Middle Eastern and Russian Journal of Plant Science and Biotechnology ©2007 Global Science Books

Selection, Characterization and Application of Microbial Antagonists for the Control of Wheat Fusarium Head Blight, Using Single and Mixture Strains of Antagonistic Bacteria on Different Cultivars

M. Javad Soleimani[1*] • Heshmatollah Rahimian[2] • Mahsa Alimi[1]

[1] Department of Plant Protection, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran
[2] Department of Plant Sciences, Faculty of Agriculture, Mazandaran University, Sari, Iran

Corresponding author: * **agrms@basu.ac.ir**

ABSTRACT

Fusarium head blight incited by *Fusarium graminearum*, is a devastating disease that causes extensive yield and quality losses to wheat (*Triticum aestivum* L.) throughout the world. One strategy to control Fusarium head blight is the use of antagonistic bacteria. In order to assess the potential of phyllospheric microorganisms in biological control of such foliage diseases, in this study one hundred and ninety isolates of antagonistic bacteria including *Pseudomonas*, *Erwinia* and *Bacillus* spp. from the phyllosphere of healthy and infected wheat, were collected. Among them, by using the dual culture method, only eight isolates with the most antagonistic ability against the growth of pathogenic fungal species (*Fusarium graminearum*) were selected and purified. According to the results of biochemical and physiological tests, they were identified as three biovars of *Pseudomonas fluorescens*, an isolate of *Erwinia herbicola* and some species of *Bacillus* like *B. subtilis* and *B. cereus*. Production of antifungal substances and volatile metabolites, 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 bacteria on disease severity and incidence caused by *F. graminearum*, by the application of bacteria were studied. Statistical analysis of data indicated that treating wheat spikes with some of the antagonistic bacteria not only reduced the disease severity and incidence when compared with the control, but also had showed a positive influence on growth and yield of wheat cultivars.

Keywords: *Bacillus subtilis*, biological control, *Erwinia herbicola*, FHB, *Fusarium graminearum*, *Pseudomonas fluorescens*

INTRODUCTION

Fusarium head blight (FHB), is responsible for significant damage in humid and semi-humid regions of the world. The primary causal agent of the disease in the north of Iran is *Fusarium graminearum*, which causes foliage diseases of matures, including pink and mouldy spots on wheat glumes of spiklets. This disease is economically important and is among the most widespread disease of wheat, especially in areas with warm and wet condition during growing season (Parry *et al*. 1995; Strange and Smith 1971). The disease is spread all over the world especially in North Dakota, Mid-western and Eastern states of the USA, as well as in Central and Eastern Canada and Brazil (Andrews 1992; Knudsen *et al*. 1995). The annual losses are estimated at US $1 billion of yields in Canada (Gilbert and Tekauz 2000; Sutton 1982). In Iran, especially in the Northern provinces that are located on the margin of the Caspian Sea, is the most devastating disease on wheat. FHB is of particular concern because of the ability of the *Fusarium* species to produce mycotoxins in the grain that are harmful to human and animal consumers (Stack and McMullen 1985).

Chemical control and resistant cultivars are potential options for reducing the severity of FHB. Most wheat cultivars currently in production are vulnerable to infection. In order to reduce the application of chemicals in diseases control, especially air-borne pathogens, some efforts in biological control of *F. graminearum* by antagonistic microorganisms, have been conducted (Ramesh-Kumar *et al*. 2002). In fact, the phyllospheric organs are the first-line of defense against air-borne pathogenic fungi (Andrews 1992). Therefore, there is an excellent opportunity to find phyllosphere-competent bacteria in the phyllosphere, 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 such as competition, antibiosis and parasitism. In recent years, fluorescent pseudomonads have drawn attention worldwide because of their ability to colonize the organ and production of secondary metabolites such as siderophores, antibiotics, volatile compounds, enzymes and phytohormones (Cook and Baker 1983).

Biological control, though currently not commercially available for FHB, would offer another option for reducing the disease. The feasibility of biologically controlling this disease (FHB) has been demonstrated (Kloepper *et al*. 1991; Knudsen *et al*. 1995; Khan *et al*. 2001). In this regard, bio-antagonistic fungi such as *Trichoderma* spp., *Chaetomium* sp. and *Gliocladium roseum* (Cook and Baker 1983; Stack and McMullen 1985; Knudsen *et al*. 1995) and antagonistic bacteria such as *Sporobolomyces roseus*, *Pseudomonas putida* and *Paenibacillus macerans* have been studied (Bleakley *et al*. 2000; Khan *et al*. 2001). Certain Plant Growth Promoting Bacteria (PGPB) degrade fusaric acid produced by *Fusarium* sp., the causal agent of wilt, and thus prevent pathogenesis. Some PGPB can also produce enzymes that can lyse fungal cells. Application of a single strain of a biological control agent only rarely leads to a level of suppression as observed so far, and positive effects with single inoculants are often inconsistent (Weller 1998; Khan *et al*. 2001). Hence, it was postulated that a concerted action of several microorganisms and mechanisms is responsible for the highly consistent disease suppressiveness (Andrews 1992). Consequently, application of a mixture of

Received: 30 July, 2007. Accepted: 9 October, 2007.

Original Research Paper

biocontrol agents is likely to more closely mimic the natural situation and may, therefore, represent a more viable control strategy. Pierson and Weller (1994) showed that combinations of several fluorescent pseudomonads have the potential for greater biocontrol activity against wheat take-all disease compared to the same strains applied individually. There are also reports stating that combinations of biological control agents do not result in improved suppression of disease as compared to the separate inoculants (Sneh *et al*. 1984). Positive and negative interactions between introduced biocontrol microorganisms or between an introduced biocontrol agent and the indigenous microflora can influence their performance in the rhizosphere.

The objectives of the present research were to select and characterize antagonistic bacteria and their antagonistic mechanisms on reducing severity and incidence of disease in the greenhouse, as well as to determine whether specific interactions between *Pseudomonas* spp. strains and other bacterial isolates influence disease suppression by combinations of these strains. Subsequently, suppression of FHB by the single strains and their combinations were investigated to determine how far interactions *in vitro* between the strains have predictive value for disease suppression by combinations of these strains *in vivo*.

MATERIALS AND METHODS

Isolation of *Fusarium graminearum* species

Spikes and rachises of collected wheat from several infected fields in 2004 that showed symptoms of pink spots on spikes, small and shrunken seeds and white heads were submerged in 0.5% sodium hypochlorite for 3-5 min. After this treatment, they were extensively washed with sterile distilled water, placed on Petri dishes containing Potato Dextrose Agar (PDA), and incubated at 22-25°C for one week. *Fusarium graminearum* was on of the most prevalent fungi that grew on the medium. Fungal recognition was on the basis of characteristics such as long falcate macroconidia, simple lateral phialides, branched conidiophores and many isolates of this fungus have floccose aerial mycelium and rose to coral pigmentation (Montealegre *et al*. 2003).

Preparation of fungal inoculum and pathogenicity test

Two hundred grams of sterile wheat stubble and barley were placed in an Erlenmeyer flask (500 ml), and were incoculated with four 5 mm mycelial disks from a five-day-old culture of fungal species, and incubated at room temperature for 96 hours on a rotary shaker at 150 rpm/min. Fungal colonies were developed, and for each pathogenicity test, suspensions of macroconidia were obtained by flooding the surface of the colonized PDA with PO_4 buffer and dislodging conidia using a sterile inoculating loop. The inoculum mixture of *F. graminearum* (5×10^5 conidia/ml) was sprayed on the wheat spikes *in vivo*. After inoculation, wheat plants were misted with water, incubated in a plastic humidity chamber for 72 h at approximately 22°C, and transferred to greenhouse benches. Plastic humidity chamber consisted of a PVC pipe frame covered with clear plastic. The disease symptoms were assessed 3 weeks after inoculation.

Isolation, selection and identification of bacteria

Antagonistic bacteria were isolated from infected and healthy wheat spikes in 2004. More than 300 spike segments of wheat were washed with tap water, 0.5 g added to 50 mL, 1% peptone and were shaken for 30 min, after which 0.1 mL of each bacterial suspension was spotted in nutrient agar (NA) medium, incubated at 22°C. Fluorescent Pseudomonads were isolated on King's B (KB) medium (Schaad *et al*. 2001). According to the methodology of Schaad *et al*. (2001) antagonistic isolates of antagonistic bacteria were selected by biochemical, physiological and biological tests (Schaad *et al*. 2001). The single colonies of purified isolates were pre-evaluated against the isolates of *F. graminearum* by using dual culture in Petri dishes containing PDA and the percentage of fungal growth inhibition were determined. Eight isolates with the most inhibition percentage, which were calculated by using the following formula (Sivan *et al*. 1987), were selected and re-purified:

% Inhibition = (1- (fungal growth / control growth)) × 100

Antagonistic mechanisms of antagonistic bacteria

Production of volatile metabolites

100 µL of each bioantagonistic bacterial suspension (5×10^9 cfu / mL) was spread on a Petri dish containing KB medium, and a 5-mm disk of a five-days-old pure culture of *F. graminearum* 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 with parafilm to isolate the inside atmosphere, preventing loss of volatile compounds. Plates were incubated at 22-25°C in the dark 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 in triplicate and repeated three times. Results are expressed as means of percentage inhibition ± S.D. of the growth of *F. graminearum* in the presence and absence of any bacterial isolates.

Production of diffusible antifungal substances

According to Montealegre (2003), 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). 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 *F. graminearum* isolate. Plates were further incubated at 22°C for one week and the growth of the pathogen was measured. Controls were run with mock-inoculated PDA containing plates on the cellophane membrane (replacing the bacterial suspension by sterile distilled water), and further inoculated with *F. graminearum*. Results are expressed as means of percentage inhibition ± S.D. of growth of *F. graminearum* in the presence and absence of any bioantagonistic bacterial isolate.

Protease production

Petri dishes containing SMA (Skim Milk Agar) culture medium including milk powder (15 g L^{-1}), yeast extract (5 g), blood agar (4 g) and agar-agar (13.5 g) were inoculated with each isolate and incubated at 27°C for 24 h. Production of a colorless hallow around each bacterial colony indicated protease activity of that strain (Montealegre *et al*. 2003). Each experiment considered a single bacterial and multiple bacteria isolate and was run in triplicate and was repeated three times.

Cellulose production

Nine mL of medium containing K_2HPo_4 (1 g), $NaNO_3$ (0.5 g), $MgSO_4$ (0.5 g), KCl (0.5 g) and FeS_4 (0.01 g) in 1 L distilled water was added to each tube. A piece of filter paper (1×9 cm^2) was placed in each tube. After sterilization, 1 mL of each bacterial suspension was added and tubes were incubated at 25°C. Up to three weeks, the change in color of filter papers was traced every day. In the 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 prepared as previously been described. The bacterial suspension was adjusted to about 10^7-10^9cfu mL^{-1}, using haemocytometer for each experiment. The bacterial cells suspensions were used for spraying on wheat heads.

Table 1 Assessment of disease severity (Horsfall-Barrett scale) (based on Stack and McMullen 1998).

Disease severity levels	% Area with discoloration	Score
Clean	0-7	1
Slight	14-21	2
Moderate	33-50	3
Hard	50-66	4
Severe	79-90	5
Strongly severe	100	6

Evaluation of disease intensity on wheat

For this purpose, the effects of fungal species on wheat growth and health were evaluated. The prepared fungal spore suspension (5×10^5 conidia/ml) was sprayed on the spikes in each pot (on phyllospheric organs). The disease intensity was scored for each tiller on five plants 3-4 weeks after spraying the suspension at the flowering stage. Disease intensity was scored using the Horsfall-Barrett scale (Stack and McMullen.1998) counting infected spiklets and expressing that as a percentage of total spiklets, which is very accurate to measure severity, as described in **Table 1**. Four replications were made for each treatment. The pots were arranged in a factorial design, and the trials were repeated at least twice with similar results.

Efficacy of spraying the suspension of antagonistic bacterial isolates

In greenhouse conditions, wheat seeds (cvs. 'Shanghay' and 'Falat') were surface sterilized in 0.5% sodium hypochlorite for 5 minutes and then air dried in a laminar flow. Suspensions of antagonistic bacteria at a total concentration of 10^7-10^9 cfu mL^{-1} were sprayed twice on the phyllospheric parts in the boot stage and a fungal spore suspension at a total concentration of 5×10^5 cfu mL^{-1} (*F. graminearum*) was sprayed on the spikes of pot-plants in the flowering stage, five times a week. Pots were maintained in the greenhouse benches at 25°C and 90% relative humidity using misfire. Control treatments were inoculated with sterile distilled water, and plants with disease symptoms were recorded after 3-4 weeks after spraying the agent of disease. The effects of treatments on wheat growth factors (such as means of 100-grain weight, and dry weight of aerial parts of each plant) and disease severity and incidence on two wheat cultivars, 'Shanghay' (moderately resistant) and 'Falat' (susceptible), were assessed.

Statistical analysis

The data obtained were subjected to analysis of variance and the means separated by using Duncan's Multiple Range Test and ANOVA. Tests were used to establish significant differences at $P < 0.05$.

RESULTS

Isolation and identification of the fungal species

The prevalent agents isolated from Fusarium head blight of wheat in wheat fields of the margin of the Caspian Sea, Iran were identified as *F. graminearum*, and have been described by Nelson *et al.* (1983). In the pathogenicity test, inoculation of spikes to the fungal inocula, in addition to significant effects on disease severity and incidence, showed a significant reduction on the 100-grain weight and means of height of both the wheat cultivars ('Shanghay' and 'Falat').

Selection and identification of antagonistic bacteria

One hundred and ninety bacterial strains were isolated from wheat anthers and used in the bioassay test against fungal isolates. Based on the biochemical and physiological tests (Schaad *et al*. 2001), six antagonistic bacteria with the greatest inhibition percentage against *F. graminearum* were identified as: three biovars of *Pseudomonas fluorescens* (1, 4 and 5), an isolate from *Erwinia herbicola* and species of *Bacillus*, including *B. subtilis* and *B. cereus*.

Antagonistic mechanisms of the antagonistic bacteria

In vitro all of the six single isolates and two mixtures of isolates tested positive in the production of volatile and diffusible antifungal metabolites against the fungal isolates. Volatile compound provided by mixture isolates followed by *B. cereus* showed the most and those, provided by *P. fluorescens* bv. 5 *and Erwinia herbicola* indicated the least inhibitory effect on the growth rate of *F. graminearum* (**Fig. 1**). *A*ntifungal substances produced by multiple isolates followed by *P. fluorescens* bv. 1 and *P. fluorescens* bv. 4 and 5 in the case of *F. graminearum* were the most effective strains (**Fig. 2**). In general, the result described in **Figs. 1** and **2** indicated that the inhibitory effects of the antifungal substances on the mycelial growth of the fungal isolates were more than of the volatile compounds.

Only *P. fluorescens* bv. 5 produced hydrogen cyanide (HCN). Biovars of *P. fluorescens* secreted both protease and cellulose enzymes, whereas, isolates of *B. subtilis* and *B. cereus* produced only protease.

Influence of the interaction of antagonistic bacteria and fungal species on the wheat growth factors

Results of the co-inoculation of wheat with the isolated antagonistic bacteria as well as the fungal species indicated that spraying the suspension of bioantagonistic bacteria, in

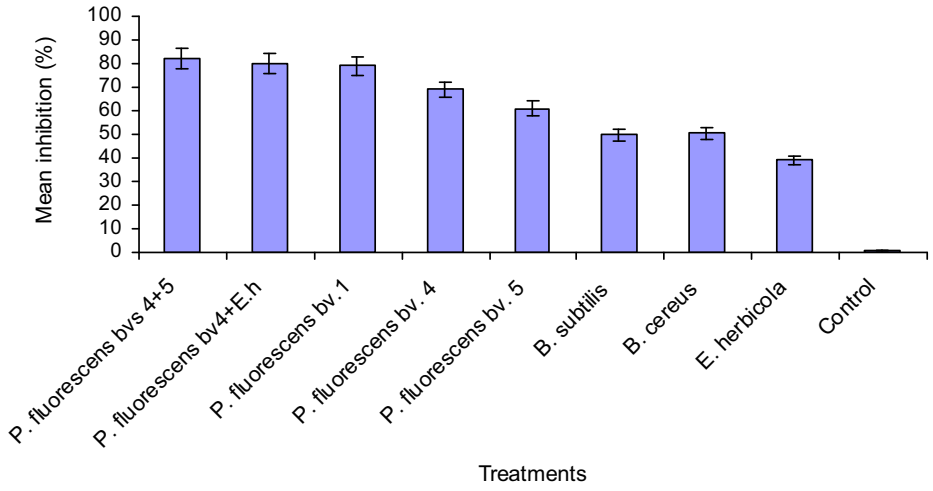

Fig. 1 The effect of bacterial volatile compounds on inhibition percentage of fungal growth on PDA.

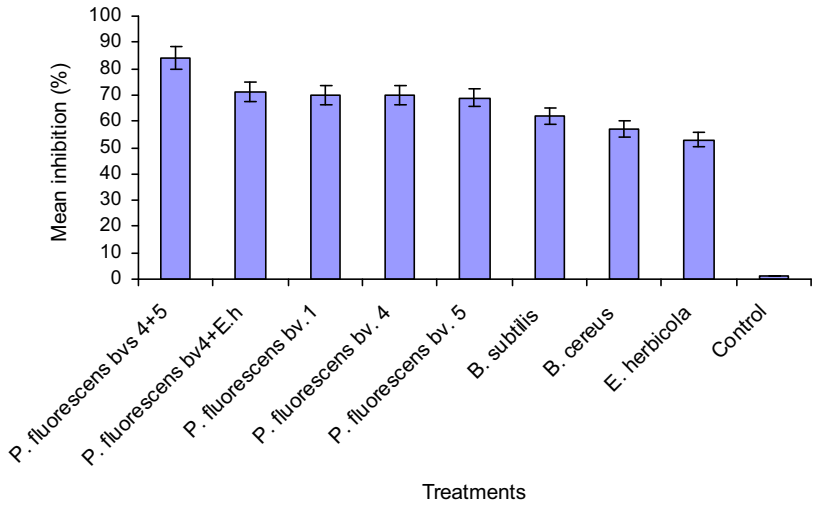

Fig. 2 The influence of bacterial antifungal substances on inhibition of fungal growth on PDA.

Table 2 Influence of the interactions of antagonistic bacteria and fungal isolates on the 100-grain weight cvs. 'Shanghay' and 'Falat'.

Treatments	*Fusarium graminearum*	
	Shanghay	Falat
P. fluorescens bv *4* + *P. fluorescens* bv *5*	3.72 ± 0.16 cd	5.097 ± 0.14 a
P. fluorescens bv *4*+ *E. herbicola*	2.98 ± 0.17 e	4.39 ± 1.30 b
P. fluorescens bv *1*	2.60 ± 0.55 f	3.73 ± 0.14 cd
P. fluorescens bv *4*	3.00 ± 0.18 e	3.72 ± 0.14 cd
P. fluorescens bv *5*	3.26 ± 0.10 e	3.05 ± 0.17 e
B. subtilis	3.95 ± 0.21 c	3.02 ± 0.16 e
B. cereus	2.59 ± 0.03 f	2.98 ± 0.20 e
E. herbicola	2.29 ± 0.17 f	3.58 ± 0.19 d
Control	3.55 ± 0.19 d	2.41 ± 0.09 f

Means followed by a common letters in a column are not significantly different according to Duncan's Multiple Range Test (p=0.01).

Table 3 Efficacy of antagonistic bacteria on disease severity on cvs. 'Shanghay' and 'Falat'.

Treatments	*Fusarium graminearum*	
	Shanghay	Falat
P. fluorescens bv *4*+ *P. fluorescens* bv *5*	1.91 ± 1.82 c	6.48 ± 7.36 c
P. fluorescens bv *4*+ *E. herbicola*	1.91 ± 1.82 c	6.48 ± 7.36 c
P. fluorescens bv *1*	2.40 ± 0.36 b	7.74 ± 8.65 b
P. fluorescens bv *4*	4.20 ± 2.98 a	7.74 ± 8.65 b
P. fluorescens bv *5*	5.20 ± 1.50 a	9.23 ± 4.79 a
B. subtilis	4.20 ± 2.98 a	7.74 ± 8.65 b
B. cereus	4.20 ± 2.98 a	7.74 ± 8.65 b
E. herbicola	4.20 ± 2.98 a	9.23 ± 4.79 a
Control	4.80 ± 1.58 a	10.02 ± 0.00 a

Means followed by a common letters in a column are not significantly different according to Duncan's Multiple Range Test (p=0.01).

addition to reduce the disease severity and incidence, caused considerable positive effects on the wheat growth factors (**Table 2**). Measured factors, especially means of 100-grain weight and disease severity and incidence were affected significantly by the fungi, bacteria and the interaction of the two (**Tables 2-4**). Spraying wheat spikes (cv. 'Falat') with mixture isolates *P. fluorescence* bv. 4 and 5 and *P. fluorescence* bv. 4 plus *E. herbicola* showed the most positive effects on the yield components of wheat cultivars (such as 100-grain weight). However, treating wheat spikes (cv. 'Shanghay') with a single strain of all isolates except *B. subtilus* caused reduction in most wheat growth factors when compared with the control (**Table 2**). Inoculation of spikes to the fungal species not only caused significant reduction effect on disease severity and incidence, but also had a significant reduction on seed germination and the means of crop height of the both wheat cultivars, 'Shanghay' and 'Falat' (data not presented).

Table 4 Efficacy of antagonistic bacteria on disease incidence on cvs. 'Shanghay' and 'Falat'.

Treatments	*Fusarium graminearum*	
	Shanghay	Falat
P. fluorescens bv *4*+ *P. fluorescens* bv *5*	22.25 ± 3.86 h	64.90 ± 1.52 bcde
P. fluorescens bv *4*+ *E. herbicola*	42.61 ± 1.97 g	66.66 ± 0.98 bcd
P. fluorescens bv *1*	50.19 ± 12.90 fg	82.28 ± 1.05 a
P. fluorescens bv *4*	55.45 ± 8.01 def	69.61 ± 1.26 b
P. fluorescens bv *5*	55.15 ± 0.75 def	87.88 ± 1.35 a
B. subtilis	56.86 ± 5.39 cdef	82.28 ± 1.05 a
B. cereus	46.59 ± 10.45 fg	68.53 ± 0.37 bc
E. herbicola	54.31 ± 5.82 efg	85.36 ± 9.84 a
Control	52.19 ± 8.44 fg	92.20 ± 3.17 a

Means followed by a common letters in a column are not significantly different according to Duncan's Multiple Range Test (p=0.01).
Distribution of data were normalized with √X+1/2
± means SE or standard error among replicates of a treatment.

Influence of antagonistic bacteria on FHB

Data presented in **Tables 3** and **4** regarding the co-inoculation of wheat with the antagonistic bacteria and the fungal species indicated that spraying with bioantagonistic bacterial suspension reduced the disease severity and incidence in both cvs. 'Shanghay' and 'Falat' (**Tables 3, 4**). In greenhouse experiments the fungal species, antagonistic bacteria and the interaction of the two affected the disease severity and incidence significantly. In general, application of mixture strains either *P. fluorescens* bv. 4 and 5 or *P. fluorescens* bv. 4 and *E. herbicola* were determined as the most effective strains in reducing the incidence of disease caused by the fungi followed by single strain application such as *B. subtilis*1 and *P. fluorescens* bv. 1 and 4. The results obtained here indicated that antagonistic effects on disease suppression due to bacterial inoculation were more remarkable in susceptible cultivar rather than in the latter one.

DISCUSSION

Application of genetic diversity of biological control agents by using mixtures of microorganisms may result in treatments that persist longer in the rhizosphere and utilize a wider array of biocontrol such as induction of systemic resistance, production of antibiotics and competition for nutrients, under a broader range of environmental conditions (Pierson and Weller 1994). The potential ability of *P. fluorescens* and *Bacillus* strains to serve as a biocontrol agent of Fusarium head blight has been described here. Members of the genera of *Pseudomonas* spp., *Erwinia* spp. and *Bacillus* spp. are well known antagonistic bacteria (van Loon *et al*. 1998; Khan *et al*. 2001). The results obtained our current

experiments also indicate a similar antagonistic potential on wheat scab disease.

When *in vitro* antagonism (**Figs. 1, 2**) is compared to *in vivo* disease suppression (**Tables 3, 4**), it appears that the *in vitro* test has some predictive value for the disease suppression by pseudomonads, *Erwinia herbicola* and *Bacillus* spp. strains. This especially accounts for *application of mixture* strains, which indicates that specific interactions between biocontrol strains can influence disease suppression by the combination of these strains. The enhanced disease suppression by the combination of strains either *P. fluorescens* bv. 4 and 5 or *P. fluorescens* bv. 4 and *E. herbicola* could be the result of a combination of different disease-suppressive mechanisms.

Different disease-suppressive mechanisms are involved in enhancing the disease suppression including, competition and parasitism (Kragelund and Nybroe 1996). It has been demonstrated that a positive relationship exists between population size of the biocontrol strain on phyllosphere and disease suppression (Pierson and Weller 1994; Smith *et al.* 1997). In general competition for nutrients supplied by the plant and occupation of sites favored for colonization are probably responsible for a small or moderate degree of disease suppression by most PGPB and are of primary importance in some strains. It is likely that the production of antifungal compounds could be responsible for enhancing the disease suppressions (**Fig. 2**). However, it has been reported that in the case of the antagonistic mechanism of *B. subtilis*, there is some evidence to suggest that its potential to providing both protease and cellulolytic enzymes seems to be involved in this regard (Pierson and Weller 1994).

Production of metabolites such as antifungal substances, siderophores and hydrogen cyanide is the primary mechanisms of biocontrol. Therefore, the disease suppression by these strains which we reported here might be due to the involvement of one of these mechanisms. 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 (Andrews 1992; Knudsen *et al*. 1995). Probably, the accumulation of antifungal products produced by *P. fluorescens* bv. 5 and other strains on the phyllosphere region due to inoculation of spikes with a high population of these strains resulted in a positive influence on the growth and yield components of wheat. The disease-suppressive mechanisms of the other strains are currently being investigated.

Spraying phyllospheric parts with mixture strains of *P. fluorescens* bv. 4 and 5 and also *P. fluorescens* bv. 4 plus *E. herbicola* showed a positive influence on most yield components of both wheat cultivars, however, spraying mixture suspension of *P. fluorescence bv* 5 and *Erwinia herbicola* caused a reduction in most wheat growth factors even when compared with the control (**Table 2**). 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. 'Shanghay' was more resistant and exhibit milder symptoms spots and shrinked seeds, comparing with cv. 'Falat' (**Tables 3, 4**). Numerous biotic and abiotic factors are likely to contribute to this inconsistent performance of biocontrol microorganisms (Andrews 1992; Knudsen *et a*l. 1995; Maurhofer *et al*. 1995).

In conclusion, in the absence of highly resistant cultivars or highly effective registered fungicides, these results indicate that, specific antagonistic bacterial agents especially in mixture could be considered as a viable option for reducing wheat scab. The results also indicated that the specific interactions between biocontrol strains can influences disease suppression by the combination of these strains, which could be considered as part of a disease control strategy like integrated pest management, thought 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. It is necessary, therefore, to further investigation the microbial interactions that enhance or detract from biocontrol in order to understand and predict the performance of (mixtures of) biocontrol agents.

ACKNOWLEDGEMENTS

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

REFERENCES

Andrews JH (1992) Biological control in the phyllosphere. *Annual Review of Phytopathology* **30**, 603-633

Bleakley BH, Draper MA, Ruden KR (2000) Controlling Fusarium head blight with biological antagonists. In: *Proceedings of the 2000 National Fusarium Head blight Forum*, pp 75-76

Cook RJ, Baker KF (1983) The nature and practice of biological control of plant pathogens. APS Press, St. Paul, 539 pp

Gilbert J, Tekauz A (2000) Recent development in research on Fusarium head blight of wheat in Canada. *Canadian Journal of Plant Pathology* **22**, 1-8

Khan NL, Schisler DA, Boehm MJ, Slininger PJ, Bothast RJ (2001) Selection and evaluation of microorganisms for biocontrol of Fusarium head blight of wheat incited by *Gibberella zeae*. *Plant Diseases* **85**, 1253-1258

Kloepper JW, Zablotowics RM, Tipping EM, Lifshitz R (1991) Plant growth promotion mediated by bacterial rhizosphere colonizers. In: Keister DL, Cregan PB (Eds) *The Rhizosphere and Plant Growth*, Kluwer Academic Publishers, pp 315-326

Knudsen IMB, Hockenhull J, Jensen DF (1995) Biocontrol of seedling diseases of barley and wheat caused by *Fusarium culmorum* and *Bipolaris sorokiniana*: Effects of selected fungal antagonists on growth and yield components. *Plant Pathology* **44**, 467-477

Kragelund L, Nybroe O (1996) Competition between *Pseudomonas fluorescens* Ag1 and *Alcaligenes eutrophus* during colonization of barley roots. *FEMS Microbiological Ecology* **20**, 41-51

Maurhofer M, Keel C, Haas D, Defago G (1995) Influence of plant species on disease suppression by *Pseudomonas fluorescence* strain CHAO with enhanced production. *Plant Pathology* **44**, 40-50

Montealegre JR, Reyes R, Perez L, Herrera MR, Silva P, Besoain X (2003) Selection of bioantagonistic bacteria been used in biological control of *Rhizoctonia solani* in tomato. *Electronic Journal of Biotechnology* **6**, 116-127

Nelson PE, Toussoun TA, maracas, WFO (1983) *Fusarium Species: an Illustrated Manual for Identification*, The Pennsylvania State University Press, University Park, 193 pp

Parry DW, Jenkinson P, Mcleod L (1995) Fusarium ear blight (Scab) in small grain. A review. *Plant Pathology* **44**, 207-238

Pierson EA, Weller DM (1994) Use of mixtures of fluorescent pseudomonads to suppress take-all and improve the growth of wheat. *Phytopathology* **84**, 940-947

Ramesh Kumar N, Thirumalai Arasu V, Gunasekaran P (2002) Genotype of antifungal compounds producing plant growth-promoting rhizobacteria, *Pseudomonas fluorescence*. *Current Science* **82**, 1463-1467

Schaad NW, Jones JB, Chum W (2001) *Laboratory Guide for Identification of Plant Pathogenic Bacteria*, APS Press, St. Paul, 373 pp

Sivan A, Ucko O, Chet I (1987) Biological control of *Fusarium* crown rot of tomato by *Trichoderma hazianum* under field condition. *Plant Diseases* **71**, 587-595

Smith KP, Handelsman J, Goodman RM (1997) Modeling dose-response relationships in biological control: partitioning host responses to the pathogen and biocontrol agent. *Phytopathology* **87**, 720-729

Sneh B, Dupler M, Elad Y, Baker R (1984) Chlamydospore germination of *Fusariun oxysporum f.sp. cucumerinum* as affected by fluorescent and lytic bacteria from a *Fusarium* suppressive soil. *Phytopathology* **74**, 1115-1124

Stack RW, McMullen MI (1985) Head blighting potential of *Fusarium* species associated with spring wheat heads. *Canadian Journal of Plant Pathology* **70**, 79-82

Stack RW, McMullen MP (1998) *A Visual Scale to Estimate Severity of Fusarium Head Blight in Wheat*, North Dakota State University Extension Services, 1095 pp

Strange RN, Smith H (1971) A fungal anther, which predisposes wheat to attack by *Fusarium graminearum*. *Physiological Plant Pathology* **4**, 277-290

Sutton JC (1982) Epidemiology of wheat head blight and maize ear by *Fusarium graminearum*. *Canadian Journal of Plant Pathology* **72**, 199-200

van Loon LC, Baker HM, Pieters CMJ (1998) Systemic resistance induced by rhizosphere bacteria. *Annual Review of Phytopathology* **36**, 453-483

Weller DM, Cook RJ (1983) Suppression of take-all of wheat by seed treatment with fluorescent Pseudomonads. *Phytopathology* **73**, 463-469

Weller DM (1988) Biological control of soilborne plant pathogens in the rhizosphere with bacteria. *Annual Review of Phytopathology* **2**, 379-407