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Research Article

The effect of biocontrol agents consortia against *Rhizoctonia* root rot of common bean *Phaseolus vulgaris*

Ali Nasir Hussein, Saeed Abbasi*, Rouhallah Sharifi and Samad Jamali

Department of Plant Protection, College of Agriculture, Razi University, Kermanshah, Iran.

Abstract: In recent years, biological control has become a promising and ecologically friendly alternative to chemical control in the management of soil-borne plant diseases and several biological control agents have been introduced as potential bio-fungicides. The aim of this study was to investigate different biological control agent consortia against *Rhizoctonia solani* root rot disease of common bean. *Bacillus pumilus* INR7, *Trichoderma harzianum* and *Rhizophagus intraradices* were used individually or in combination. There were two application methods: simultaneous application of biocontrol agents with the plant pathogen, and pre-inoculation of biocontrol agents one month before the pathogen. Treatments containing *B.pumilus* INR7 were the best treatments for suppression of the disease in the simultaneous application method, where *B. pumilus* INR7 + *T. harzianum* reduced the disease up to 54%. However, in pre-inoculation method *T. harzianum* alone was the only treatment that reduced disease severity up to 49% compared to the infected control; other treatments did not haveany significant effect on disease severity. In current study, combination of *T. harzianum* and *R. intraradices* was unable to decrease disease severity and improve plant growth. This phenomenon was common in both simultaneous and pre-inoculation experiments. However, results showed that *B. pumilus* INR7 and *R. intraradices* were compatible with each other. Their combination not only decreased the disease, but also improved the dry weight of common bean in both application methods. Our results revealed that *B. pumilus* INR7 had positive interaction with *T. harzianum*. This combination increased their ability to suppress root rot disease and improve plant health, significantly. Overall, combinations of biocontrol agents have good potential to be applied in modern agriculture, but such combinations need to be checkedin advance for their compatibility in greenhouse and field experiments.

Keywords: Bean, Biocontrol, *Rhizoctonia solani*, Root rot

Introduction

Root and crown rot caused by *Rhizoctonia solani* J. G. Kühn, is one of the most serious diseases in Beans throughout the world. This pathogen causes seed decay,damping off, crownrot, root rot and web blight (Matloob and Juber, 2013). Integrated disease management (IDM) strategies are considered essential for reducing disease pressure (Schwartz, 2011). Management strategies are often based on agronomic practices such as crop rotation and good soil drainage (Habtegebriel and Boydom, 2016). Crop rotation with resistant crops such as barely, wheat, oats, alfalfa, and corn is the best treatment to reduce population of *R. solani*

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* **Corresponding author**, e-mail: abbasikhs@yahoo.com
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in common beans (Schwartz, 2011). Fungicideshave also been used in IDM of this disease. Fungicides like pyrimidine derivatives have had good activity against *R. solani* (Liu *et al.*, 2001). Soil treatment with fungicides such as benomyl has also reduced the rate of damage by *R. solani*in beans under field conditions.

In recent decades, several microbial biocontrol agents have been reported for suppression of soil-borne pathogens (Sharifi and Ryu, 2017, Haas and Defago, 2005). Isolates of *Bacillus* spp., *Pseudomonas* spp., *Burkholderia* spp., *Trichoderma* spp. as well as arbuscular mycorrhizal fungi (AMF) were able to suppress *Rhizoctonia* root rot of Bean under greenhouse and field condition (Martinez *et al.*, 2004, Ghanbarzadeh *et al.*, 2016, Sharifi *et al.*, 2006, de Jensen *et al.*, 2002, Hwang and Benson, 2002). *Trichoderma harzianum* Rifai treatment was able to decrease root rot incidence of faba bean plants better than the fungicide Rizolex-T (Abdel-Kader *et al.*, 2011). *T. harzianum* suppresses fungal pathogens through several mechanisms (Matloob and Juber, 2013). *Bacillus subtilis* is also an effective biocontrol agent against *R. solani* (Estevez de Jensen *et al.*, 2002, Yobo *et al.*, 2011). *Bacillus* strains produce resting spores that let them survive in adverse environmental conditions and permit easy formulation and storage of the commercial products. Thereby, *Bacillus* based biopesticides alone cover more than 85% of commercial products (Pérez-García *et al.*, 2011). The AMF have a good potential in suppression of soil-borne fungi especially *Fusarium* spp. and *R. solani* (Sohrabi *et al.*, 2015, Martínez-Medina *et al.*, 2010, Filion *et al.*, 2003, Akköprü and Demir, 2005). The colonization of bean roots with *Rhizophagus intraradices* (N. C. Schenck & G. S. Sm.) C. Walker & A. Schüßler.improved growth and yield parameters at the same time significantly reduced the negative effects of *R. solani* (Abdel-Fattah *et al.*, 2011).

However, each biocontrol strain exploits a specific set of mechanisms to suppress plant diseases and improve plant growth. Therefore, it is possible to develop strain mixtures with multifaceted mechanisms of action. Combining different microbial biocontrol agents with different types of action will increase their capacity for suppression of disease beyond the contribution of the individual isolates (Palmieri *et al.*, 2017, Ghanbarzadeh *et al.*, 2016). For instance, it has been demonstrated that some bacteria named "mycorrhiza helper bacteria" (MHB) can enhance mycorrhizal development (Duponnois, 2006). Co-inoculation of *R. intraradices* and *Azotobacter chroococcum* was found to be more effective than each one of them alone in decreasing disease severity of *R. solani* on beans under greenhouse and field conditions (Matloob and Juber, 2013). Several reports also indicated that beneficial bacteria and the fungus *Trichoderma* have positive interaction in biological control of plant pathogens. Co-inoculation of *Trichoderma* with *B. subtilis* showed the highest ability to suppress rice sheath blight caused by *R. solani* and enhance the growth of plants (Ali and Nadarajah, 2013). However, interactions are not always synergistic e. g. *T. harzianum* can decrease the efficiency of the mycorrhiza *R. intraradices* (Rousseau *et al.*, 1996) *R. intraradices* also reduces both the population development and the metabolic activity of *T. harzianum* (Green *et al.*, 1999). Application of *G.mossease*to soil two weeks earlier reduced the population of *T. koningii* (McAllister *et al.*, 1994b). In contrast to above reports there are reports indicating the conidial germination of *T. harzianum* increased in the presence of the AM fungal extract (Filion *et al.*, 1999). The combination of *T. harzianum* and *R. intraradices* greatly reduced disease severity of *Fusarium* crown and root rot in tomato (Datnoff *et al.*, 1995).

The aim of current study was to check the ability of the bacterium *Bacillus pumilus* INR7 (YieldShiled®a. i.), the mycoparasite *T. harzianum* and the commercial AM fungus, *R. intrardices,* againstbean damping-off disease. Biocontrol agents were used alone, two by two and all together to check their antagonistic or synergistic effect on suppression of *R. solani*. Finally, possible consortia to develope new biofungicdewill be evaluated.

Materials and Methods

Isolates of pathogen and biocontrol agents

Fungal pathogen *Rhizoctonia solani*, AG 2-2 isolated from diseased common bean plants and identified based on morphological and molecular characteristics was provided by fungal collection, Department of plant protection, Razi University, Kermanshah. The isolate was stored in test tubes on Potato Dextrose Agar (PDA) slants or sterile vermiculite+Potato Dextrose Broth (2: 1) for long term storage at 4 °C and activated by placing on water agar to induce the fungus growth and detect possible contamination by bacteria or other fungi. After three days of growth at room temperature, a 6 mm agar plug was transferred to PDA or PSA to use for inoculum preparation.Fungal isolate of *T. harzianum* was provided by Agriculture and Natural Resourse Research and Education center of Kermanshah, Iran. The isolate was stored using the same method and conditions as previously described for *R. solani* isolate. *R. intrardices* inoculum (soil containing spores and hyphae) was provided by Turan Biotechnology Company, Semnan province, Iran (http://turanbiotech.ir). The inoculum was stored at 4 °C. Bacterial strain of *B.pumilus,* INR7 was provided by Professor Joseph W. Kloepper, Department of Entomology and Plant Pathology, Auburn University, USA. The strain was preserved in a glycerol stock for long-term storage. Two ml of the overnight bacterial culture on Nutrient Brothwas mixed with 2 ml of 40% glycerol in a 5 ml cryovial to prepare glycerol stock and stored at -20 °C.

Inocula preparation

To prepare the inoculum of *R. solani*, a mixture of 100 ml vermiculite with 50 ml Potato Dextrose Broth was transferred to a 500-ml Erlenmeyer flask and autoclaved twice for 20 min during two consecutive days. The sterilized mixture was inoculated with four agar plugs (6-mm-diameter) taken from an actively growing culture of fungal isolate. The flasks were then incubated at 25 °C for two weeks.

T. harzianum was grown on PDA medium in Petri dish (9cm) for seven days in incubator at 25 °C. Wheat bran and peat moss (v: v 1: 1) were mixed with hand, then substrate moisture was adjusted to 50% (w/w) with distilled water and transferred to flask then autoclaved at 121 °C for 20 min twice in two consecutive days. The sterile Wheat bran + peat moss mixture was then inoculated with four agar plugs (6-mm-diameter) taken from an actively growing culture of *T. harzianum*. The flasks were then incubated at 25 °C for two weeks, according to Sivan *et al.* (1984).

To prepare the inoculum of *B. pumilus* INR7, fresh cells were obtained from stock cultures stored at (-20 °C) and grown in NA medium overnight at room temperature; then each 250ml flask containing 150 ml nutrient broth was inoculated with fresh bacterial cells and kept for 48 h at room temperature on a rotary shaker. The supernatant was discarded and washed bacterial cells were re-suspended in sterile distilled water. The concentration of bacterial suspension was adjusted to 1×10^8 CFU/ml and used for seed bacterization and drenching the soil.

Pot experiments

Two experiments were designed to evaluate the interactions of *T. harzianum*, *B. pumilus* and *R. intraradices*as biocontrol agents against *R. solani.* In one experiment inoculation of pathogen was at the same time as application of biocontrol agents. In another experiment, biocontrol agents were used at the time of planting seeds in pots, but inoculation of pathogen was delayed and applied one month after sowing. The treatmentsare shown in table 1. Common bean (*P. vulgaris*), cultivar Chitti, was used in this study. The seeds were provided by Department of agronomy and plant breeding, Razi University, Kermanshah. Seeds were surface sterilized in ethanol 70% for 1min and sodium hypochlorite 2% for 40 second, then rinsed 3-4 times with sterile distilled water.

Table 1 Soil treatment with biocontrol agents in the presence of soil-borne pathogen, *Rhizoctonia solani.*

Treatment	*Rhizoctonia solani*	*Rhizophagus intrardices*	*Trichoderma harzianum*	*Bacillus pumilus*
Rs	*			
Rs × R	*	*		
Rs × T	*		*	
Rs × B	*			*
Rs × R × T	*	*	*	
Rs × R × B	*	*		*
Rs × T × B	*		*	*
Rs × R × T × B	*	*	*	*

Rs = *R. solani,* T= *T. harzianum,* B = *B. pumilus* INR7, and R = *R. intraradices*.
*: Indicate significant at $p < 0.05$.

Application of biocontrol agents pre-inoculation of pathogen

The pasteurized soil of each plastic pot (15 × 15 cm) was treated with one of the biocontrol agents or a combination of two or all three of them in the presence of pathogen. Inoculum of *R. intraradices*, was completely mixed with the autoclaved soil at a rate of 1: 15 (w/w). The inoculum of *T. harzianum* was added at a rate of 1: 100 (w/w) just below the seed bed. Four uniform germinated seed were sown in each pot. Bacterial suspension at a concentration of 1×10^8 was used as a post-plant drench treatment at a rate of 1: 15 (v/v). Control pots received the inoculum substrate instead of inoculum. The soil infestation was conducted one month after planting. For inoculation, soil in the center of each pot was carefully removed without damaging the roots, then 10 g of inoculum was added and the soil was replaced. Control pots contained non-infested mixture of vermiculite/PDB. All pots were kept under greenhouse conditions (day temperature 25 ± 5 °C, night temperature 20 ± 5 °C, 16 h photoperiod) and watered when necessary. Pots were arranged in a completely randomized design with 4 replicates. All sixteen plants (four pots) of each treatment were carefully harvested two months after sowing time, washed under running water to remove soil particles and evaluated for the following growth parameters: shoot dry weights (g), root dry weights (g) and number of seeds per plant upon drying for 48 h at 60 °C**.** Severity of symptoms on root caused by *R. solani* was rated according to a modified CIAT scale of (Schoonhoven, 1989) as follows: 0 = healthy plant, 2 = necrotic lesions in the hypocotyl, 4 = 25% of the hypocotyl area with lesions, 6 = 50% of hypocotyl area affected and root rot, 8 = dead plant.

The collected data were statistically analyzed using SAS software. Data were subjected to analyses of variance and treatment means were compared by Least Significant Difference (LSD) test at $P < 0.05$.

Simultaneous application of biocontrol agents and pathogen

In this experiment, the inocula of biocontrol agents and pathogen were added at the same time. The experiment was conducted under the same conditions as previously described. The experimental design and the amount of inocula were also the same as described in the previous section.

Root colonization with *Rhizophagus intraradices*

Root colonization of *R. intraradices* was determined using the method of Phillips and Hayman (1970). Two months after sowing, root pieces, approximately 2cm in length were mounted in lactophenol and the chlamydospores and mycelia were observed under stereomicroscope with the magnitude of 10-40X. For each inoculation method, Ninety randomly selected stained root in the treatment, Rs × R (Table 1) were mounted on slides and examined microscopically for estimation of mycorrhizal root colonization. The percentage colonization of Mycorrhizal Fungi in roots were calculated by the following formula: % Root colonization = (No. of root segments infected/Total no. of root segments studied) × 100 (Sohrabi *et al.*, 2015).

Results

Simultaneous application assay

The effect of biocontrol agents on disease severity were checked under greenhouse condition. *T. harzianum* alone and in combination with *R.*

intraradices did not suppress the disease severity. All other treatments significantly reduced symptoms of stem and root rot caused by *R. solani*. Treatments of *B. pumilus* alone (41%), in combination with *T. harzianum* (56%) or *R. intraradices* (51%) were the best in suppression of *R. solani* disease severity. These results revealed that *B. pumilus* interacts positively with both fungal biocontrol agents. In contrast, *T. harzianum* and mycorrhiza had negative interaction (Fig. 1). It should be mentioned that combination of all biocontrol agents was not good enough.

The effect of biocontrol agents singly or their combination on plant growth factors was assessed in presence of the pathogen. None of the treatments improved plant shoot and root dry weight (Table 2). In contrast, most of the treatments increased seed production significantly. Combination of all three biocontrol agents caused significant increase in number of seeds per plant, up to 36%, compared to control (Table 2). Indeed, treatment that included *B. pumilus* were statistically similar. Number of seeds per plant in treatments with *R. intraradices* alone or in combination with *T. harzianum* were same as infected control.

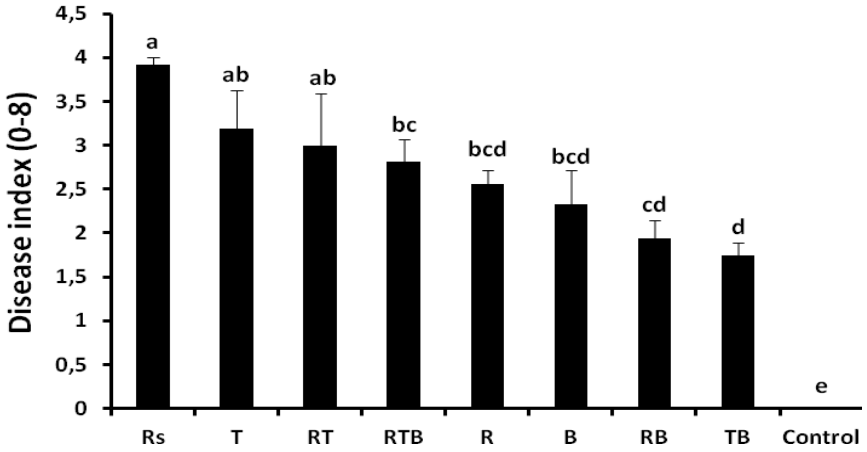

Figure 1 Effects of the biocontrol agents *Rhizophagus intraradices*, *Bacillus pumilus* INR7, and *Trichoderma harzianum* individually or their combinations on disease severity of common beans stem and root rots caused by *Rhizoctonia solani*. The inocula of biocontrol agents and pathogen were added at the same time. Means comparison analysis was done by Fisher protected LSD. Means with the same letters do not have significant difference.
Rs = *R. solani* (infected control), T = *T. harzianum*, B = *B. pumilus* INR7, and R = *R. intraradices*.

Table 2 Effect of root treatment with *Bacillus pumilus* INR7, *Rhizophagus intraradices* and *Trichoderma harzianum* and their combinations on plant shoot dry weight, root dry weight and number of seeds per plant.

Treatments	Simultaneous application assay			Pre-inoculation assay		
	Shoot dry weight (g)	Root dry weight (g)	No. of seeds / plant	Shoot dry weight (g)	Root dry weight (g)	No. of seeds / plant
Healthy	6.18 a	2.00 ab	6.75 ab	8.88 a	1.50 bc	6.00 a
Infected	5.84 a	2.02 ab	5.00 c	7.97 ab	1.06 cde	5.25 a
T	5.28 a	1.58 b	6.25 ab	8.37 ab	1.99 a	6.00 a
B	5.51 a	1.72 ab	6.75 ab	8.03 ab	0.97 de	5.75 a
R	5.38 a	1.71 ab	5.33 bc	8.46 ab	0.97 de	4.50 a
TB	6.18 a	2.24a	7.25 a	6.38 b	0.71 e	5.75 a
TR	5.88 a	1.98 ab	5.50 bc	6.36 b	1.17 cde	5.00 a
BR	5.71 a	1.69 ab	7.25 a	7.40 ab	1.69 ab	5.25 a
TBR	6.29 a	2.06 ab	7.75 a	7.12 ab	1.31 bcd	4.75 a

Mean followed by the same letters in each column are not significantly different (LSD test, $P \leq 0.05$).
T = T. harzianum, B = B. pumilus INR7, and R = R. intraradices.

Pre-inoculation assay

In this experiment, efficiency of biocontrol agents on common bean stem and root rot disease severity was checked under greenhouse conditions. Biocontrol agentswere introduced to the soil one month before pathogen inoculation to guarantee biocontrol agent establishment. Soil treatment by *T. harzianum* alone reduced disease severity up to 49% compared to the infected control (Fig. 2). However, the other treatments did not show any significant effect on stem and root rot disease severity compared to the control.

Furthermore, The effect of biocontrol agents alone or incombination, on the plant growth factors were assessed in the presence of pathogen. The application of *T. harzianum* alone caused significant increase in root dry weight up to 47% compared with infected control (Fig. 3). This treatment acted better than the healthy control. Combination of *R. intraradices* and *B. pumilus* also increased root dry weight up to 37% (Table 2, Fig. 3). However, *T. harzianum* and *B. pumilus* had negative interaction on root dry weight. In contrast, none of the biocontrol agents alone or in combination were effective on shoot dry weights and number of seeds per plant (Table 2).

Root colonization with *R. intraradices*

Root colonization by arbuscular mycorrhizal fungi were estimated under greenhouse condition. Ninety root pieces were inspected for presence of chlamydospore, hyphae, vesicles and arbuscules of *R. intraradices.* Microscopic inspection showed that nearly 45% of root pieces were colonized by Mycorrhiza when it was applied one month before pathogen inoculation. In the simultaneous experiment, presence of pathogen reduced root colonization rate by *R. intraradices* and about 25% of roots were mycorrhizal (Fig. 4).

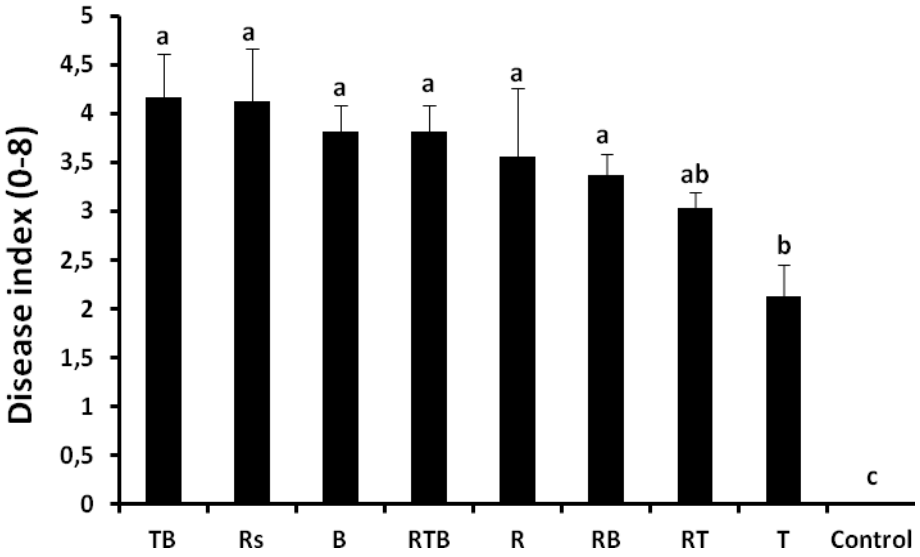

Figure 2 Effects of the biocontrol agents *Rhizophagus intraradices, Bacillus pumilus* INR7, and *Trichoderma harzianum,* applied singly or their combinations, on disease severity of common bean stem and root rot caused by *R. solani.* Biocontrol agents were used right at the time of planting seeds in pots, but inoculation of pathogen was done one month later. Mean comparison analyses were done by Fisher protected LSD. Means with the same letters have no significant difference**.**

Rs = *R. solani* (infected control), T = *T. harzianum*, B = *B. pumilus* INR7, and R = *R. intraradices.*

Figure 3 Effect of biocontrol agents on common bean growth. Plant inoculated with *Trichoderma harzianum*(a), *Rhizophagus intraradices* + *Bacillus pumilus* INR7 (b), *Rhizophagus intraradices* + *Bacillus pumilus* INR7 + *Trichoderma harzianum* (c) and *Rhizoctonia solani* infected control (d).

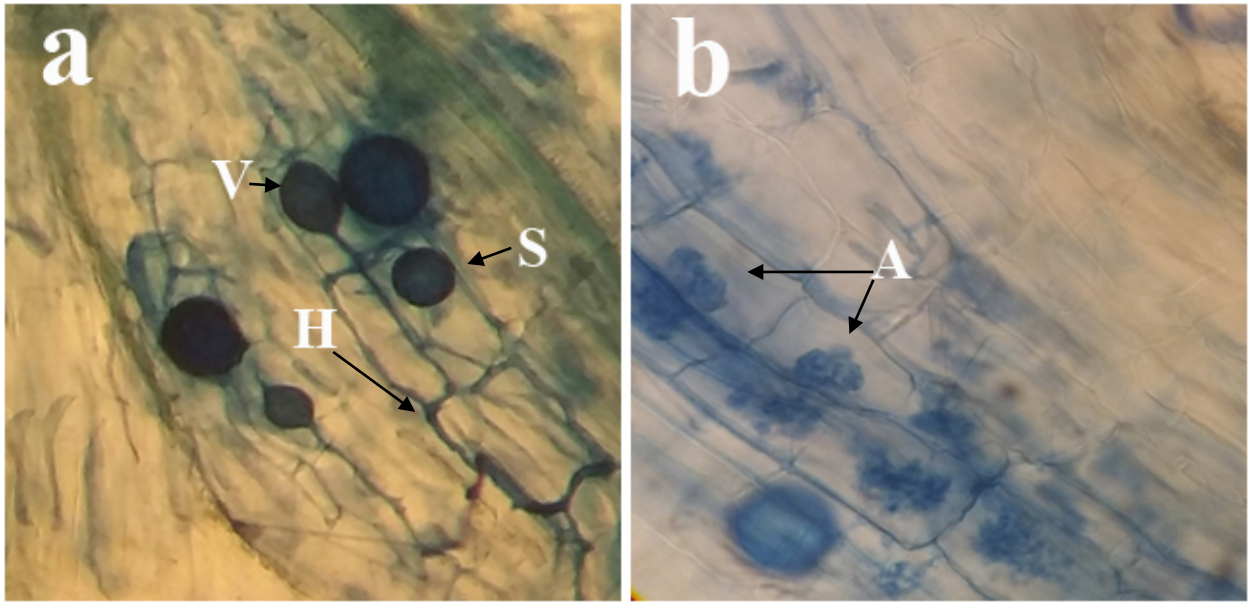

Figure 4 Endomycorrhizal sturctures of *Rhizophagus intraradices* in common bean roots pieces.
A: spore (S), hyphae (H), vesicle (V); b: arbuscules (A).

Discussions

In recent decades, biological control has become of interest in integrated disease management. Several biological control agents have been introduced as potential biofungicides. These microorganisms are applied as active ingredients in several commercial bio-inoculants. Of course, Each product has special set of mechanisms for plant

growth promotion and biocontrol of plant pathogens. For example *Trichoderma* spp. parasitize other fungi and induce systemic resistance in host plants (Harman, 2011), bacteria produce siderophore and provide N, P and Fe for plant growth and release several antibiotics and volatiles for suppression of plant pathogens (Sharifi *et al.*, 2010, Sharifi and Ryu, 2016), and mycorrhizae improve plant health and compete with plant pathogens by colonizing root tissues (Ghanbarzadeh *et al.*, 2016). Therefore, different biological control agent consortia were investigated in this study to determine whether they would be compatible or incompatible.

In this study, biological control agents, alone or in combination, were used for suppression of common bean root and stem rot. There were two application methods, simultaneous application of plant pathogen and biocontrol agents and pre-inoculation of biocontrol agents one month before pathogen application. The aim of these experiments was to check whether biocontrol establishment is necessary for their optimum activity. Treatments containing *B. pumilus* INR7 were the best for suppression of disease in the simultaneous application method. However, combination of bacteria with *T. harzianum* and mycorrhizae improved their biocontrol activity. In contrast, just *T. harzianum* was able to suppress *R. solani* when pathogen was applied one month later. *R. solani* is highly aggressive to hypocotyl of plants and mainly show canker symptom in this area. Thereby, the ability of biocontrol agents to colonize hypocotyl parts of plants improve their performance to suppress *R. solani* crown canker (Khateri, 2002). In pre-inoculation tests *R. solani* inoculum was introduced in top layer of soil near plant hypocotyl. These seedlings were more prone to infection. Bacteria and Mycorrhiza mainly colonize root system but not hypocotyl. *Trichoderma* mycelia can growto soil surface and reach hypocotyl area. That may be the reason why only *Trichoderma* was effective in pre-inoculation test.

In the current study, combination of *Trichoderma* and *Rhizophagus* was unable to improve plant growth mainly in pre-inoculationtests. It is considered that co-inoculation of *Rhizophagus* and *Trichoderma* decrease the mycorrhizae root colonization (Ghanbarzadeh *et al.*, 2016, Green *et al.*, 1999). Interestingly, *Rhizophagus* also decreased the activity of *Trichoderma* through competition for nutrients such as phosphorous (Green *et al.*, 1999). *Trichoderma* decreased lettuce and maize root colonization by Mycorrhizae when they were applied in the same time or *Trichoderma* was applied earlier. In contrast, *Rhizophagus* decreased *Trichoderma* population when it was applied earlier (McAllister *et al.*, 1994a). *Trichoderma* spp. exploit several mechanisms for inhibition of *Rhizophagus* growth and can reduce spore germination in *Rhizophagus* (Martinez *et al.*, 2004). *Trichoderma* hypha can colonize and lyse spore and mycelium of *Rhizophagus*. Electeron microsopy inspection has showed that *Trichoderma* can parasitize *Rhizophagus* hypha and degradate its cell wall. Furthermore, *Trichoderma* and *Rhizophagus* may have antagonistic effects on plant growth and health by modulating plant hormone signaling. *Trichderma* has been shown to increase salycilic acid (SA) and Jasmonic acid (JA) concentration in response to *Fusarium* disease of melon (Martinez-Medina *et al.*, 2010), but co-inoculation with *Rhizophagus* diminshed these effects. Gene experesion analysis showed that *Rhizophagus* does not induce direct change in plant defence hormones SA and JA but prime plant defence potential which means that, JA increases more rapidly just after pathogen attack. When *Trichoderma* and *Rhizophagus* were applied alone, *Trichoderma* decreased disease sevirity but *Rhizophagus* decreased the disease slightly. Interestingly, combination of *Rhizophagus* and *Trichoderma* provided more suppression on plant disease. Gene expresion data showed that this combination reduced the expresssion of Abscisic acid and Ethylene hormones. These hormones are essential in susceptibility of plant to *Fusarium* (Martinez-Medina *et al.*, 2010).

Results showed that *B. pumilus* INR7 and *Rhizophagus* were compatible witheach other. Their combination not only decreased plant disease but also improved common bean dry weight in both application methods. Similar results have been reported in biological control of *R. solani* in Celery by combination of *Bacillus* and *Rhizophagus* (Nemec *et al.*, 1996). Other biocontrol bacteria such as *Rhizobium*, *Pseudomonas* and *Azetobacter* also have compatible interaction with *Rhizophagus* in improving plant health and suppressing plant diseases (Akköprü and Demir, 2005, Hassan *et al.*, 1997, Matloob and Juber, 2013). Some strains of *Bacillus* and *Pseudomonas* increased spore germination of *R. intraradices*in Chickpea rhizoasphere (Akhtar and Siddiqui, 2008). There are several examples of mutual bacteria/Mycorrhiza interactions, these bacteria are known as Mycorrhizae helper bacteria or MHB (Bonfante and Anca, 2009).

Our results revealed that *B. pumilus* INR7 had positive interaction with *T. harzianum*. This combination increased their abilty to suppress root rot diseasse and improve plant health, significantly. *B. pumilus* INR7 + *T. harzianum* was the best treatment in suppression of *R. solani* and improvement of root dry weight. Antogonistic bacteria and fungi exploit different mechanisms in biological control of plant disease. So, in most cases there is not a cross-talk between their biocontrol mechanisms. In cucumber, bacteria and *Trichoderma* induced different signaling pathway against *F. oxysporum*. So their combination showed synergitic effect on suppression of disease (Alizadeh *et al.*, 2013). However, *B. pumilus* INR7and *T. harzianum* interaction was not positive in pre-inocuulation test. If fact, they release a set of antibiotics to the rhizospher which may have negative effect on their survival over time (Yobo *et al.*, 2011).

In conculusion, combinations of biological control agents receive more attention in recent years. In some cases, same group of microbes are combined to make a microbial consortia. These consortia mostly show a better ability compared to individual agents. There are reports in consortia of *Bacillus* and *Pseudomonads* for biological control of plant diseases (Kumar and Jagadeesh, 2016, Thakkar and Saraf, 2015). However, same bacteria or fungi share same future in most cases and researchers do not expect high synergetic effect. On the other hand, there are examples of consortia containing biocontrol agents from different taxonomic groups. There are reports on synergitic and antagonistic interaction of these agents on each other. for examples, Mycorrhiza and *Trichoderma* had negative interaction on each other (Martinez *et al.*, 2004, McAllister *et al.*, 1994a). However, based on *Trichoderma* species or strains, these interactions may not be always antagonistic (Dehariya *et al.*, 2015, Chandanie *et al.*, 2006). So, we can screen several strains to find compatible interactions.

Most case studies on combination of bacteria with *Trichoderma* report synergetic interaction. These biocontrol agents exploit different strategies in promotion of plant growth and suppression of plant pathogens. So, it is easier to screen and introduce synergistic combinations. In the case of bacteria/mycorrhizae combination, it is better to seek for mycorrhizae helper bacteria. These bacteria improve spore germination, hyphal growth and rhizosphere competence of mycorrhizae (Bonfante and Anca, 2009). Overall, the combination of biocontrol agent has agood potential to be applied in agriculture. This combination is a double edge sword which means that if we do not check their compatibility in greenhous and field conditions they can increase suceptibility to some pathogens. But if researchers investigate their compatibility, these biocontrol consortia are promising products in biological control of plant diseases in modern agriculture.

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اثر ترکیب عوامل کنترل بیولوژیک علیه پوسیدگی ریزوکتونیایی ریشه لوبیا *Phaseolus vulgaris*

علی‌ناصر حسین، سعید عباسی*، روح‌الله شریفی و صمد جمالی

گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه رازی، کرمانشاه، ایران.
* پست الکترونیکی نویسنده مسئول مکاتبه: abbasikhs@yahoo.com
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چکیده: در سال‌های اخیر کنترل بیولوژیک به‌عنوان یک روش امیدبخش و طبیعت دوستانه برای جایگزینی روش‌های شیمیایی در مدیریت بیماری‌های خاکزاد مطرح می‌شود و تعداد زیادی از عوامل کنترل بیولوژیک به‌عنوان قارچ‌کش‌های با قابلیت بالا وارد بازار شده‌اند. هدف از این پژوهش، بررسی ترکیب عوامل کنترل بیولوژیک مختلف در کنترل بیماری پوسیدگی ریشه لوبیا با عامل *Rhizoctonia solani* بود. عوامل *Bacillus pumilus* INR7، *Trichoderma harzianum* و *Rhizophagus intraradices* به‌صورت مجزا یا در ترکیب با هم به‌کار برده شدند. دو روش کاربرد استفاده شد، کاربرد هم‌زمان عوامل بیوکنترل و قارچ بیمارگر و روش پیش‌تیمار عوامل بیوکنترل یک ماه قبل از مایه‌زنی بیمارگر. در روش کاربرد هم‌زمان، تیمارهای حاوی *B. pumilus* INR7 بهترین تیمارها در مهار بیماری بودند. ترکیب *B. pumilus* و *T. harzianum* میزان بیماری را ۵۴ درصد کاهش داد. اما در روش پیش تیمار، کاربرد مجزای *T. harzianum* تنها تیمار مؤثر بود و شدت بیماری را در مقایسه با شاهد آلوده ۴۹ درصد کاهش داد. تیمارهای دیگر اثر معنی‌داری در کاهش شدت بیماری نداشتند. در مطالعه حاضر، ترکیب *T. harzianum* و *R. intraradices* قادر به کاهش بیماری و افزایش رشد گیاه نبود. این پدیده در هر دو روش کاربرد هم‌زمان و پیش‌تیمار مشاهده شد. در مقابل، نتایج این پژوهش نشان داد که *B. pumilus* و *R. intraradices* با هم سازگار بودند. ترکیب آنها نه‌تنها شدت بیماری را کاهش داد بلکه وزن خشک لوبیا را در هر دو نوع روش کاربرد بهبود بخشید. علاوه بر این، باکتری *B. pumilus* تعامل مثبتی با *T. harzianum* داشت. ترکیب این دو عامل به‌صورت معنی‌داری باعث هم‌افزایی توانایی آنها در مهار بیماری پوسیدگی ریشه و بهبود سلامت گیاه شد. در مجموع، اگرچه کاربرد ترکیب عوامل بیوکنترل از قابلیت بالایی در کشاورزی مدرن برخوردار است ولی لازم است که سازگاری آنها در شرایط گلخانه و مزرعه با دقت مورد ارزیابی قرار گیرد.

واژگان کلیدی: پوسیدگی ریشه، کنترل بیولوژیک، لوبیا، *Rhizoctonia solani*