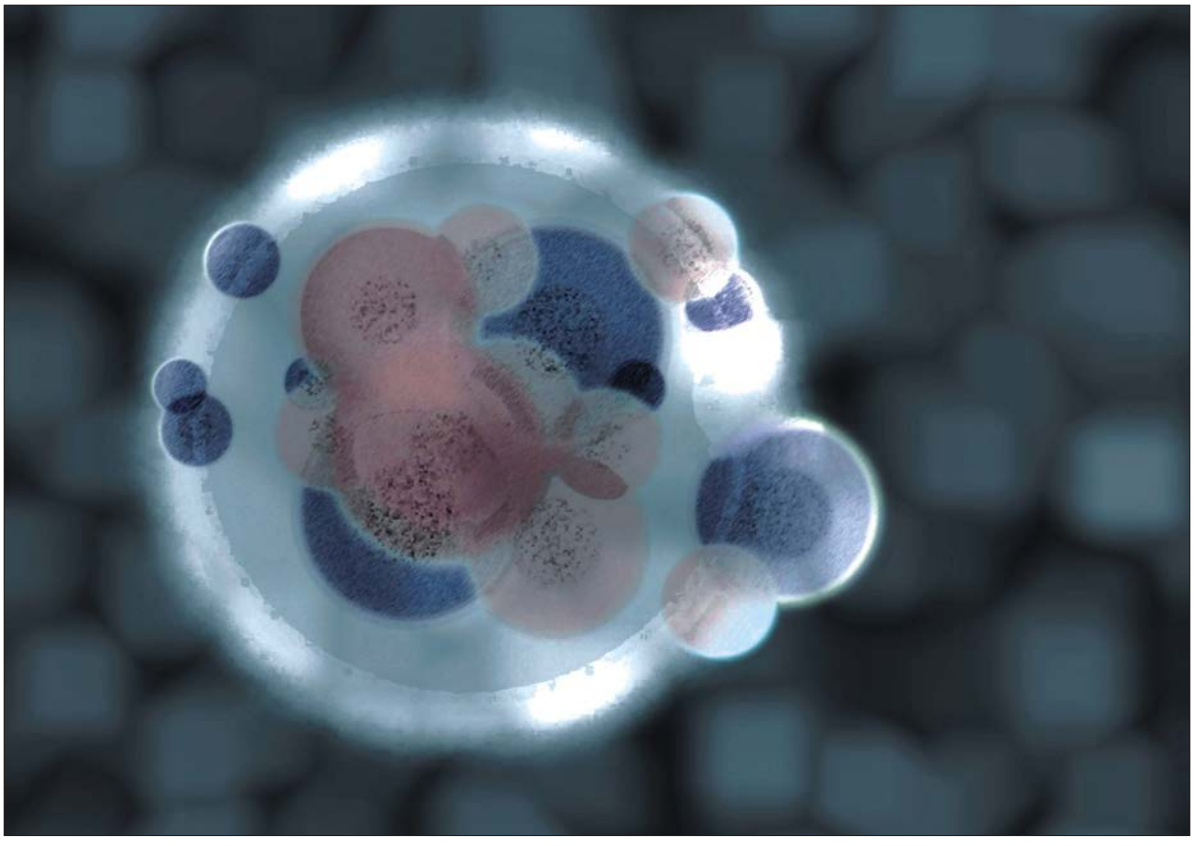

Journal of Biological Sciences

ISSN 1727-3048

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Journal of Biological Sciences 5 (3): 347-353, 2005
ISSN 1727-3048

Biological Control of Stem and Root-rot of Wheat Caused by *Bipolaris* spp. by using Antagonistic Bacteria, Fluorescent *Pseudomonads* and *Bacillus* spp.

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Abstract: Stem and root-rot caused by *Bipolaris* spp., lead to significant yield losses of wheat (*Triticum aestivum* L.) in Hamadan province, Iran. One strategy to control stem and root-rot is the use of antagonistic, root-colonizing rhizobacteria. In order to assess the potential of rhizospheric microorganisms in biological control of such soil-borne diseases, in this study one hundred eighty isolates of both *Pseudomonas* and *Bacillus* spp. from rhizoplane and surrounding soil of healthy and infected wheat, were collected. Among them, by using the dual culture method, only 9 isolates with the most antagonistic ability against the growth of two pathogenic fungal species (*Bipolaris australiensis* and *B. sacchari* (were selected and purified. According to the results of biochemical and physiological tests, they were identified as three biovar of *Pseudomonas fluorescens* and some species of *Bacillus*; *B. subtilis, B. coagulans*, *B. licheniformis*, *B. megaterium* and *B. brevis*. Production of antifungal substances and volatiles metabolites, siderophores and secretion of lytic enzymes such as protease and cellulase as the inhibitory mechanisms *in vitro* were evaluated. Furthermore, in greenhouse conditions the effects of antagonistic rhizobacteria on disease severity and incidence caused by *Bipolaris australiensis* and *B. sacchari*, using seed coating and soil drenching were studied. Statistical analysis of data indicated that, treating wheat seeds with some of the antagonistic rhizobacteria, not only reduced the disease severity and incidence, comparing with the control, but also had showed positive influence on growth and yield of wheat cultivars.

Key words: Biological control, stem and root rot, *Bipolaris australiensis*, *Bipolaris sacchari, Pseudomonas fluorescens*, *Bacillus subtilis*

INTRODUCTION

The pathogen *Bipolaris* spp. causes different seed and soil-borne diseases of seedlings, including brown foot-rot, root-rot and seedling blight on cereals and grasses. These diseases are economically important and are among the most widespread disease of wheat, especially in areas with warm and wet condition during growing season. The pathogen is most often localized in the pericarp or conidia may be carried externally on the testa. Infection can take place through stomata on the hypocotyls, from where the fungus progresses to the root, shoot and coleoptile[1]. Worldwide disease occurrence of this fungi in leaf spot and stem and root rot of wheat, specially in China, North and Latin America, Brazil and much less frequently in parts of Europe have been reported[2-8]. In order to reduce the application of chemicals in diseases control, especially soil-borne pathogens, some efforts in biological control of *Bipolaris* spp. by rhizospheric microorganisms, have been conducted[9,10]. In fact the rhizosphere is the first-line defense for roots against soil-borne pathogenic fungi[11]. Therefore, there is an excellent opportunity to find rhizosphere-competent bacteria in the rhizosphere which are potential biocontrol agents. A successful biocontrol agent efficiently suppresses the pathogen and reduces disease incidence. Biocontrol agents act against pathogens by such forms of antagonism as competition, antibiosis and parasitism. In recent years, fluorescent pseudomonads have drawn attention worldwide because of their ability to colonize rhizosphere and production of secondary metabolites such as siderphore, antibiotics, volatile compounds, HCN, enzymes and phytohormones[9]. They also could protect plants against a wide range of important agronomic fungal diseases such as blotch root-rot of tobacco, root-rot of pea and wheat, damping-off of sugar beat[10]. In order to control of this soil-borne fungi, bioantagonistic fungi such as

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Trichoderma spp., *Chaetomium* sp. and *Gliocladium roseum*[1,12,13] and rhizobacteria such as *Stenotrophomonas malthophilia* C3, *Pseudomonas chlororaphis* MA342 and *Bacillus* sp. have been studied[14].

One of the mechanisms involved in preventing the proliferation of phytopathogens, is through their ability to produce siderophores for sequestering iron. The secreted siderophores binds to the Fe^{3+} that is available in the rhizosphere and thereby effectively prevent growth of the pathogens in that region. Some other PGPR synthesize antifungal antibiotics, e.g. *P. fluorescens* produces 2, 4-diacetyl phloroglucinol, which inhibits growth of phytopathogenic fungi[13]. Certain PGPR degrade fusaric acid produced by *Fusarium* sp. causal agent of wilt and thus prevents the pathogenesis. Some PGPR can also produce enzymes that can lyse fungal cells.

The objectives of the present research, were to determine the effects of coating wheat seeds with bioantagonistic rhizobacteria, e.g. fluorescent *Pseudomonads* and some species of *Bacillus*, on reducing severity and incidence of stem and root rot caused by *Bipolaris* spp. *in vitro* and *in vivo* and their characterization in terms of antagonistic mechanism used to control the pathogen and conditions for growth similar to those present in the field.

MATERIALS AND METHODS

Isolation of *Bipolaris* species: Stem base and root of collected wheat from several infected fields in 2002, that showed symptoms of root rot and necrosis on crown were submerged in 0.5% sodium hypochlorite for 3-5 min. After this treatment, they were extensively washed with sterile distilled water and placed on petri dishes containing PDA and incubated at 22-25°C for one week. *Bipolaris* spp. were on of the most prevalent fungi that grown on medium. Their recognition was on the basis of characteristic such as conidia and number of pseudosepta, conidiophores, their arrangement and hilum on Tap Water Agar (TWA) medium plus sterile pieces of wheat stubble[15].

Preparation of fungal inoculum and pathogenicity test: After inoculating two hundreds gram sterile wheat stubble were put in Erlenmeyer, with four 5 mm mycelial disk from a five days old culture of each fungal species, incubated at room temperature for three weeks. The colonies of fungi were developed and for each pathogenicity test the inoculum was added to the sterile soil in 1% (w/w).

Isolation, selection and identification of bacteria: Rhizospheric bacteria were isolated from infected and healthy wheat root in 2002. Root segments of wheat were washed with tap water, (0.5 g) added to 50 mL sterile distilled water and were shaken for 30 min, 0.1 m L of each bacterial suspension were spotted in Nutrient Agar (NA) medium, incubated at 22°C. Fluorescent Pseudomonads were isolated on King's B (KB). According to the methodology of Schaad *et al.*[16] antagonistic isolates of rhizobacteria were identified by biochemical, physiological and biological tests. The purified isolates were pre-evaluated against the isolates of *Bipolaris australiensis* and *B. sacchari* by using dual culture in petri dishes containing PDA and the percentage of fungal growth inhibition were determined. Nine isolates with the most inhibition percentage, which was calculated by using the fallowing formula[17], were selected and re-purified.

$$\% \text{ Inhibition} = (1 - (\text{fungal growth}/\text{control growth})) \times 100$$

Antagonistic mechanisms of rhizobacteria

Production of volatile metabolites: Hundred microliter of each bioantagonistic bacterial suspension (5×10^9 cfu mL^{-1}) were scattered on petri dish containing KB medium and a 5 mm disk of a five days old pure culture of *Bipolaris* spp. was placed at the center of another petri dish containing PDA. Both plates were placed face to face preventing any physical contact between the pathogen and the bacterial suspension and were sealed to isolate the inside atmosphere, preventing loss of the volatiles compound. Plates were incubated at 22-25°C for one week and the growth of the pathogen was measured and compared to controls developed in the absence of the bioantagonists. Each experiment considering a single bacterial isolate was run triplicates and was repeated three times. Results are expressed as means of % inhibition ±SD of the growth of *Bipolaris* spp. in the presence and absence of any bacterial isolates.

Production of diffusible antifungal substances: According to Montealegro[18], PDA plates covered with a cellophane membrane, were inoculated in the center with 100 μL of a bioantagonistic bacterial suspension (5×10^9 cfu mL^{-1}). After incubation for 72 h at 22°C, the membrane with the grown bacterial isolate was removed and the plate was inoculated in the middle with a 5 mm disk a pure culture of *Bipolaris* isolates. Plates were further incubated at 22°C for one week and the growth of the pathogen was measured. Controls were run with mocked inoculated PDA containing plates on the cellophane membrane (replacing the bacterial suspension by sterile distilled water) and further inoculated with *Bipolaris* isolates. Results are expressed as means of % inhibition ±SD of growth of *Bipolaris* spp. in the

presence and absence of any bioantagonistic bacterial isolate.

Effect of Fe^{3+} on antagonism levels: This effect was tested according to Weller and Cook[19]. Placing a 0.5 mm disk of fungi in the face of each bacterial isolate on King B medium and King B plus 1000 μm $FeCl_3$ and incubating one week at 22°C. After this period, the mycelial growth of fungi was compared in both media. Also, inoculating bacterial strains on Chrome Azurol S (CAS) medium[20] and changing the color of medium from blue to orange showed the siderophore production.

Hydrogen cyanide production: After providing a suspension from pure culture of each bacteria in 4-5 mL sterile distilled water and scattering 100 μL from that on NA medium, some pieces of filter paper were suspended in HCN indicator solution (5 mg cupper ethyl acetoacethate and 5 mg 4, 4-methylene-bis-(N,N-dimethyl aniline) in 2 cm^3 chloroform). Changing the color of the filter papers placed on petri dish's caps after 3-18 h indicated the HCN production[21]. Each experiment considering a single bacterial isolate was run triplicates and was repeated three times.

Protease production: Petri dishes contained SMA (Skim Milk Agar) culture medium including milk powder (15 g), yeast extract (5 g), blood agar (4 g) and agar-agar (13.5 g), inoculated with each isolate and incubated at 27°C for 24 h. Production of a colorless hallow around each bacterial colonies indicated protease activity of that strain[22]. Each experiment considering a single bacterial isolate was run triplicates and was repeated three times.

Cellulose production: The medium including K_2HPo_4 (1 g), $NaNO_3$ (0.5 g), $MgSO_4$ (0.5 g), KCl (0.5 g) and $FeSO_4$ (0.01 g) in 1 L distilled water, was provided and scattered 9 mL of it to each tube. A piece of filter paper in dimension of 1×9 cm^2 was put in each tube. After sterilization, 1 mL of each bacterial suspension was added to them and incubated at 25°C. Until three weeks, the color changing of filter papers was traced every day. In control, 1 mL of $MgSO_4$ solution (0.1 M) was added.

Greenhouse experiments

Preparation of bacterial inocula: Cells of antagonistic bacteria for use in greenhouse experiments were grown King's Medium B broth (KMB) to late exponential phase at 25°C with shaking at 150 rpm. Cells were harvested by centrifugation (3000 rpm/min 10°C, 15 min), washed twice and re-suspended in $MgSO_4 \cdot 7H_2o$ solution (0.1 M). The bacterial suspension was adjusted to about 10^7-10^8 cfu mL^{-1}, using haemocytometre for each experiment. The bacterial cells suspensions were used for seed coating and soil drenching.

Table 1: Assessment of disease severity (SCI-Index)

Disease severity levels	% area with discoloration	Score
Clean	0	1
Slight	1-25	2
Moderate	26-50	3
Severe	51-100	4

Evaluating the disease intensity on wheat: For this purpose the effects of fungal species on wheat growth and health were evaluated. The prepared fungal inoculum was added to the sterile soil in 1% (w/w) in each pot (25 cm in diameter). The disease intensity was scored for each tiller in the 5 plants at 75 days after seeding, in the heading growth stage. Disease intensity was scored using Singleton *et al.*[23] sub-crown internode index (SCI-Index), as described in Table 1. Five replications were maintained for each treatment. The pots were arranged in factorial design and the trials were repeated at least twice with similar results.

Efficacy of seed coating and soil drenching with the rhizobacterial isolates: In greenhouse conditions wheat seeds (cvs. Alvand and Roushan) were surface sterilized in 0.5% sodium hypochlorite for 5 minutes and then air dried in a laminar flow and they were soaked in antagonistic bacterial suspensions overnight. Inoculum of two fungal species (*Bipolaris australiensis* and *B. sacchari*) was added to the soil in pot and after 24 h, 8 inoculated wheat seed per pot were sown. Separately, at the same time, soil in pot were drenched with suspensions of the antagonistic bacteria at a total concentration of 10^7-10^9 cfu mL^{-1}. Pots were maintained in the greenhouse under 25°C and 90% relative humidity condition. Control treatments were inoculated with sterile distilled water and plants with disease symptoms were recorded 75 days after planting. The effects of treatments on wheat growth factors (such as germination percentage, means of plant height, number of tillers and dry weight of aerial parts of each plant) and disease severity and incidence on two wheat cultivars (Alvand and Roshan) were assessed.

Statistical analysis: The data obtained were subjected to analysis of variance and the means separated by using Duncun's Multiple Range Test and ANOVA. Tests were used to establish significant differences.

RESULTS

Isolation and identification of the fungal species: According to the Sivanesan[15], two of the prevalent agents isolated from wheat stem-base and roots in wheat

Table 2: Inhibition percentage of rhizobacterial strains on the mycelial growth rate of the fungal species

	Bipolaris australienis		*Bipolaris sacchari*	
Treatments	Volatile comp.	Antifungal sub.	Volatile comp.	Antifungal sub.
B. subtilis1	0.68i	86.21c	41.16d	82.14a
P. fluorescens bv 1	44.77e	46.55f	6.14d	69.23b
B. coagulans	55.23c	62.07e	60.29a	82.14a
B. subtilis2	12.95h	91.38a	13.36f	82.14a
P. fluorescens bv 5	22.73g	82.76d	23.1e	82.14a
B. licheniformis	24.32f	89.66b	9.75g	82.14a
P. fluorescens bv 4	51.59d	91.38a	45.85c	82.14a
B. megaterium	68.18a	89.66b	47.65b	50.0c
B. brevis	65.91b	91.38a	1.44i	82.14a
Control	0.00i	0.00g	0.00j	0.00d

Table 3: Influence of the interactions of rhizobacteria and fungal species on the wheat yield components (Cv. Alvand)

	Bipolaris australienis			*Bipolaris sacchari*		
Treatments	Germination percentage	Means of height	Dry weight	Germination percentage	Means of height	Dry weight
B. subtilis1	8.93a	4.42j	1.61c-e	7.95ab	4.4jk	1.72a-d
P. fluorescens bv 1	7.76a-c	4.4k	1.7b-e	7.3a-d	4.37L	1.91a
B. coagulans	7.33a-c	4.66c	1.61c-e	7.3a-d	4.63d	1.77a-d
B. subtilis2	5.8cd	4.22o	1.6b-e	8.16ab	4.28m	1.67b-e
P. fluorescens bv 5	5.3d	4.39k	1.8a-d	8.16ab	4.26n	1.61c-e
B. licheniformis	8.56ab	4.25n	1.47de	8.06ab	4.5h	1.59de
P. fluorescens bv 4	7.55a-c	4.96a	1.82a-c	7.1a-d	4.59e	1.92a
B. megaterium	7.3a-d	4.87b	1.7a-d	8.13ab	4.1p	1.61c-e
B. brevis	6.56b-d	4.52g	1.8a-d	7.3a-d	4.55f	1.78a-d
Control	8.56ab	4.45i	1.6de	5.72cd	4.08q	1.84ab

Table 4: Influence of the interactions of rhizobacteria and fungal species on the wheat yield components (Cv. Roshan)

	Bipolaris australienis			*Bipolaris sacchari*		
Treatments	Germination percentage	Means of height	Dry weight	Germination percentage	Means of height	Dry weight
B. subtilis1	8.9a-f	4.97ab	2.25ab	10.03a	4.99ab	1.8bc
P. fluorescens bv 1	7.8f	4.6hi	2.1a-c	9.85ab	4.72c-h	1.95a-c
B. coagulans	9.5a-d	4.88b-f	2.2a-c	8.4d-f	4.72e-h	2.12a-c
B. subtilis2	9.3a-d	4.78b-h	1.85bc	4.96a-d	9.5a-c	1.77bc
P. fluorescens bv 5	9.85ab	4.92a-e	2.16ab	4.77ef	8.16b-h	1.81bc
B. licheniformis	9.3a-e	4.35j	1.77bc	4.87a-f	8.96b-g	2.44a
P. fluorescens bv 4	8.9a-f	4.95a-d	2.2a-c	4.42a-c	9.68ij	2.05a-c
B. megaterium	8.56c-f	5.14a	2.1a-c	4.66a-f	8.93f-I	2.21ab
B. brevis	10.03a	4.72d-h	1.63c	4.63a-e	9.31g-I	2.11a-c
Control	8.78b-f	4.45ij	1.79bc	8.57c-f	4.57h-j	1.86bc

fields of Hamadan, Iran were identified as *Bipolaris australiensis* and *B. sacchari*. In the pathogenicity test, inoculation of soil to the fungal species, in addition to significant effects on disease severity and incidence, especially in the case of *B. sacchari*, showed significant reduction on seed germination and means of height of the both wheat cultivars (Alvand and Roushan).

Selection and identification of rhizobacteria: Based on the biochemical and physiological tests[16], nine rhizobacteria with the most inhibition percentage against *Bipolaris* spp. were identified as, three biovars of *Pseudomonas fluorescens* (1, 4 and 5) and some species of *Bacillus, B. subtilis*1 and 2, *B. coagulans*, *B. licheniformis*, *B. megaterium* and *B. brevis*.

Antagonistic mechanisms of the rhizobacteria: *In vitro* all of the 9 isolates were positive in production of volatile and diffusible antifungal metabolites against the fungal species. Volatile compound provided by *B. megaterium* and *B. coagulans* showed the most and those, provided by *B. subtilis*1 and *B. brevis* indicated the least inhibitory effect on the growth rate of the fungal species, *B. australiensis* and *B. sacchari*, respectively (Table 2). *A*ntifungal substances produced by *B. brevis, B. subtilis*2 and *P. fluorescens* bv. 4 in the cace of *B. australiensis* and *Bacillus subtilis*1 *and B. coagulans* in the case of *B. sacchari,* were the most effective strains (Table 2). In general, the result described in Table 2 indicates that, the inhibitory effects of the antifungal substances on the mycelial growth of the fungal species were more than of the volatile compounds.

In addition of three biovars of *P. fluorescens*, also *B. megaterium*, *B. brevis* and *B. subtilis* were able to produce siderophores. Only *P. fluorescens* bv. 5 produced hydrogen cyanide (HCN). Isolates of

Table 5: Efficacy of rhizobacteria on disease severity caused by fungal species on Cvs. Alvand and Roshan

	Bipolaris australienis		*Bipolaris sacchari*	
Treatments	Alvand	Roshan	Alvand	Roshan
B. subtilis1	1.86abc	1.46c	1.58cd	2.04a
P. fluorescens bv1	1.68c	1.77ab	2.04ab	1.68bc
B. coagulaus	1.77bc	1.94ab	1.86a-c	1.68bc
B. subtilis2	1.34d	1.87ab	1.87a-c	2.04a
P. fluorescens bv 5	1.86a-c	2.04a	1.68c	2.04a
B. licheniformis	1.58cd	1.86ab	2.12a	1.68bc
P. fluorescensbv 4	1.68c	1.94ab	1.68c	2.04a
B. megaterium	1.68c	1.95ab	1.56d	2.12a
B. brevis	1.58cd	2.12a	2.04ab	2.04a
Control	1.68c	1.94ab	1.87a-c	2.12a

Table 6: Efficacy of rhizobacteria on disease incidence caused by fungal species on Cvs. Alvand and Roshan

	Bipolaris australienis		*Bipolaris sacchari*	
Treatments	Alvand	Roshan	Alvand	Roshan
B. subtilis1	5.31f	5.31c	7.25b-e	8.44ab
P. fluorescens bv1	8.27a-d	9.37a	7.55a-e	7.81ab
B. coagulaus	7.54b-e	8.28ab	6.85de	7.83ab
B. subtilis2	7.74a-e	8.75a	7.75a-e	9.13a
P. fluorescens bv 5	7.83a-d	9.2a	8.2a-e	9.21a
B. licheniformis	7.08c-e	7.64ab	6.18ef	6.68bc
P. fluorescens bv 4	8.46a-d	9.2a	7.55b-e	8.99a
B. megaterium	8.72a-c	9.51a	7.51b-e	8.87a
B. brevis	8.63a-c	9.37a	6.84d-f	8.42ab
Control	8.84ab	9.53a	9.29a	9.28a

- Means followed by a common letter(s) in a column are not significantly different according to Duncan's Multiple Range Test (p=0.05).
- Distribution of data were normalized with √X+1/2

B. subtilis, *B. coagulans* and *B. licheniformis* secreted both protease and cellulose enzymes, whereas, *P. fluorescens* bv. 5 produced only protease and *P. fluorescens* bvs. 1 and 4 produced cellulase enzyme.

Influence of the interaction of rhizobacteria and fungal species on the wheat growth factors: Results of the co-inoculation of wheat with the isolated rhizobacteria and the fungal species indicated that, seed treating with bioantagonistic rhizobacteria, in addition to reduction in disease severity and incidence, caused considerable positive effects on the wheat's growth factors (Table 3 and 4). Measured factors, especially means of height, dry weight and disease severity and incidence were affected significantly by the fungi, bacteria and the interaction of the two (Table 3-6). Coating seeds with *B. subtilis*1, *B. licheniformis* and *P. fluorescence* bv. 4 showed positive effects on the yield components of wheat cultivars (such as seed germination, means of height, number of tillers and dry weight of aerial parts of the plant), however, treating of seeds with *P. fluorescence* bv. 5 and *B. subtilis* 2 caused reduction in the most wheat growth factors even in comparing with the control (Table 3 and 4). Inoculation of soil to the fungal species, not only caused significant reduction effect on disease severity and incidence, especially in the case of *B. sacchari*, but also had a significant reduction on seed germination and the means of height crop of the both wheat cultivars, Alvand and Roushan (Table 3 and 4).

Influence of the antagonistic rhizobacteria on root-rot disease: Data presented in Table 5 and 6 regarding of the co-inoculation of wheat with the rhizobacteria and the fungal species indicated that, seed treating with bioantagonistic rhizobacteria, have reduced the disease severity and incidence on both cvs. Alvand and Roushan (Table 5 and 6). In the greenhouse experiments the disease severity and incidence were affected significantly by the fungal species, rhizobacteria and the interaction of the two. In general, *B. subtilis*1 and *B. licheniformis* were determined as the most effective strains in reducing the incidence of disease caused by the fungi. Results obtained here, regarding the coefficient correlations of these factors showed that only among means of height with dry weight and disease incidence with dry weight in the case of infection of wheat to *Bipolaris australiensis* correlation were significant (r = 0.64) and among other determinants even severity and incidence of disease in this case and among all of the determinants when wheat were inoculated *with B. sacchari*, no significant correlation was seen.

DISCUSSION

Application of fluorescent *Pseudomonads* and other rhizobacteria have drawn attention worldwide because of the production of the secondary metabolites such as siderphore, antibiotics volatile compounds, HCN, enzymes and also induction of systemic resistance[22,24,25].

The potential ability of the *P. fluorescens* and *Bacillus* strains to serve as biocontrol agent of wheat stem-base disease is described here. Members of the both genera of *Pseudomonas* spp. and *Bacillus* spp. are well known antagonistic rhizobacteria[25]. The result obtained from current experiments also indicates similar antagonistic potential.

When *in vitro* antagonism (Table 2) is compared to *in vivo* disease suppression (Table 5 and 6), it appears that the *in vitro* test has some predictive value for the disease suppression by both pseudomonads and *Bacillus* spp. strains. This especially accounts for *Bacillus megaterium* and *B. coagulans* strains. Different disease-suppressive mechanisms are involved in enhancing the disease suppression. For example, competition for limited carbon sources or iron in the soil can influence the outcome of root colonization[26] and consequently disease suppression. It has been demonstrated that a positive relationship exists between population size of the biocontrol strain on root and disease suppression[27,28]. In general competition for nutrients supplied by roots and seeds and occupation of sites favored for colonization probably are responsible for a small or moderate degree of disease suppression by most PGPR and are of primary importance in some strains. It is likely that the production of volatile compounds could be responsible for enhancing the disease suppressions (Table 2). However, it has been reported that the productive activity of *B. megareium* might be due to its potential for providing the phosphorus through the soil-phosphate mineralization[29]. In the case of antagonistic mechanism of *B. coagulans*, as been demonstrated in the result, there is some evidence to suggest that, its potential to providing both protease and cellulase enzymes seems to be involved in this regards[30].

In addition of three biovars of *P. fluorescens*, also *B. megaterium*, *B. brevis* and *B. subtilis* were able to produce siderophores. Methalophores or siderphores are low-molecular-weight molecules that are secreted by microorganisms to take up iron from the environment and their mode of action in suppression of disease were thought to be solely based on competition for iron with the pathogen[11]. Production of metabolites such as antifungal substances, siderophores and hydrogen cyanide is the primary mechanisms of biocontrol. So, the disease suppression by these strains which been reported here, might be due to involvement of this mechanism.

Production of secondary metabolites such as Phenazine-1-carboxilic Acid (PCA), 2, 4-diacetyl phloroglucinol (phl), pyoleutorin (plt), pyrrolnitrin, oomycin and Hydrogen Cyanide (HCN) are also characteristic features of biocontrol agents[11,13]. Probably, accumulation of HCN produced by *P. fluorescens* bv. 5 on the roots region due to inoculation of seeds with high population of this strain, is resulted in negative influence of this strain on the growth and yield components of wheat.

Seed treating with *B. subtilis*1, *B. licheniformis* and *P. fluorescens* bv. 4 showed positive influence on the most yield components of wheat cultivars, however, treating of seeds with *P. fluorescence bv.* 5 and *B. subtilis* 2 caused reduction in the most wheat growth factors even in comparing with the control (Table 3 and 4). Also comparing disease severity and incidence in wheat cultivars, confirmed the differences between sensitivity of cultivars to pathogenic agents such as the mentioned fungi, since in these experiments, *cv.* Roshan was more resistant and exhibit milder symptoms of necrosis and root rot, comparing with *cv.* Alvand (Table 5 and 6). Numerous biotic and abiotic factors are likely to contribute to this inconsistent performance of biocontrol microorganisms[11,13,22].

In conclusion, these results indicate that, specific rhizobacterial agents can influence disease suppression and could be considered as part of a disease control strategy like integrated pest management which offers a successful approach for the deployment of both agro-chemicals and biocontrol agents. However, no uniformity in the results of using biocontrol agents especially in natural situation and fields, emphasize the necessity of the continual study in this regard as well as their ecological interactions.

ACKNOWLEDGMENT

Financial support for this study by the Research Council of Bu-Ali Sina University, Hamadan, Iran, is gratefully acknowledged.

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