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Full Length Research Paper

Evaluation of some antagonistic bacteria in biological control of *Gaeumannomyces graminis* var *tritici* causal agent of wheat take-all disease in Iran

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Take-all is a disease of wheat root caused by the soil borne fungus, *Gaeumannomyces graminis* (Sacc) Von Arx and Oliver var *tritici* Walker (Ggt) all over the word. There has been considered biological control using the microorganisms departed from suppressive soils or wheat roots, widely. In this study, strains of *Bacillus* (*B. subtilis and B. pumilus*), *Pseudomonas* (*P. fluorescent*, *P. putida* and *P. aeruginosa*) and *Chromobacteria* sp., separated from wheat rhizosphere, were assessed for their ability to control this disease. Three fungal isolates (23, 35 and 45) were used in all experiments. Out of 45 bacterial strains in laboratory conditions, 15 strains were selected for isolates 23; 14 strains for isolates 35, and 25 strains for isolates 45 based on dual culture tests. In volatile metabolites test, for isolate 45 the most effective bacteria were strains 132 (*P. fluorescens*) and 159 (*P. putida*) and for isolates 23 and 35, strains 73 (*P. fluorescens*) and 10 (*P. putida*) were the best, respectively. Antibiotic production results indicated that for isolate 23 strains 93, 196, 142 (*P. fluorescens*) and 106 (*P. putida*); isolate 35, strain 10 (*P. putida*) and isolate 45, strain 66 (*B. subtilis*), 68, 103, 159 (*P. putida*), 173, 93 (P. *fluorescens*) and 78 (*P. aeruginosa*) reduced pathogene growth significantly ($p<0.05$). Regarding siderophore production, among fluorescent pseudomonads, strains 189, 87, 5 and MP were selected. The effectiveness of bacteria on take-all was assessed in test tubes and greenhouse by seed treatment, too. Disease severity was assessed based on 0-6 scale and in all three fungal isolates, there were significant differences between treatments ($p<0.01$). In growth chamber experiments for isolate 35, *P. fluorescens* (VN) was the best one and for isolates 23, wheat treated with *P. fluorescens* strains VN and 196 showed less disease. Finally, for isolates 45, the most effective strains were VN, 132, 39, 73, 189, 196, MP (*P. fluorescens*), 53, 103 and 147 (*P. putida*). According to greenhouse results, in isolate 35 wheat treated with strains 10 (*P. putida*) and VN (*P. fluorescens*) showed no infection indicating bacterial effectiveness in fungal inhibition. Isolate 23, strains 100 (*P. putida*), 196, 5, VN (*P. fluorescens*) and 65 (*B. pumilus*) and in isolate 45, strains VN, 132, 93 (*P. fluorescens*) and 53 (*P. putida*) were the most effective ones. In these cases there was no infection as well. The role of bacteria in promoting plant growth was assessed. There was no difference in growth indices between intact plant and plant treated with bacteria (without any fungal inoculation), so bacteria cause growth enhancement by disease control.

Key words. *Gaeumannomyces graminis* var *tritici, Bacillus, Pseudomonas, Chromobacteria,* take-all, biological control.

INTRODUCTION

Take-all is a disease of wheat root caused by the soilborne fungus, *Gaeumannomyces graminis (Sacc) Von Arx and Oliver var tritici* Walker (Ggt) all over the word. There have been some reports of this disease in Iran as well, in provinces of Fars, Golestan, Mazandaran, Markazi and Tehran (Sadeghi et al., 2009; Ghalandar et al., 2000). Although infection can occur from the seedling stage onward, the disease is often observed soon after heading. The foliage turns to pale green and the heads become bleached and ripen prematurely. These heads are specifically white and sterile. Blackening of root which extends even inside the crown and basal up to stem is another symptom of take-all. Stem in base becomes weak and sometimes tilted and the plant falls on the ground. Root system in infected plants is brittle, weak and sparse and in this stage plant can easily draw out of the ground (Christensen and Hart, 2008). Disease control is difficult due to the lack of commercial resistant cultivars and limited effect of fungicides. The 3 to 4 year crop rotations in an effective way can somehow control this disease, but because of economic pressure to grow 2 to 3 wheat crops, this technique is not used in most areas of wheat production. Tillage and Stubble burning which cause the reduction of disease, does not take place to protect soil and environment (Duffy et al., 1996). Severity of take-all is often suppressed, sometimes leading to a condition known as 'take-all decline', which is strongly associated with the development of antagonistic microorganisms in wheat rhizosphere (Nasraoui et al., 2007). Biological control by the microorganisms departed from suppressive soils or wheat roots, is considered widely, because there are no recognized substituted control methods. Disease was controlled using species of *Bacillus sp*. and some strains of fluorescent pseudomonads and other gram-negative bacteria, because they have the capability of forming endospores and a wide range of antifungal metabolites named antibiotics and volatile compositions which are considered as mechanisms of biological control (Kim et al., 1997). Based on FAO's reports, with 13.5 milion tones wheat production, Iran took the 12^{th} place in the world in 2009. Iran followed Argentina as the second country in terms of wheat production growth rate. These data show the importance of disease management in this area. Take-all is a disease that is in progress and biological control is expected to be useful to control the disease. In this study, some strains of *Bacillus* (*B. subtilis* and *B. pumilus, Pseudomonas (P. fluorescent, P. putida* and *P. aeruginosa) and Chromobacterium* sp*.* separated from wheat rhizosphere were assessed for their ability to control this disease.

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MATERIALS AND METHODS

Fungal isolates and inoculum preparation

Fungal isolates of *Ggt* named 23, 35 and 45 with high disease severity from Fars, Mazandaran and Tehran provinces respectively (Iran), isolated from wheat rhizosphere, received from Tarbiat Modarres University Collection (sadeghi et al., 2009). Inoculum for tube assays and greenhouse tests was prepared according to Weller and Cook (1983).

Bacteria and seed treatment

Strains of *Pseudomonas fluorescens* (87, 120, 99, 173, 196, 189, 93, 73, 6, 5, 71, 132, 153, 142, 161, VN, MP), *P. putida* (112, 143, 53, 41, 147, 10, 56, 68, 165, 103, 9, 100, 113, 159, 106, 122, 39), *P. aeruginosa* (78, 35, 46), which have been separated by Sedghiani accompanied by *Bacillus subtilis* (66**),** *B. pumilus* (65) and *Chromobacterium sp.* (99, 23, 102, 80, 3, 62) which have been separated and identified by Zeidabadi, were chosen as antagonist bacteria. Bacteria-coated seeds, based on Duffy et al. (1996) were used in all experiments.

In vitro inhibition

Bacteria were initially selected for their ability to inhibit *G. graminis var tritici in vitro* through dual culture according to Hagedron et al. (1989) method. Antibiotic (Kraus and Lopper, 1990), volatile metabolites (Montealegre et al., 2003) and siderophore production by bacteria (Weller and Cook, 1983) were tested in 9 cm petri dishes, too. Percentage of fungal growth inhibition was calculated according to the following equation:

% inhibition= (Fungal growth- Control growth / Control growth) ×100

Each treatment was replicated three times in a completely randomized design. The entire experiment was performed twice.

Growth chamber tube assay

In this experiment, test tubes (2 cm diameter x 20 cm long) were loaded by 6 cm deep layer of sterile soil and the fungal inoculum was added to the tubes at the rate of 1% (w/w) in the form of colonized wheat kernels. About 1 g of sterile soil was added. Then, three seeds treated with bacteria were sown per tube and covered with sterile dry soil. The rack of tubes was kept in the growth chamber at 25°C and 16 h photoperiod. Each tube rece ived 5 ml of water whenever it was needed. Roots were evaluated after 4 weeks for disease severity, on a scale of 0- 6 (Pierson and Weller, 1994). Each treatment was replicated three times in a completely randomized design. The whole experiment performed twice.

Greenhouse test

The effectiveness of bacteria on take-all was assessed in pots by seed treatment. The role of bacteria in promoting plant growth was investigated, too. Sterile soil was amended with inoculums in the rate of 1% (w/w). Infested soil (600 g) was placed in the pots with a 2 cm layer of non infested soil spread over it. Seven seeds treated with bacteria were put on the soil surface while covered with 5 cm of non infested soil. Plants were grown under 20±3°C and 16 h photoperiod. The irrigation was done monotonously through pot

trays (Nasraoui et al., 2007). After 5 weeks, roots were washed and disease severity was assessed on a scale of 0- 6. Each treatment was replicated three times in a completely randomized block design and the entire experiment was performed twice.

RESULTS

In vitro inhibition

Out of 45 bacterial strains in laboratory conditions, 15 strains were selected for isolates 23, 14 strains for isolates 35, and 25 strains for isolates 45 based on dual culture tests. In volatile metabolites test, for isolate 45 the most effective bacteria were strains 132 (*P. fluorescens*) and 159 (*P. putida*) and for isolates 23 and 35 strains 73 (*P. fluorescens*) and 10 (*P. putida*) were the best, respectively. Antibiotic production results indicated that for isolate 23 strains 93, 196, 142 (*P. fluorescens*) and 106 (*P. putida*); isolate 35, strain 10 (*P. putida*) and isolate 45, strains 66 (*B. subtilis*), 68, 103, 159 (*P. putida*), 173, 93 *(P. fluorescens)* and 78 (*P. aeruginosa*) reduced pathogene's growth significantly ($p<0.05$). Regarding siderophore production, among fluorescent pseudomonads, strains 189, 87, 5 and MP were selected.

Effectiveness of bacterial strains in growth chamber experiments

Infected plants showed symptoms of yellowish from the 3rd week of inoculation and root blackening and stunting were observed at 4th week. Disease severity was assessed based on a 0-6 scale and there were significant differences among treatments ($p<0.01$) in all three fungal isolates. For isolate 35, *P. fluorescens* (VN) was the best one (Table 1) and for isolates 23, wheat treated with *P. fluorescens* strains VN and 196 showed less disease (Table 2). Finally, for isolates 45, the most effective strains were VN, 132, 73, 189, 196, MP (*P. fluorescens*), 53, 103, 39 and 147 (*P. putida*) (Table 3).

Effectiveness of bacteria in greenhouse experiments

In greenhouse condition plants showed symptom from 3rd week and stunting and root and crown blackening were observed. The analysis of variance showed that treatments were significantly different ($p<0.01$) in all three fungal isolates. In isolate 35, wheat treated with strains 10 (*P. putida*) and VN (*P. fluorescens*) showed no infection indicating bacterial effectiveness in fungal inhibition (Table 1). For isolate 23, strains 100 (*P. putida*), 196, 5, VN, 173 (*P. fluorescens*) and 65 (*B. pumilus*) (Table 2) and for isolate 45, strains VN, 132, 93, 173 (*P. fluorescens*) and 53 (*P. putida*) were the most effective ones (Table 3). In these cases, there was no infection, too.

DISCUSSION

This study indicated the ability of *P. fluorescens, P. putida, P. aeruginosa, B. subtilis* and *B. pumilus* for biological control of take-all when applied as seed treat-ment. Bacterial selection, first of all, was laid at laboratory conditions (*in vitro*) by dual culture and then employed for other tests like tube assay and greenhouse conditions. Researchers like Weller and Cook (1983) believe that laboratory tests are suitable for primary selection of antagonists, while Fravel (1988) believes that the most important problem of laboratory methods is the difference between these methods and field evaluation and in addition, direct generalizing of results from laboratory conditions to normal conditions is impossible.

In volatile metabolites test, for isolate 23, strain 73; isolate 35, strain 10, and isolate 45, strains 132 and 159 were the most effective ones ($p<0.01$). Kucuk and Kivanc (2003) believe that the effect of volatile metabolites to inhibit the pathogenic fungi is very small compared to steady metabolites. In the experiment of antibiotic production for fungal isolate 23, strains 93, 106, 196 and 142; isolate 35, strain 10 and isolate 45, strains 66, 68, 78, 173, 93, 103 and 159 showed the most inhibitory effects on fungal growth ($p<0.01$). Weller (1988) and Weller et al. (1997) believed that the production of antibiotics is one of the most important specifications of bacteria for disease control in wheat. Regarding siderophore test results, strains MP, 189, and 5 secrete less siderophore. Weller and Cook (1983) pointed out that the production of siderophore is an important mechanism of growth reaction. Nevertheless, this is probably not the only complicated mechanism. Although some of bacteria have the capability of take-all inhibitory through producing both antibiotic and siderophore in laboratory conditions, more research is needed for determining their role in take-all inhibitory. In isolate 35, strains VN and 10 were more effective than the others; measurement of growth indices confirms this finding, too (Table 1). Since there was no difference in growth indices between intact plant and plant treated with bacteria (without any fungal inoculation), bacteria cause growth enhancement by disease control. In isolate 23, strains VN and 196 reduced the disease in tube bioassay considerably. Although this inhibition was significant, these strains could not prevent disease establishment. In greenhouse condition, these two strains accompanied by strains 100, 65, 46, 173 and 5, prevented fungal esta-blishment. In this way, they appeared to perform the best. Laboratory experiments also showed that strains VN and 196 compete with fungal isolate by siderophore production. Investigation of bacterial effect on growth indices revealed that they are mostly effective on disease control rather than growth indices directly. Seed treated with strains 93 and VN resulted in plants with greater root length than non treated control, so strain VN can influence root growth besides disease control (Table 2). These two strains accompanied by strain 113 increased

Table 1. Influence of bacterial strains applied as seed treatment, on disease severity and growth indices applying fungal isolate 35.

Parameter	Disease severity (0-6)[1]		Growth index[2]					
	Tube assay	greenhouse test	Shoot fresh weight (g)	shoot dry weight (g)	Stem length (mm)	Root length (mm)	Root fresh weight (g)	Root dry weight (g)
Healthy Plant[3]	0d[6]	0c	7.70b	1.39ab	273.83a	176.66a	12.71a	2.38a
Fungi Control[4]	2.67a	3a	4.10d	1.07e	238.16e	142.83e	4.70f	1.06d
10	2b	0c	8.33a	1.40ab	255.16c	157.16bc	11.65b	2.23ab
68	2b	1b	6.34c	1.25cd	246.50d	146.66de	8.43d	1.45c
MP	2b	1b	7.23b	1.32bc	244.66de	147.33de	8.76d	1.47c
VN	1.33c	0c	8.36a	1.40ab	264.50b	159b	11.38b	2.22ab
10b[5]	-	-	7.14b	1.27c	275.16a	180a	11.37b	2.26ab
68b	-	-	6.36c	1.18d	247.66d	157.66bc	9.58c	1.35c
MPb	-	-	4.66d	1.43a	244.16de	152.5cd	7.30e	1.15d
VNb	-	-	7.50b	1.32bc	273.83a	182.16a	11.11b	2.09b

[1]-disease severity: 0= healthy plant, 1=lesions confined to seminal roots, 2= lesions on seminal roots and the subcrown internode, 3= lesions up to the first node, 4= lesions up to the second node, 5= plant severely stunted and yellow, and 6= plants dead.
[2]-meausured in greenhouse.
[3]-control with no treatment.
[4]-plant infected with fungal isolates with no bacterial treatment.
[5]-b indicates seed treated with bacteria and no fungal inoculation.
[6]-mean in the same column followed by the same letter are not significantly different ($p<0.01$) according to Duncan's multiple range test. Each value is the mean of three replications.

Table 2. Influence of bacterial strains applied as seed treatment, on disease severity and growth indices applying fungal isolate 23.

Parameter	Disease severity (0-6)[1]		Growth index[2]					
	Tube assay	greenhouse test	Shoot fresh weight (g)	shoot dry weight (g)	Stem length (mm)	Root length (mm)	Root fresh weight (g)	Root dry weight (g)
Healthy Plant[3]	0e[6]	0c	8.23a	1.44ab	273.83ab	176.66b	12.71a	2.38ab
Fungi Control[4]	2.83a	2.66a	3.26m	1.13ij	231.16o	142.66o	4.66j	1.15f
5	1.83bc	0c	7.65abc	1.36abcd	245.83ijklmn	154.50jklmn	11.05c	2.14cd
39	2.16b	2b	5.81ijk	1.25defghij	240mn	151mn	7.76hi	1.31ef
46	2b	0c	7.33bcde	1.30bcdefg	257.66efg	162.16ef	10.83c	2.16 cd
65	2b	0c	6.21fghij	1.29bcdefgh	246ijklmn	156hijklm	8.36fgh	1.40e
66	1.83bc	2b	5.60jkl	1.16ghij	241.16lmn	150n	7.58hi	1.35ef
73	2b	2b	5.96hijk	1.27cdefghi	239.66n	150.33n	7.81ghi	1.34 ef
93	2b	2b	5.98hijk	1.20efghij	243.83jklmn	153.33klmn	7.71hi	1.35 ef
100	1.83bc	0c	7.98ab	1.37abcd	260.83def	164.66e	11.10c	2.17 cd

Table 2. Cont.

102	2b	2b	5.33kl	1.14hij	244.33jklmn	152.33lmn	7.36i	1.31 ef
106	2b	2b	5.76ijk	1.26cdefghij	245jklmn	154.16jklmn	8ghi	1.37 e
142	2b	2b	6hijk	1.23defghij	248.16hijklm	156.16hijklm	8.03ghi	1.39e
173	1.9bc	0c	7.65abc	1.36abcd	266.167	175.16bc	11.38c	2.22 bcd
196	1.33d	0c	7.91ab	1.37abcd	257.33efg	161.66efg	11.50bc	2.25 abcd
MP	2b	2b	5.40kl	1.23defghij	242.83klmn	152.83lmn	7.24i	1.28ef
VN	1.50cd	0c	8.36a	1.46a	266.66bcd	161.66efg	11.50bc	2.33abc
5b[5]	-	-	5.84ijk	1.30bcdefg	250.16ghijk	160efghi	9ef	1.30ef
39b	-	-	6.04ghijk	1.19efghij	246.33ijklmn	155ijklmn	9.10def	1.34ef
46b	-	-	6.72defg	1.26cdefghij	261.66cdef	169.83d	11.25c	2.39ab
65b	-	-	6.25fghij	1.18fghij	250.83ghijk	160.83efgh	9.88d	1.38e
66b	-	-	5.45kl	1.34abcde	248.66hijkl	156.83ghijkl	8.40fgh	1.27ef
73b	-	-	6.34fghi	1.18fghij	251.83ghij	162.33ef	9.67de	1.37e
93b	-	-	8.23a	1.41abc	277.50a	184a	12.33ab	2.14cd
100b	-	-	6.80bcde	1.30bcdefg	254fghi	159.16fghij	11.17c	2.34abc
102b	-	-	6.66efgh	1.26cdefghij	255fgh	163.16ef	9.92d	1.42e
106b	-	-	7.05cde	1.29bcdefgh	257.83efg	169.83d	11.25c	2.43a
142b	-	-	5.75ijk	1.11J	248.66hijkl	158.50fghijk	8.66fg	1.29ef
173b	-	-	7.75abc	1.37abcd	269.16bc	177b	11.73bc	2.30abc
196b	-	-	6.80def	1.25cdefghij	264cde	170.66cd	11.03c	2.17cd
MPb	-	-	5l	1.43ab	244.16jklmn	152.50lmn	7.30i	1.15f
VNb	-	-	7.38bcd	1.32abcdef	273.83ab	182.16a	11.11c	2.09d

[1]disease severity: ˙ 0= healthy plant, 1=lesions confined to seminal roots, 2= lesions on seminal roots and the subcrown internode, 3= lesions up to the first node, 4= lesions up to the second node, 5= plant severely stunted and yellow, and 6= plants dead.
[2]meausured in greenhouse.
[3]control with no treatment.
[4]plant infected with fungal isolates with no bacterial treatment.
[5]b indicates seed treated with bacteria and no fungal inoculation.
[6]mean in the same column followed by the same letter are not significantly different ($p<0.01$) according to Duncan's multiple range test. Each value is the mean of three replications.

root length in plant infected with isolate 45, too (Table 3). In this fungal isolate, strains VN, MP, 196, 189, 147, 132, 103, 73, 53 and 39 resulted in plants with significantly less take-all than non treated control in tube assays. In greenhouse condition, strains VN, 132, 93, 173 and 53 were more suppressive and extensively prevented disease establishment. Strains 132 and 93 competed the fungal isolate, by siderophore production *in vitro*. In all three fungal isolates, the efficiency of strains obtained from laboratory and test tubes to disease control at greenhouse, suggests that these methods can be used for rapid bacterial screen. Tube assay was suggested as a rapid method for the first time by Weller et al. (1985). In our study, there were some conflict in which, *in vitro* selected bacteria had no efficiency in greenhouse and *in vitro* eliminated bacteria were able to control the disease in greenhouse. This is due to different conditions of laboratory and rhizosphere. By investigation of selected strains in field condition,

Table 3. Influence of bacterial strains applied as seed treatment, on disease severity and growth indices applying fungal isolate 45.

Parameter	Disease severity (0-6)[1]		Growth index[2]					
	Tube assay	Greenhouse test	Shoot fresh weight	shoot dry weight	Stem length	Root length	Root fresh weight	Root dry weight
Healthy Plant[3]	0j[6]	0g	8.23a	1.39ab	273.83ab	176.66bcd	12.71a	2.38ab
Fungi Control[4]	3.83a	3.3a	1.54u	0.56p	192.83u	120x	2.08x	0.96r
5	2.83defg	1f	5.89klmn	1.22fghijkl	268.33abcd	149.16stu	8.10 pq	1.67hi
39	2.50fghi	2.33cd	4.35qr	1.25defghij	231.50st	153.33opqrst	5.60 v	1.12pqr
53	2i	0g	5.33nop	1.09mn	243.33jklmnopq	150.50qrstu	9.93 ijkl	2.05def
65	3.66ab	1f	5.49mnop	1.16ijklmn	246.66jklmnop	161jklmn	7.95 pq	1.47jklm
66	3.08bcdef	2.33cd	4.10rs	1.28bcdefgh	238.66nopqrst	144.33uv	4.97 v	1.56ijkl
68	3.50abc	2de	5.31nop	1.12klmn	244.16jklmnopq	160.83jklmno	6.60 tu	1.26nop
71	3.33abcd	1f	5.72lmno	1.17ijklmn	252.66ghijk	153.50nopqrs	7.93 pq	1.66hij
73	2.58efghi	1.66e	5.10op	1.07n	238.50nopqrst	161.83ijklm	7.40 qrs	1.22nopq
78	2.83defg	1f	4.87pq	1.12lmn	240.83lmnopqrs	163.33ghijkl	7.94 pq	1.28mnop
87	2.66efgh	2.33cd	4.44qr	1.12lmn	237pqrst	151.16pqrstu	5.03 v	1.09pqr
93	2.66efgh	0g	6.96cdef	1.29 bcdefgh	253.66fghij	160.50klmno	10.48 efghi	2.05def
103	2.33ghi	2de	3.70s	1.10mn	251.16ghijkl	162.33hijklm	3.96 w	1.13pqr
106	3cdef	1f	3.01t	0.97o	231.33st	139vw	7.94 pq	1.04qr
113	3cdef	2.66bc	2.94t	0.95o	232.66rst	144.33uv	7.94 pq	1.04qr
120	2.66efgh	2.33cd	4.10rs	0.97o	244.66jklmnopq	149.66rstu	5.35 v	1.27mnop
122	3.16bcde	2de	4.88pq	1.08mn	245.50jklmnop	163.66ghijkl	6.75 stu	1.22nopq
132	2i	0g	6.53efghijk	1.25defghij	229.16t	169.16efgh	10.33 fghij	2.05ef
147	2.33ghi	1f	5.53mnop	1.11lmn	237.50opqrst	160.16klmno	7.31 qrst	1.21nopq
159	3cdef	1f	6.80defg	1.26cdefghi	240.33mnopqrs	156.50lmnopqrs	9.33 lmn	2fg
161	2.3ghi	1f	6.26ghijkl	1.31abcdef	250.66hijklm	170.33defg	10.15 hijk	2.06def
173	2.66efgh	0g	6.66efgh	1.24efghij	261.16defg	169.83defg	10.60efghi	2.10cdef
189	2.33ghi	2de	4.95pq	1.07n	239.50nopqrs	158.16lmnop	6.38 u	1.18nopq
196	2.16hi	1f	5.34nop	1.15ijklmn	247jklmnop	155.33mnopqrs	7.55 qr	1.24nopq
MP	2.58efghi	2.33cd	4.12rs	0.95o	246.66jklmnop	146.16tu	5.28 v	1.10pqr
VN	2i	0g	7.10cde	1.30abcdef	247.83 ijklmno	134.33w	10.48 efghi	2.14cdef
5b[5]	-	-	5.84lmn	1.30abcdef	250.16hijklm	160lmno	9 no	1.30mnop
39b	-	-	6.04hijklm	1.19ghijklm	246.33jklmnop	155mnopqrs	9.10 mno	1.34mno
53b	-	-	7cdef	1.29bcdefgh	264.50bcde	169.83defg	11.11 cdef	2.14cdef
65b	-	-	6.25ghijkl	1.18hijklmn	250.83hijkl	160.83jklmno	9.88 ijklm	1.38klmn
66b	-	-	5.45mnop	1.34abcde	248.66ijklmn	156.83lmnopqr	8.40 op	1.27mnop
68b	-	-	6.31fghijkl	1.18hijklmn	247.66ijklmno	157.66lmnopq	9.58 jklmn	1.35mno

Table 3. Cont.

71b	-	-	6.31fghijkl	1.17ijklmn	250hijklm	158.50lmnop	9.36 klmn	1.35mno
73b	-	-	6.34fghijkl	1.18ghijklmn	251.83ghijk	162.33hijklm	9.67 jklmn	1.37lmn
78b	-	-	6.57efghijk	1.22fghijkl	260defgh	167.50fghijk	10.36 fghij	2fg
87b	-	-	6.58efghij	1.21fghijkl	265.66bcde	174.66cdef	10.05 hijkl	1.57ijk
93b	-	-	8.23a	1.41a	277.50a	184a	12.33 ab	2.14cdef
103b	-	-	5.97ijklmn	1.11lmn	265.66bcde	169.83defg	10.54 efghi	2.06def
106b	-	-	7.05cde	1.29bcdefg	266.66bcde	169.83defg	11.25 cde	2.43a
113b	-	-	7.51bc	1.34abcd	263.16cdef	178.33abc	11.51 cd	2.10cdef
120b	-	-	7.55bc	1.35abcd	242.33klmnopqr	173.83cdef	11.45 cd	2.20bcdef
122b	-	-	7.95ab	1.38ab	234.66qrst	178.50abc	11.80 bc	2.15cdef
132b	-	-	7.42bcd	1.32abcdef	266.66bcde	175.33bcde	11.48 cd	2.26abcd
147b	-	-	6.62efghi	1.23efghijk	259.16defgh	170.83defg	10.29 ghij	1.83gh
159b	-	-	5.91jklmn	1.14jklmn	257.50efghi	168efghij	10.67 efghi	2.02efg
161b	-	-	5.33nop	1.09mn	234.66qrst	153.33opqrst	6.91rstu	1.20nopq
173b	-	-	7.75ab	1.37abc	269.16abcd	177bcd	11.73 bc	2.30abc
189b	-	-	6.20ghijkl	1.16ijklmn	257.50efghi	168.66efghi	10.86 defgh	2.23bcde
196b	-	-	6.80defg	1.25defghi	264bcde	170.83defg	11.03 cdefg	2.17cdef
MPb	-	-	5pq	1.21fghijkl	244.16jklmnopq	152.50pqrst	7.30 qrst	1.15opqr
VNb	-	-	7.38bcd	1.32abcdef	273.83ab	182.16ab	11.73 bc	2.10cdef

[1.] disease severity: 0= healthy plant, 1=lesions confined to seminal roots, 2= lesions on seminal roots and the subcrown internode, 3= lesions up to the first node, 4= lesions up to the second node, 5= plant severely stunted and yellow, and 6= plants dead.
[2-] meausured in greenhouse.
[3-] control with no treatment.
[4-] plant infected with fungal isolates with no bacterial treatment.
[5-] b indicates seed treated with bacteria and no fungal inoculation.
[6-] mean in the same column followed by the same letter are not significantly different ($p<0.01$) according to Duncan's multiple range test. Each value is the mean of three replications.

we can access more reliable results.

Weller and Cook (1983) used the fluorescent pseudomonads as seed and soil treatment in the form of individual and combined strains in greenhouse and field conditions. They found out that the combination of strains caused more inhibition of take-all and resulted in increase of plant height and number of heads and reduction in root disease, compared to individual ones. Biological control through seed treatment was suggested as a new point of view for disease control. On the other hand, because of the growth habit of vulnerable ectotrophic fungi on roots, take-all is an ideal disease for biological control through seed treatment by antagonistic bacteria that colonize roots (Weller and Cook, 1983). Similarly, our investigation showed that *Bacillus* sp. and *Pseudomonas* strains have the capability of take-all control. However, for reassurance of these strains efficiency towards disease control, we can test them in field conditions as well.

Capper and Higgins (1993) used two *P. fluorescens* strains (2-79, 13-79) towards biological control of wheat take-all as seed treatment. All treatments reduced crown and root infection, but only one of these strains had significant effect.

In Iran, Shirzad et al. (2008) isolated pseudomonads bacteria from wheat rhizosphere in Azar-shahr (Tabriz) and investigated their inhibitory effect on *G. graminis var tritici* by dual culture method. They indicated that out of 42, just 12 strains were effective. Reyhany Tabar et al. (2003) investigated the abundance and dispersion

of fluorescent pseudomonads in Tehran province wheat fields. They showed that 26 of 40 selected strains inhibited *G. graminis var tritici* from 0 to 70%.

Sari et al. (2006 b), using *P. fluorescens* (CHAO, bio III (21 p), bio III (22 p), biov (5 km, 32 j) towards biological control of *Ggt* in greenhouse conditions, recognized that all applied strains were able to reduce the disease severity; although CHAO and bio III (21 p) were more effective than the others. Also, in micro plot assessment, all strains reduced disease but similar to greenhouse results, CHAO and bio III (21 p) were the more effective ones.

It is noteworthy that *P. putida*, after *P. fluorescens* could be effective in biological control. In general, one can propound that out of under-test bacteria in laboratory conditions, most fluorescens bacteria, by production of different compounds play the major role in disease control. Kim et al. (1997), demonstrated that in field conditions, the population of *Bacillus* sp. (L324- $92R_{12}$) in rhizospher; at least, were less than *P. fluorescens* (2-$79RN_{10}$)150 days after winter plow of wheat and after that, they nearly became unique. In comparison with spermosphere, the population of both strains gradually reduced so that population of strain 2- $79RN_{10}$ in each section of root was more than that of L324- $92R_{12}$. Howeverf, Capper and Campbell (1986) proved that at field conditions, applying *B. pumilus* in fall or spring time (and *B. mycoides* lonely in spring) as biocontrol agents, causes disease reduction. They concluded that bacterial application in ploughing time has got the best security compared to their usage afterwards.

In Iran, Sari et al. (2006 a) also used *B. subtilis* (1 j), *B. pumillus* (7 km, 4 km) and *B. licheniformis* (b3n) isolates in greenhouse and field conditions for biological control of *Ggt*. They found that disease severity was reduced, by applying *B. pumillus* (7 km) and *B. subtilin* (commercial antagonist formulated with *B. subtilis*) was reduced. Also in microplot experiments, *B. pumillus, B. subtilin* and *B. licheniformis* (b3n) isolates increased the thousand kernel weights in comparion to not treated control.

Namazifard et al. (2004), evaluated antagonistic effect of 5 strains, *Bacillus* sp., *B. licheniformis, B. cereus, Streptomyces* sp. and 2 commercial strains (B6, B7) on two fungal isolates of *G. graminis var. tritici* (G1, G2) in the greenhouse. They showed that strains B5 and B7 were the most effective ones, and there would be a meaningful reduction in root disease severity and an increase in plant's weight.

As in other countries, plant pathologists in Iran are using fungal isolates for Ggt control. Zafari et al. (2008) used *Trichoderma virens* isolates and Trichodermin B and subtilin (commercial bioproducts) by seed and soil treatment in greenhouse condition. They showed that seed treatment was more suppressive than soil treatment. They also indicated that isolates T97 and T65, Trichodermin B and a mixture of eight different *Trichoderma* isolates showed significant suppression of
take-all compared to the other ones.

It seems that there are a lot of promising fungi and bacteria as biological agents toward Ggt. More investigation is needed and it seems that having evaluated the selected agents in field condition, we can formulate them as biofungicide seed treatment.

REFERENCES

Capper AL, Higgins KP (1993). Application of *Pseudomonas fluorescens* isolates to wheat as potential biological control agents against take-all. Plant Pathol., 42: 560-567.

Capper L, Campbell R (1986). The effect of artificially inoculated antagonistic bacteria on the prevalence of take-all disease of wheat in field experiments. J. Appl. Bacteriol., 60: 155-160.

Christensen NW, Hart JM (2008). Combating take-all of winter wheat in western Oregon. Oregon State University Extension Service, EC 1423-E. pp. 1-7.

Duffy BK, Simon A, Weller DM (1996). Combination of *Trichoderma koningii* with fluorescent pseudomonads for control of take-all on wheat. Phytopathology, 86: 188-194.

Fravel DR (1988). Role of antibiosis in the biocontrol of plant disease. Ann. Rev. Phytopathol., 26: 75-91.

Ghalandar M, Jafarpur B, Falahati Rastegari M (2000). Consideration and determination in the dispersion of *Gaeumannomyces graminis* on the wheat fields of Markazi province. In: Abstracts of the 14th Iranian Plant Protection congress, Esfahan, Iran, p. 2.

Hagedron C, Gould DW, Bardinelli TR (1989). *Rhizobacteria* of cotton and their repression of seedling disease pathogens. Appl. Environ. Microbiol., 55: 2743-2797.

Kim DS, Weller DM, Cook RJ (1997). Population dynamics of *Bacillus* sp. L324-92R12 and *Pseudomonas fluorescens* 2-79RN10 in the rhizosphere of wheat. Phytopathology, 87: 559-564.

Kraus J, Lopper JE (1990). Biocontrol of *Pythium* damping-off of cucumber by *Pseudomonas fluorescens* Pf-5: mechanistic studies. In: Second Intenational Work Shop on Plant Growth-Promoting *Rhizobacteria*, Interlaken, Switzerland, pp. 172-175.

Kucuk C, Kivanc M (2003). Isolation of *Trichoderma spp*. and determination of their antifungal, biochemical and physiological features. Turk. J. Biol., 27: 247-253.

Montealegre JR, Reyes R, Perez LM, Herrera R, Silva P, Besoain X (2003). Selection of bioantagonistic bacteria to be used in biological control of *Rhizoctonia solani* in tomato. Electro. J. Biotechnol., 6: 116-127.

Namazifard S, Etebarian HR, Khodakaramian GH, Torabi M (2004). The possibility of biological control of take-all disease of wheat by antagonistic bacteria. In: Abstracts of the 16th Iranian Plant Protection congress, Tabriz, Iran, p. 46.

Nasraoui B, Hajlaoui MR, Gargouri S, Kremer RJ (2007). Biological control of wheat take-all disease: II- Rapid screening for selection of bacteria suppressive to *Gaeumannomyces graminis var. tritici* in laboratory with greenhouse and field confirmation trials. Tunis. J. Plant Prot., 2: 35-46.

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.

Reyhany Tabar A, Saleh Rastin NM, Mohammadi M, Alikhani HA (2003). The occurrence and distribution of fluorescent pseudomonads in wheat fields in tehran province along with a study on their repressive effect against *Gaeumannomyces graminis var. tritici* , Causal Agent of Take-all. Iranian, J. Agric. Sci., 34 (3): 571-578.

Sadeghi L, Alizadeh A, Safaie N, Ghalandar M (2009). *Gaeumannomyces graminis var tritici* using morphological, molecular and pathological studies. Plant Pathol., 45: 23-26.

Sari E, Etebarian HR, Roustaei A, Aminian H (2006a). Biological control of *Gaeumannomyces graminis* on wheat with *Bacillus* spp. Plant Pathol., 3: 307-314.

Sari E, Etebarian HR, Roustaei A, Aminian H (2006b). Biological control *Gaeumannomyces graminis var tritici* on wheat with some isolation of *Pseudomonas fluorescens*. Pak. J. Biol. Sci., 7: 1205-1211.

Shirzad A, Sharifi Tehrani A, Behbodi K, Ahmadzadeh M (2008). Quantification of the fluorescent pseudomonads population in wheat fields of Azarshahr region of Tabriz (Iran) and evalution of the inhibitory effects of its isolates on the growth of *Gaeumannomyces graminis var tritici*. In: Abstracts of the 18th Iranian Plant Protection congress, Hamedan, Iran, p. 248.

Weller DM (1988). Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Annu. Rev. Phytopathol., 26:379-407.

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

Weller DM, Raaijmakers JM, Thomashow LS (1997). The rhizosphere ecology of antibiotic producing pseudomonads and their role in take-all decline plant growth promoting rhizobacteria. In: Ogoshi A, Kabayashi Yhamma K, Kodama F, Kondo N, Akino S (eds). Present status and future prospects. Sapporo, Japan, pp. 58-64.

Weller DM, Zhang BX, Cook RJ (1985). Application of a rapid screening test for selection of bacteria suppression to take-all of wheat. Plant Dis., 69: 710-713.

Zafari DS, Mehrabi Koushki M, Bazgir E (2008). Biocontrol evaluation of wheat take-all disease by *Trichoderma* screened isolates. Afr. J. Biotechnol., 7(20): 3653-3659.