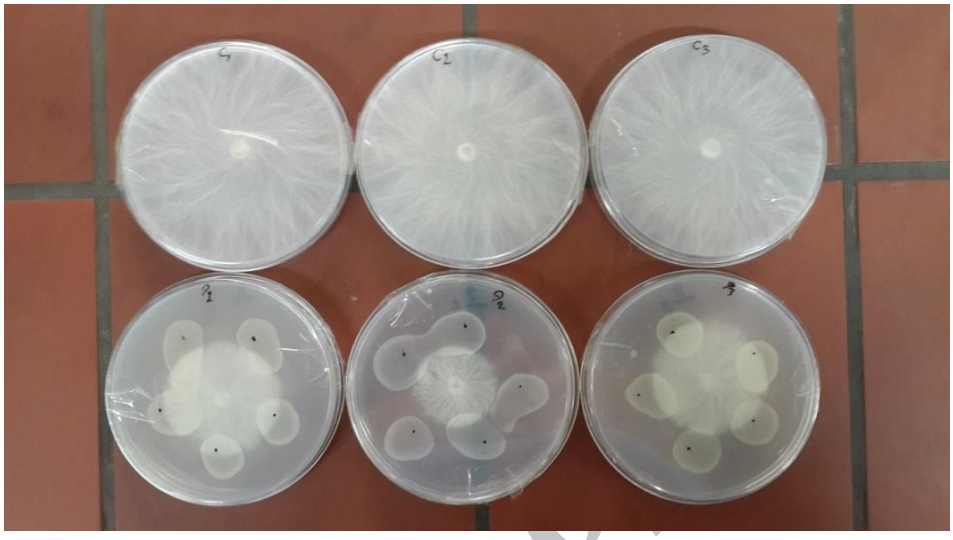

Figure 2 *Sclerotium rolfsii* radial growth in absence (above) and presence (below) of *Bradyrhizobium* Br9 strain in dual culture method (3 replications).

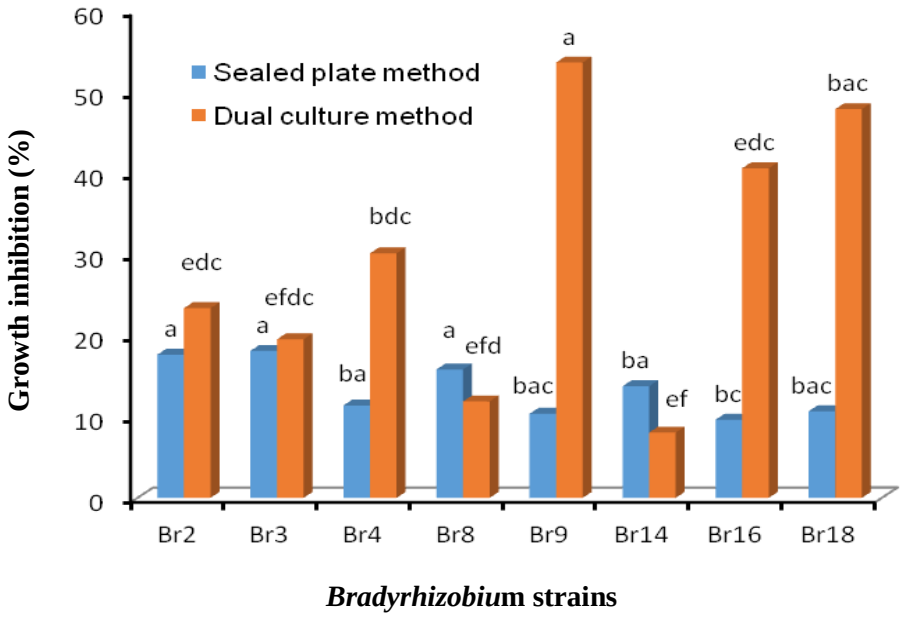

Figure 3 Mean comparison of the *Bradyrhizobium* strains inhibitory effect (%) on *Sclerotium rolfsii* radial growth ($P \leq 0.01$).

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Antagonistic activity of the isolates in greenhouse conditions

The studied *Bradyrhizobium* strains showed good ability in preventing stem rot disease in greeenhouse conditions (Fig. 4). Mean comparison of shoot wet and dry weight showed significant difference between the plants treated with the fungus and the bacterial strains simultaneously and the plants treated with the fungus alone (Fig. 5), but no significant differences were observed between the treatments for root wet and dry weight and the plant height ($P \leq 0.01$).

Br16 and Br18 showed high ability in preventing the stem rot disesae and the plants treated with these strains had the least lesion length, stem area affected with the lesion (%) and disease index at $P \leq 0.01$ (Fig. 6, Fig. 7 and Fig. 8). The other bacterial strians also had inhibitory effect on the disease development under greenhouse conditions, although their effects were lower than the two mentioned strains.

Figure 4 Peanut plants 45 days after seeding as treated with *Sclerotium rolfsii* and *Bradyrhizobium* Br16 strain (Left) and *S. rolfsii* alone (Right).

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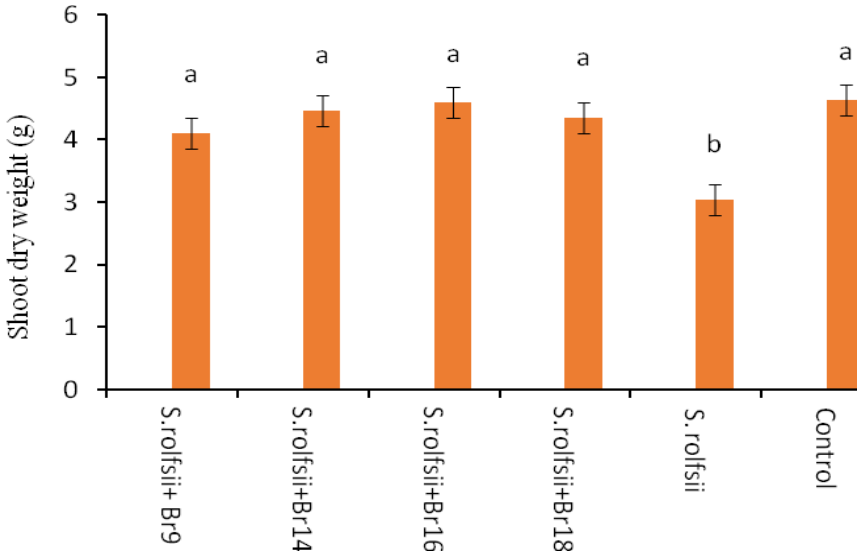

Figure 5 Mean comparison of the shoot dry weight of the peanut plants infected with *Sclerotium rolfsii* alone or treated with *S. rolfsii* and *Bradyrhizobium* strains simultaneously. The control plants are without any treatment.

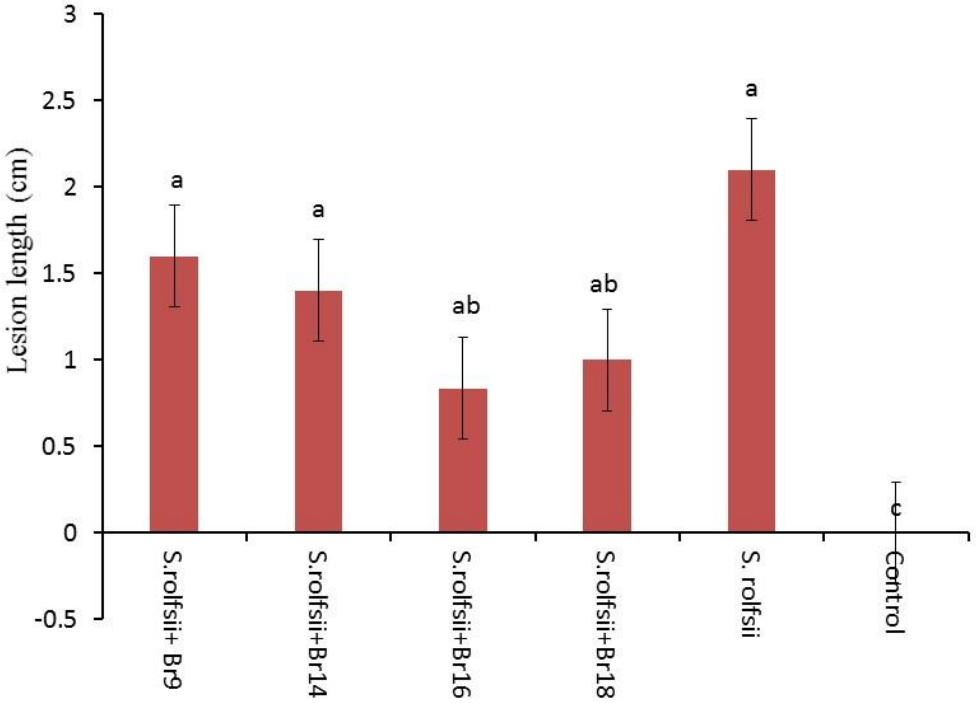

Figure 6 Mean comparison of the lesion length on the peanut plants infected with *Sclerotium rolfsii* alone or treated with *S. rolfsii* and *Bradyrhizobium* strains simultaneously. The control plants are without any treatment.

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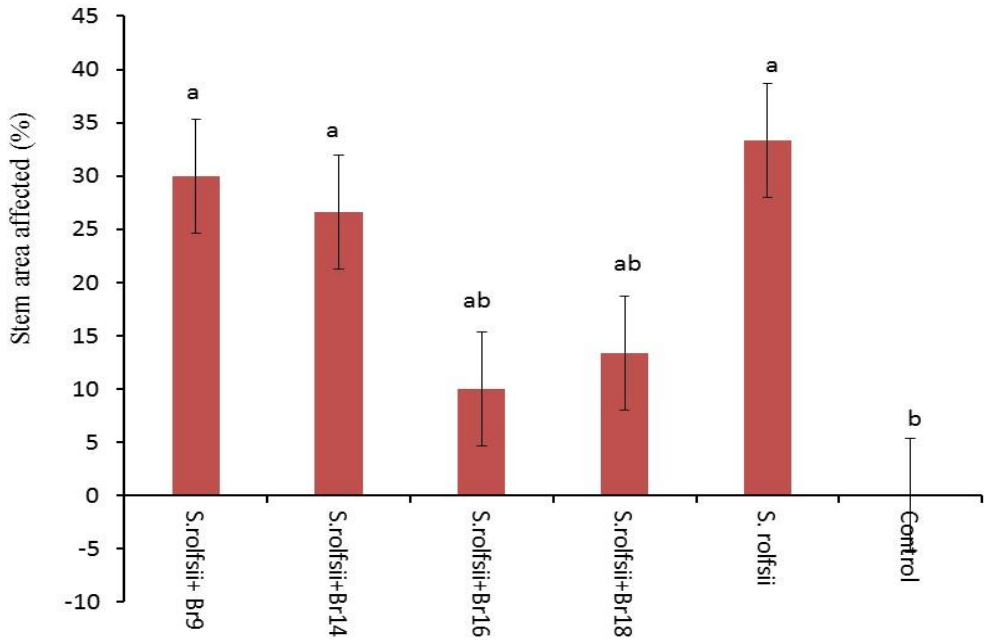

Figure 7 Mean comparison of the stem area affected (%) on the peanut plants infected with *Sclerotium rolfsii* alone or treated with *S. rolfsii* and *Bradyrhizobium* strains simultaneously. The control plants are without any treatment.

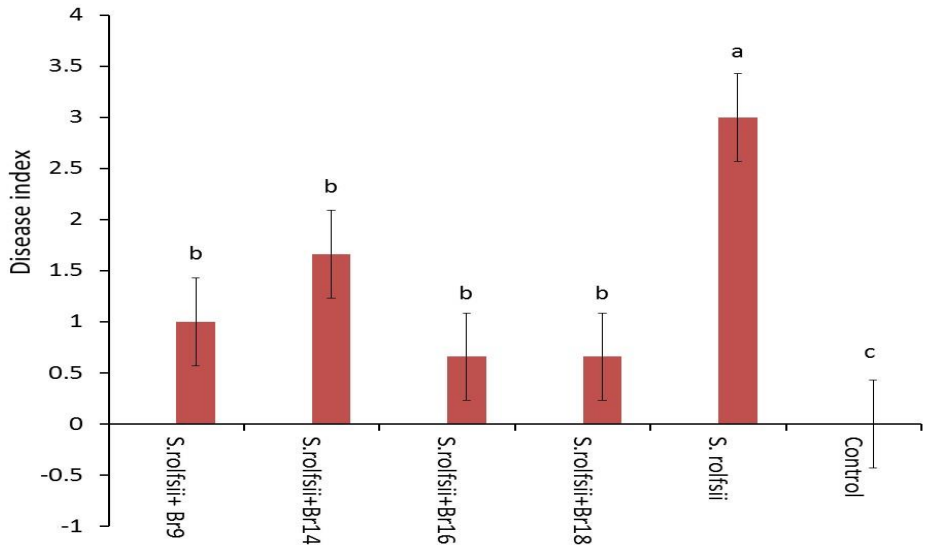

Figure 8 Mean comparison of the stem rot disease index on the peanut plants infected with *Sclerotium rolfsii* alone or treated with *S. rolfsii* and *Bradyrhizobium* strains simultaneously. The control plants are without any treatment.

Discussion

In the current study, the antagonistic effects of *Bradyrhizobium* strains, isolated from peanut root nodules was investigated on *S. rolfsii* the causal agent of peanut white stem rot. The studied bacterial strains inhibited the *S. rolfsii* radial growth significantly in both dual culture

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and sealed plate methods and also showed good ability in preventing stem rot disease in greenhouse conditions ($P \leq 0.01$). These results are consistent with findings of other researchers who investigated the biocontrol activity of the rhizobial strains on different plant pathogens (Shaban and El-Bramawy, 2011; Hemissi *et al*., 2011a, b; Al-Ani *et al*., 2012; Khalifa *et al*., 2013; Srinivasan *et al*., 2013; Figueredo *et al*., 2014). As the first studies in this area, Tu (1978) observed that the rhizobial strains had the ability to reduce root rot of soybeans caused by *Phytophthora megasperma.* Antoun *et al*. (1978) studied antagonism of 49 strains of *Sinorhizobium meliloti* towards *Fusarium oxysporum* and reported that they inhibited fungal growth by 5-50%. In another study, Chakraborty and Purkayastha (1984) reported that some rhizobiotoxine-producing strains of *Bradyrhizobium japonicum* protected soybeans from the infection by *Macrophomina phaseolina*, the charcoal rot fungus of leguminous crops. Malajczuk *et al.* (1984) also isolated rhizobia from the root nodules of *Acacia pulchella* and observed that these bacteria significantly reduced the survival of *Phytophthora cinnamomi* zoospores. In another investigation, Buonassisi *et al.* (1986) inoculated the seeds of bean with strains of *Rhizobium leguminosarum* bv. *phaseoli* antagonistic to *Fusarium solani* f.sp. *phaseoli*. A significant reduction in root rot was observed in the bean plants grown in pasteurized soil artificially infested with the fungal pathogens.

Growth inhibition of seven pathogenic microorganisms of soybean by twenty *B. japonicum* strains was studied by Balasundaram and Sarbhoy (1988). Chao (1990) also tested six different *Rhizobium* strains for their antagonistic activity against 10 fungal isolates and found that all the tested strains inhibited the growth of fungi. *R. leguminosarum* bv. *phaseoli* 6-3 showed the highest antagonistic activity. In another study, antagonistic rhizobia and bradyrhizobia used as seed dressing or as soil drench were observed to reduce the infection of *M. phaseolina*, *Rhizoctonia solani* and *Fusarium* spp. in both leguminous and non-leguminous plants in field conditions (Enteshamul-Haque and Chaffar, 1993).

Perdomo *et al*. (1995) evaluated 64 strains of *Rhizobium* for their antifungal activity against *M. phaseolina*. They found that the expression of inhibition varied among strains and was dependent on growth media and screening methods. In another investigation, *B. japonicum* was used alone or in combination with *Bacillus subtilis* and *Glomus fasciculatum* for biocontrol of *Heterodera cajani* and *Fusarium udum* on pigeonpea and resulted in the increased nodulation and decreased nematode multiplication (Siddiqui and Mahmood, 1995).

Nautiyal (1997) also observed that *Rhizobium* sp. NBRI9513 inhibited growth of *Fusarium oxysporum* f.sp. *ciceri, Rhizoctonia bataticola* and *Pythium* sp. under *in vitro* conditions. In another study, twenty one *Rhizobium* and *Bradyrhizobium* strains were tested *in vitro* against the mycelial growth of three soilborne root infecting fungi *(F. solani, M. phaseolina* and *R. solani)* on solid and liquid media. All tested rhizobia and bradyrhizobia significantly suppressed the growth of these pathogens. The three most active *Rhizobium* and *Bradyrhizobium* strains were tested under greenhouse conditions for their ability to protect one leguminous (soybean) and two non-leguminous (sunflower and okra) seedlings from root rot caused by the tested pathogens and were shown to provide significant suppression of disease severity (Omar and Abd-Alla, 1998).

R. leguminosarum bv. *trifolii* R39 strain showed *in vitro* antagonistic activity against the soil borne plant root pathogens, *Fusarium* spp., *R. solani*, *Helminthosporium sativum* and *Gaeumannomyces graminis* (Hoflich, 2000). In other studies, several species of *Rhizobium* and *Bradyrhizobium* have been reported to restrict the growth of *M. phaseolina* (Arora *et al*., 2001; Deshwal *et al*., 2003a).

The antagonistic activity of 21 *Rhizobium* isolates were tested *in vitro* in dual culture, and *in vivo* under greenhouse and field conditions against *F.oxysporum* f.sp. *ciceri.* In dual

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culture, 14 isolates inhibited the mycelial growth of the pathogen more than 30%. Greenhouse experiments revealed the effectiveness of five strains which reduced the percentage of wilted plants from 12.5 to 54.6% in the susceptible cultivar and 8.3 to 29.1% in the moderately resistant cultivar of chickpea (*Cicer arietinum*). In field experiments none of the 14 *Rhizobium* isolates reduced the percentage of wilted plants of the susceptible cultivar, although in the moderately resistant cultivar, eight of the isolates significantly reduced wilt incidence (Arfaoui, 2006).

Integrated management of stem rot disease of groundnut using a combined application of *Rhizobium* and *Trichoderma harzianum* (ITCC -4572) was performed by Ganesan *et al*. (2007). The results indicated that the application of these native microorganisms successfully decreased the stem rot incidence and also increased the growth of the groundnut plants. Shaban and El-Bramawy (2011) also reported that the *Rhizobium* spp. can be used in biological control of some important soil borne fungal diseases of legumes under greenhouse conditions.

Hemissi *et al*. (2011a) evaluated the antagonistic effect of *Rhizobium* isolates against *Fusarium culmorum*, the causal agent of wheat foot rot. The results demonstrated the effectiveness of *Rhizobium* in the inhibition of *F culmorum* both *in vitro* and *in vivo.* Hemissi *et al*. (2011b) also studied the biocontrol effect of *Rhizobium* on *R. solani*, causal agent of root rot in Chickpea. Among the 42 rhizobial strains tested, 24 isolates had effective control on *R. solani in vitro*. In pot trials, chickpea plants inoculated with different rhizobia showed significant reduction of root rot symptoms compared to the control growing in uninoculated soil.

The activity of *Rhizobium japonicum* against the soil-borne pathogens *F. solani* and *M. phaseolina* as causative agents of soybean root rot disease was evaluated by Al-Ani *et al*. (2012) in both culture medium and soil. Rhizobial culture filtrate caused an inhibition of the radial growth of *F. solani* and *M. phaseolina* on potato dextrose agar medium amended with the filtrate compared with control. The addition of rhizobial culture suspension to the soil contaminated by the two pathogens or sowing of rhizobial coated seeds, improved seed germination percentages and reduced the root rot disease index significantly in pot and field experiments.

In a study by Khalifa *et al*. (2013) under both greenhouse and field conditions, *Rhizobium* soil inoculation enhanced efficacy of seed and/or soil fungicides for controlling soil borne diseases of peanut,damping-off, root rot and pod rot, compared with untreated control.

Srinivasan *et al.* (2013) also demonstrated the biocontrol effect of a *Rhizobium* strain against *S. rolfsii* in rainfed groundnut. The rhizobial strain reduced the incidence of *S. rolfsii* to a high level when compared to control.

Figueredo *et al.* (2014) reported the biocontrol effect of the peanut nodulating strain *Bradyrhizobium* sp. SEMIA 6144 on *S. rolfsii* too.

In the current research, the studied *Bradyrhizobium* strains inhibited the mycelial growth of *S. rolfsii* on PDA medium in both dual culture (20-50%) and sealed plate (10-20%) methods which is comparable with the results of Antoun *et al.* (1978), Perdomo *et al.* (1995), Nautiyal (1997), Arfaoui (2006) and Al-Ani *et al.* (2012) studies. The studied strains also reduced stem rot disease index in greenhouse experiments as mentioned by Ganesan *et al.* (2007), Al-Ani *et al.* (2012), Srinivasan *et al.* (2013) and Figueredo *et al.* (2014).

The *Bradyrhizobium* strains with high inhibitory effect which were identified in this study need to be tested in field conditions for verification of their biocontrol effect on *S. rolfsii* and also the other peanut fungal pathogens in Guilan province.

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کارایی باکتری‌های هم‌زیست گره‌زای ریشه بادام زمینی در کنترل بیماری پوسیدگی سفید ساقه

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چکیده: بیماری پوسیدگی سفید ساقه با عامل *Sclerotium rolfsii* یک بیماری مهم بادام زمینی در استان گیلان محسوب می‌شود. این مطالعه با هدف اثبات خاصیت بازدارندگی سویه‌های بومی باکتری‌های هم‌زیست گره‌زای ریشه بادام زمینی روی قارچ *Sclerotium rolfsii* در شرایط آزمایشگاه و گلخانه صورت گرفت. سویه‌های باکتریایی زیادی از ریشه‌های بادام زمینی جمع‌آوری شده از مزارع، جداسازی و خالص‌سازی شدند. با انجام آزمون گره‌زایی، ۸ سویه به‌عنوان سویه‌های هم‌زیست گره‌زا شناخته شدند. براساس بررسی ژن ۱۶S rDNA و انجام آزمون‌های بیوشیمیایی مختلف، هر ۸ سویه به‌عنوان جنس *Bradyrhizobium* تشخیص داده شدند. اثر بازدارندگی این سویه‌ها روی میزان رشد شعاعی پرگنه قارچ در شرایط آزمایشگاه با استفاده از دو روش کشت متقابل و تشتک پتری برعکس مورد بررسی قرار گرفت. سویه‌ها به‌طور معنی‌داری از رشد شعاعی میسلیوم قارچ در سطح محیط کشت PDA جلوگیری کردند. سویه‌های Br9، Br18 و Br16 به‌عنوان بازدارنده‌های قوی و سویه Br14 به‌عنوان سویه ضعیف در آزمون کشت متقابل شناسایی و برای آزمایش‌های گلخانه‌ای انتخاب شدند. توانایی سویه‌های منتخب در کنترل بیماری پوسیدگی ساقه، کاهش پارامترهای بیماری و افزایش فاکتورهای رشدی بادام زمینی در شرایط گلخانه مورد بررسی قرار گرفت. سویه‌های مورد بررسی در کاهش شاخص پوسیدگی سفید و افزایش وزن خشک بوته‌ها در شرایط گلخانه تأثیر معنی‌داری داشتند ($P \leq 0.01$).

واژگان کلیدی: *Bradyrhizobium*، کشت متقابل، بادام زمینی، تشتک پتری برعکس، بلایت جنوبی

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