Plant Pathology Journal

ISSN 1812-5387

science alert

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Plant Pathology Journal 5 (3): 307-314, 2006
ISSN 1812-5387

Biological Control of *Gaeumannomyces graminis* on Wheat with *Bacillus* spp.

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Abstract: *Bacillus pumilus* (4 km), *B. pumilus* (7 km), *B. subtilis* (1j), *B. licheniformis* (b3n) were evaluated as potential biological agent for wheat take-all caused by *Gaeumannomyces graminis* var. *tritici in vitro* and *vivo*. Dual culture, volatile metabolite and cell free culture test showed that all isolates of *Bacillus* tested inhibited growth of the pathogen. Inhibition varied from 41 to 87% in dual culture, from 85 to 96% in volatile metabolite and from 95 to 98% in cell free culture test. The seed soaking treatment with Subtilin (commercial antagonist formulated from *Bacillus subtilis*) and *Bacillus pumilus* (7 km) were the most effective in reducing disease index and also promoted root and shoot weight in glasshouse and field experiment. The weight of 100 grains from plants treated with pathogen+Subtilin, *Bacillus pumilus* (7 km) and *B. licheniformis* (b3n) were significantly greater than in controls inoculated with pathogen alone in micorplot test. These results indicate that *Bacillus pumilus* (7 km) could be an important new biological control for take-all of wheat.

Key words: Biocontrol, wheat disease, soil borne pathogen, take-all, *Bacillus* spp.

INTRODUCTION

Take-all disease of wheat (*Triticum aestivum* L.) caused by *Gaeumannomyces graminis* (Sacc) Von Arx and Oliver var. *tritici* Walker (*Ggt*), is one of the most serious diseases of wheat worldwide. Take-all could reduce yield of wheat by as much as 75% (Rovira, 1990). Historically, control of take-all has been difficult due to the lack of resistant cultivar and effective chemical treatments (Hornby *et al*., 1993). Baytan (triadimenol) which is the only fungicide registered for take-all in the USA, is not widely use because it performs inconsistently. Crop rotation is an effective method to control the disease, but because of economic factors, grower often need grow wheat every other year or several years in a row before a break crop (Cook and Weller, 1987). This disease is managed best by taking advantage of the development of suppressive soils, a phenomenon referred to as take-all decline (Asher and Shipton, 1981). Suppressiveness has been related to various bacteria including *Pseudomonas* and *Bacillus* spp. (Weller, 1988; Weller and Cook, 1983).

Attempts to develop a biological control method for take-all using organism isolated from suppressive soil or from wheat roots have included the addition of these organisms to seed or to the soil. In most cases, the biological seed treatments have utilized various bacteria especially those that have been found to move from the seed and effectively colonize the host roots (Weller, 1988). A range of different microorganisms have been investigated as potential biological control agent for take-all.

There have been many reports that bacterial isolate from rhizosphere soil or plant roots reduced take-all severity. Scientific and technical advance have also been made in the use of biocontrol strain introduced on or with seed and subsequently expected to colonize the root as the grow in to the soil and survive for as long as necessary to protect against root infection from soil-born inoculum. *Bacillus* species as a group, offer several advantages over fluorescent pseudomonads and other gram negative bacteria as seed inoculants for protection against root pathogens, including longer shelf life, because their ability to form endospore and the broad spectrum activity of their antibiotic. There are a number of reports on the potential of *Bacillus* species as a biological control agent against fungal pathogen (Ryder *et al*.,1999; Kim *et al*., 1997; Mapleston and Campbell, 1989; Capper and Campbell, 1986; Czaban *et al*., 2004).

Production of antifungal metabolites such as antibiotic and hydrogen cyanide are primary mechanism by which these bacteria suppress diseases. Because of expensiveness and unusefulness of usual approach of control, biological control is the best item for controlling of wheat take-all.

The purpose of this research was to evaluate the potential of some isolates of *Bacillus* isolated from wheat rhizosphere in Mazandaran and Markazi Province for biological control of *Gaeumannomyces graminis* var. *tritici* the agent of wheat take-all and comparing them with fungicide triadimenol and formulated antagonist Subtilin.

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MATERIALS AND METHODS

Pathogen and *Bacillus* isolates: *Bacillus pumilus* (4 km), *Bacillus pumilus* (7 km), reduced growth of *Bipolaris sorokiniana* (Mohammadi, 2004), *Bacillus subtilis* (1j) inhibited growth of *Fusarium graminearum* (Nourozian, 2003) and *Bacillus licheniformis* reduced take-all severity in glasshouse study (Namazifar, 2003). All these bacteria isolated from rhizosphere of wheat and obtained from Department of Plant Protection of Abourayhan Campus, Tehran University. Isolate of *Gaeumannomyces graminis* var. *tritici Ggt*1 obtained by M. Ghalandar, Agricultural Research Center of Markazi province was used in this investigation. Bacterial and fungal isolates were maintained in Sterilized Distilled Water (SDW) and Potato Dextrose Agar (PDA) at 5°C, respectively. Subtilin as a commercial antagonist formulated from *Bacillus subtilis* (10^8 cfu) by Talfigh Dane Company, Tehran, was used in this experiment.

Effect of *Bacillus* isolates on mycelial growth of *Ggt*: Dual culture (Dennis and Webster, 1971; Etebarian *et al*., 2003), cell free culture (Weller *et al*., 1988) and volatile metabolite test (Fiddaman and Rossall, 1993) were used to observe the effect of *Bacillus* isolates on *Gaeumannomyces graminis* var. *tritici*. All antagonist-pathogen combinations were examined on 10-15 mL of potato dextrose agar in 9 cm petri plate with four replicates per treatment. The plates were incubated 5 days for dual culture and 9 days for cell free culture and volatile metabolites test at 25°C. The percentage of growth inhibition was calculated using the formula n = (a-b)/a×100, where n is the % growth inhibition; a is the colony area of uninhibited *Gaeumannomyces graminis* var *tritici* and b is the colony area of treated *Ggt*.

Glasshouse test: The ability of *Bacillus* isolates to reduce the incidence and severity of take-all in wheat grown in glasshouse was investigated. Soil prepared by mixing of field soil, leaf compost, sand, organic manure by rate 1:2:2:2. Soil sterilized by autoclaving at 121°C for 30 min for 2 successive days. Inoculum of pathogen was prepared as follow. Wheat seed were soaked for 24 h in water at room temperature and then transferred to Erlenmyer flask and autoclaved for 15 min at 121°C on two successive days. *Ggt*1 isolate was grown on Potato Dextrose Agar (PDA) and when they were grown, piece of culture 5×10 mm in size were added to each Erlenmyer flask containing of autoclaved wheat, mixed with wheat and incubated at 20°C for 25 days. Wheat infested with pathogen was blended in SDW to make a slurry. The resultant inoculum were mixed by soil at ratio of 1% (w/w) (Etebarian *et al*., 2000). Bacterial isolates were grown in Potato Dextrose Broth (PDB) on a rotary shaker at 150 r min^{-1} for 2 days at room temperature. Cell were pelletized by centrifugation at 2500×g for 10 min, suspended in 0.01 M phosphate buffer (pH = 7), repelletized and resuspended in buffer and mixed with equal volume of 1% methylcellulose and added to disinfected seed (sodium hypochloride 0.5% for 3 min) at 8 mL/100 g seeds. The initial population on the seeds was determined by dilution-plating immediately following the treatment. The initial population of bacterial cells on the seed was about 10^9 colony per seed. Spring wheat cultivar Shiraz was used in this experiment. Triadimenol was used as effective fungicide for chemical control of take-all by seed treatment at rate of 0.2% (w/w). Treated seed were dried in laminar flow hood for 1 h. Ten seed were planted in 10 cm diameter pot (Mathre *et al*., 1986; Milus and Rothrock, 1997).

Pots were watered at 2 days intervals until emergence and daily thereafter. There were four replicates per treatment, arranged in a randomized complete design with 16 treatments (Table 2). The experiment was carried out in a glasshouse at day temperature 32°C and night temperature 25°C with natural day light without supplementary lighting from September to October 2005 in Tehran. Plants were harvested 5 weeks after sowing and severity of disease on roots was assessed using a 0-5 scale modified from Rothrock (1986). Root height, root fresh weight, root dry weight, shoot height, shoot fresh weight and shoot dry weight also determined.

Microplot trial: Microplot trial was conducted at Abourayhan Campus University of Tehran, Pakdasht in Tehran Province from March to June 2005. Three pots (each, 20 cm diameter and 25 cm height) was used as a microplot. Seed treatment and preparing of inoculum were done by methods previously noted in glasshouse test. The amount of inoculum which was applied to potting mix 10 g infested wheat per kg of soil (Czaban *et al*., 2004). The field soil (loam soil with 49% sand, 36.8% silt, 14.2% clay) was mixed with organic manure at 2:1 rate (v/v). Approximately 60 seeds of spring wheat cultivar Shiraz were sown in each microplot. There were three replicates per treatment, arranged in randomized complete block design with 14 treatments (Table 3). Microplots were watered at 7 days intervals at early spring and 3 days intervals when the air temperature increased at late spring. Chemical control of aphids and leafhopper was carried out by insecticide and weed control was done mechanically.

All plants in any microplot harvested 14 weeks after sowing when plants given to ripening period. Observation were consisted of disease severity rating (0-5) as mentioned above, percentage of blackended crowns, root

fresh weight, root dry weight, shoot dry weight and weight of 100 grains.

Statistical analysis: Data on percentage inhibition of growth of the pathogens *in vitro* were subjected to arcsin square root transformation before analysis. These data were analyzed using the MSTAT-C and means were compared by Duncan's Multiple-Range Test at $p<0.05$ (Little and Hills, 1978). In harvesting process of microplot test, all plants in each microplot were harvested and the number of plant in each microplot calculated as a covariate. Therefore means of weighted observation corrected by software and covariance analysis exert for this data.

RESULTS

Effect of *Bacillus* isolates on mycelial growth of *Ggt*: All bacterial antagonists tested inhibited mycelial growth of *Gaeumannomyces graminis* var. *tritici* in dual culture, volatile metabolite and cell free culture test, however, there were significant differences among bacterial isolates. Inhibition varied from 41 to 87% in dual culture, from 85 to 96% in volatile metabolite test and from 95 to 98% in cell free culture test. In dual culture test, percentage of growth inhibition of *Ggt* by *Bacillus pumilus* (4 km) was significantly greater than those of other isolates tested (Table 1). In volatile test, mycelial growth of pathogen was numerically reduced more by *Bacillus pumilus* (4 km) and *B. licheniformis* (b3n) than the other isolates. Cell free metabolites of *B. pumilus* (7 km), *B. licheniformis* (b3n), *B. pumilus* (4 km) and *B. subtilis* (1j) reduce growth of *Ggt* by 98.1, 98.4, 96.4 and 95.9, respectively.

Glass house test: The result of seed soaking with *Bacillus* isolates indicated that take-all was reduced by some *Bacillus* isolates tested. Disease severity in plant inoculated with pathogen+Subtilin and pathogen+*B. pumilus* (7 km) was significantly less than pathogen control. *Bacillus pumilus* (7 km) and Subtilin promoted root height, fresh and dry weight of roots compared to *Ggt* alone. There were no significant differences between *Ggt* only and Triadimenol+*Ggt* treatments in disease severity rating. There were no differences among *Ggt* only, *Ggt*+methylcellulose and *Ggt*+triadimenol treatments in all observations (Table 2).

Microplot trial: All the *Bacillus* isolates significantly reduced disease incidence of wheat take-all compared to *Ggt* only control. *Bacillus pumilus* (7 km) and Subtilin were more effective in reducing take-all than the other isolates tested. The *Ggt*+fungicide treatment differ from *Ggt* only control and reduced disease severity and percentage of infected crowns. *Bacillus pumilus* (7 km) and Subtilin promote fresh and dry weight of root and dry weight of shoot compared to *Ggt* only control.

Table 1: Percentage of growth inhibition of *Ggt* by *Bacillus* isolates

Treatments	Dual culture	Volatile metabolite	Cell free culture
B. pumilus (4 km)	87.2a	95.6a	96.4b
B. pumilus (7 km)	54.5b	85.4c	98.1a
B. subtilis (1j)	46.8c	92.1b	95.9b
B. licheniformis(b3n)	41.2c	96.4a	98.4a

Significant differences are denoted by different letters within each column according to Duncan,s Multiple Range Test at $p<0.05$. Data are expressed as % of control colonies without antagonist and values are average of 4 replicates. Data were subjected to arcsin square root transformation prior to analysis of variance. The percentage of growth inhibition was caculated using the formula $n = (a-b)/a \times 100$, where n is the % growth inhibition; a is the colony area of uninhibited *Gaeumannomyces graminis* var *tritici*; and b is the colony area of treated *Ggt*

Table 2: Effect of *Bacillus* isolates on *Ggt* in glasshouse condition

Treatments	Disease severity (0-5)	Root height (cm)	Root fresh weight (g)	Root dry weight (g)	Shoot height (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
Healthy control	-	14.3bc	7.2ab	2.6a	31.1b	31.6bc	5.2b
Healthy control+ Methyl cellulose	-	13.5c	7.3a	2.5a	32.1b	32.6bc	5.4b
Subtilin	-	17.9a	7.0abc	2.9a	34.8b	33.8b	5.5b
B. pumilus (4 km)	-	15.2bc	6.1c	2.7a	36.7a	37.9a	5.5b
B. pumilus (7 km)	-	16.5ab	7.0abc	2.7a	34.5b	34.3b	6.3a
B. subtilis (1j)	-	13.7c	6.2bc	2.7a	37.6a	34.5b	5.2b
B. licheniformis (b3n)	-	14.2bc	6.4abc	2.7a	37.2a	34.7b	6.3a
Fungicide (Triadimenol)	-	14.0c	6.4abc	2.8a	34.5ab	30.1c	5.1b
Ggt only	4.7a	12.4ef	3.2ef	1.0c	20.9d	18.5fg	3.1d
Ggt only+Methyl cellulose	4.2a	11.9f	3.0ef	1.1c	21.5d	19.8efg	2.9d
Ggt+ Subtilin	2.1c	14.6de	4.7d	1.9b	25.4c	23.8d	4.2c
Ggt+ *B. pumilus* (4 km)	4.0ab	11.7f	2.4f	1.0c	23.2cd	22.7de	2.8d
Ggt+ *B. pumilus* (7 km)	2.0c	15.6d	5.1d	1.9b	22.9cd	24.0d	4.3c
Ggt+ *B. subtilis* (1j)	4.0ab	12.0f	2.7ef	1.0c	26.2c	20.2ef	2.7d
Ggt+ *B. licheniformis* (b3n)	3.2b	12.5ef	3.02ef	1.1c	26.6c	17.9fg	2.8d
Ggt+ Fungicide (Triadimenol)	4.3a	12.0f	3.5e	1.1c	24.6cd	16.5g	3.1d

Significant differences are denoted by different letters within each column according to Duncan,s Multiple Range Test at $p<0.05$ and values are average of 4 replicates, *Ggt* = *Gaeumannomyces graminis* var. *tritici*, Disease severity rating (0-5): 0 = root with no symptom, 1 = lesion on <25% of the root, 2 = lesion on 25% to <50% of the root, 3 = lesion on 50% to <75% of the root, 4 = lesion on 75% to 100% of the root, 5 = lesion on 100% root by blackended crown. (Rothrock, 1986), Subtilin = commercial formulation of *Bacillus subtilis* (10^8cfu) synthesized by Talfigh Dane Company, Tehran

Table 3: Effect of *Bacillus* isolates on *Ggt* in microplot trial

Treatments	Disease severity (0-5)	Infected crowns (%)	Root fresh weight	Root dry weight	Shoot dry weight	Weight of 100 grains
Healthy control	-	-	83.4ab	18.0bc	40.7ab	2.4ab
Fungicide (Triadimenol)	-	-	61.9abcd	14.9cde	33.5bcd	2.4b
B. pumilus (4 km)	-	-	59.9bcd	15.9cd	34.2bc	2.5ab
B. pumilus (7 km)	-	-	83.9ab	18.0bc	43.3ab	2.5ab
B. subtilis (1j)	-	-	70.2abc	16.9cd	36.6bc	2.4b
B. licheniformis (b3n)	-	-	75.4ab	25.6a	37.8bc	2.5a
Subtilin	-	-	84.9a	22.1ab	46.9a	2.5a
Ggt only	4.2a	85.1a	14.53f	6.6g	8.7g	1.1f
Ggt+ Fungicide (Triadimenol)	2.0c	45.1bc	27.9ef	9.6fg	14.3efg	1.3e
Ggt+ *B. pumilus* (4 km)	2.5b	53.8b	28.1ef	10.7efg	15.6efg	1.1f
Ggt+ *B. pumilus* (7 km)	1.4d	35.1cd	42.8de	12.4def	18.9ef	2.1c
Ggt+ *B. subtilis* (1j)	2.4b	35.9cd	11.8f	8.1fg	6.4g	1.2f
Ggt+ *B. licheniformis* (b3n)	2.0c	49.5b	29.4ef	8.4fg	10.4fg	1.3e
Ggt+ Subtilin	1.2d	30.5d	48.5cde	12.8cdef	22.9de	1.7d

Significant differences are denoted by different letters within each column according to Duncan,s Multiple Range test at $p<0.05$ and values are average of 3 replicates. Percentage of infected crowns were subjected to arcsin square root transformation prior to analysis of variance, *Ggt* = *Gaeumannomyces graminis* var. *tritici*, Disease severity rating (0-5): 0 = root with no symptom, 1 = lesion on <25% of the root, 2 = lesion on 25% to <50% of the root, 3 = lesion on 50 to <75% of the root, 4 = lesion on 75% to 100% of the root, 5 = lesion on 100% root by blackended crown (Rothrock, 1986), Subtilin = Commercial formulation of *Bacillus subtilis* (10^8cfu) synthesized by Talfigh Dane Company, Tehran

Seed inoculation with *Bacillus pumilus* (7 km) *B. pumilus* (4 km) and Subtilin significantly promoted weight of hundred seed compared to pathogen control (Table 3).

DISCUSSION

Take-all decline develops in a field when wheat grown continuously for several year in the presence of *Ggt*. The actual length of monoculture required varies from field to field, but may range from only a couple of years to more than 10 (Cook and Weller, 1987; Shipton, 1975). The four isolates used in this study chosen from collection that had been screened for the ability to suppress root pathogen of wheat. All isolates obtained from wheat rhizosphere on continuously growing wheat field. Thus these isolates may be some of agent cause take-all decline under field condition. Also these isolates have ability for suppressing other soil-borne pathogens of wheat such as *Bipolaris sorokiniana*, *Fusarium, graminearum* under glasshouse condition (Mohammadi, 2004; Nourozian 2003). This subject supported this hypothesis which these isolates have a promise for control of take-all and other soil-borne pathogen of wheat under field condition.

All the *Bacillus* isolates reduce mycelial growth of pathogen by means dual culture, cell free culture and volatile metabolite test. Zone of inhibition were observed between the colonies of pathogen and bacteria. The inhibition zone could be due to the effect of diffusible inhibitory substances produced by the bacteria, which suppressed the growth of *Ggt*. Weller (1988) and Weller *et al*. (1997) claimed that the antibiotic production was one of the most important feature of bacterial with regard to take-all control on wheat. Analysis of mutants of *Bacillus cereus* shows a significant quantitative relationship between disease suppressiveness and the production of two antibiotics, Zwitermicin A and Kanosamine. (Silo-Such *et al*., 1994; Milner *et al*., 1996). Also *Bacillus* species have been used as biocontrol agent against pathogenic fungi, by producing lipopeptide antibiotic such as Iturin A and Macrolacin (Fravel, 1988; Kim and Kim, 1994; Han *et al*., 2005). Other mechanism of diseases suppressing are volatile metabolites (especially HCN) and siderophore may involved in diseases suppression (Fiddaman and Rossall, 1993; Bsat *et al*., 1998).

Bacillus pumilus (4 km) produces large inhibition zone on PDA indicating that they synthesize compounds that are highly active against the pathogens. This isolate although was powerful strain *in vitro* but under glasshouse and microplot condition show lower biocontrol activity. It, s inability to promote growth of wheat in comparing with *Ggt* only treatment is not surprising. For example it may not be able to grow effectively or produce antibiotic in sufficient quantity in wheat rhizosphere (Ryder and Rovira, 1993). However It should be noted that the size of the inhibition zone is not a reliable indicator of inhibition strength because the inhibition zone is based the mobility of the antifungal compounds. This can be influenced by the polarity of the compound moving through the agar or the molecular size of the compound and because of the complexity and number of antifungal metabolites that are produced by antagonistic isolates (Etebarian *et al*., 2005). Other possibility is that the bacterial isolates depleted the

nutrient in the agar surrounding them and thereby inhibited *Ggt* (Nourozian *et al*., 2006). Further research will be needed to identify and characterize the antifungal compounds produced by isolates tested.

Although bacilli have received less attention as potential biocontrol agents than have the pseudomonads, evidence indicating that they may promote effective diseases suppression (Handlesman and Stabb, 1996). The bacilli are particularly attractive for practical use because they produce stable endospore, which can survive the heat and desiccation condition that may be faced by Biocontrol agent (Turner and Backman, 1991; Lumsden *et al*., 1995; Osburn *et al*., 1995).

Bacillus pumilus (7 km) showed low percentage of mycelial growth inhibition in dual culture test and volatile metabolite test than *B. pumilus* (4 km), however in glasshouse and microplot study this isolate named the best isolates. In cell free culture test *Bacillus pumilus* (7 km) named best isolate. This results show that mobility of antifungal metabolite of *Bacillus pumilus* (7 km) on PDA is lower than *B. pumilus* (4 km).

The result of glasshouse and field experiment indicated that, Subtilin, *Bacillus pumilus* (7 km) were effective in suppression of take-all in field and glasshouse condition. *Bacillus pumilus* (7 km) also previously suppressed *Bipolaris sorokiniana* under glasshouse condition (Mohammadi, 2004). Bacterial isolates tested here had lower effect in glasshouse than microplot study. Microorganism population and physiochemical properties of soil may cause of take-all decline in our microplot study. The relative importance of antibiotics and siderophore in diseases suppression by a strain may depend on environmental conditions. Factors such as soil matric potential (Howei *et al*., 1987, Weller, 1988) and rhizosphere pH influence the colonization of wheat roots by introduced bacteria and it is likely that the regulation of antibiotic and siderophore production would be even more sensitive to soil physical and chemical factors. Baker (1968) pointed out that soil pH has an indirect role in siderophore-mediated diseases suppression because pH affects the amount of iron available to plants and microorganism. Further studies on the effect of soil physical and chemical factor on diseases suppression by *Bacillus* isolates tested are greatly needed. Induction of local or systemic resistance in wheat to pathogen following bacterial isolate root colonization is yet another possible explanation for suppression take-all (Bakker *et al*., 2003). For example, *Bacillus mycoides* isolate Bac J, a non-pathogenic, phyllosphere-inhabiting bacterium, reduces Cercospora leaf spot (*Cercospora beticola* Sacc.) of sugar beet by 38-91% in both glasshouse and field experiments. Disease control is attributed to the bacterium's ability to induce systemic resistance (Bargabus *et al*., 2002). Rhizobacteria activate defense gene encoding chitinase, Beta-1,3- glucanase, peroxidase and enzymes involved in synthesis of phytoalexins (Van peer *et al*., 1991; Mpiga *et al*., 1997). The best evidence for rhozobacteria-mediated induce systemic resistance is obtained when the rhizobacterium have lower effect than the other isolates *in vitro* test, but in glasshouse and field condition shows best biocontrol activity (Van loon *et al*., 1998). In this case further study on the effect of *Bacillus pumilus* (7 km) on triggering defense mechanism are greatly needed.

Result of glasshouse experiments showed that, *Bacillus pumilus* (4 km) when used as seed treatments alone increased shoot height and shoot fresh weight compared with control without bacteria (Table 2), this is in accordance with results showing that some fungal isolate and bacterial strain are capable of promoting plant growth in glasshouse (Susloun, 1982; Salehpour *et al*., 2005). Plant growth promotion due to PGPR has been attribute to the increase of mineral nutrition available to root during the growth activity of PGPR isolates in the rhizosphere (Smith and Goodman, 1999).

Ryder *et al*. (1999) used of strains of *Bacillus* isolated in China to suppress take-all and Rhizoctonia root rot and promote seedling growth of glasshouse-grown wheat in Australia soil. Kim *et al*. (1997) applied *Bacillus* sp. L324-92 for biological control of *Gaeumannomyces graminis* var. *tritici* and also Czaban *et al*. (2004) protect winter wheat against take-all by the use of rhizobacteria *Pseudomonas fluorescens* and *Bacillus mycoides*. All of these reports confirm our results and show that *Bacillus* isolates are capable for diseases management under wheat field.

In both glasshouse and microplot experiments there were no significant difference between *Ggt* only and Triadimenol+*Ggt* treatment in disease severity index. This result show that chemical seed treatment to control soil born root rots of plant rarely have been successful because the root emerge from the zone protection soon after germination. A successful integrated control program has been reported by Beale and Pitt (1990) for fusarium basal rot of Narcissus where dual treatment with fungicide and micro-organism improved the levels of disease control over treatments with the biological control agent or fungicide alone.

It is likely that most of naturally occurring biological control result from mixture of antagonists rather than from high population of a single antagonist. Similarly application of mixture of introduced biocontrol agent

would more closely mimic the natural situation and might broaden spectrum of biocontrol activity and enhance the efficacy and reliability of control (Duffy and Weller, 1995).

In the future, ability of bacterial isolates to protect wheat against *Ggt* should be verified in field condition on different soil and with other strains of *Ggt*. Wong (1994) stated that the result of such field studies are inconsistent, with good response in some years and on some site but not on others.

In conclusion *Bacillus pumilus* (7 km) similar with commercial formulated antagonist Subtilin reduced disease severity in glasshouse and field condition, but further fundamental, multicomponent research into the interaction between host, pathogen, antagonist and environment is required.

ACKNOWLEDGMENTS

The authors thank M. Ghalandar for providing *Gaeumannomyces graminis* var. *tritici* isolate 1. The assistance of V. Azad, H.Yarhoseini, M. Mohamadifar and M. Ghadiri are gratefully acknowledged. The research presented here is part of M.Sc. Thesis at Department of Plant Protection, Abourayhan Campus, University of Tehran.

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