

EVALUATION OF *TRICHODERMA* ISOLATES FOR BIOLOGICAL CONTROL OF WHEAT *FUSARIUM* FOOT AND ROOT ROT

Abdoreza Foroutan

Department of Plant Protection of Agricultural and Natural Resources Research Center
of Mazandaran, Iran. Email: foroutan2000@yahoo.com

ABSTRACT

Trichoderma harzianum strains 22, 35, 43, 122, and *T. virens* 63 and 85 were evaluated as potential biological agents for control of wheat *Fusarium* root rot caused by *F. graminearum*. Mycelial growth of *F. graminearum* B-63 was reduced by cell free and volatile metabolites of *Trichoderma harzianum* and *T. viride* strains by 23.12 to 43.22%. *Trichoderma harzianum* strain 43 significantly ($P \leq 0.05$) reduced the incidence (7.66%) and severity (3%) of disease, 42 days after inoculation and increased the 1000 grain weight in greenhouse conditions by 64% over the *Fusarium* inoculated control, almost at the level of non inoculated control. For confirmation of the greenhouse tests, the selected antagonists were re-examined in field trials. The *Trichoderma harzianum* strain 43 also reduced the disease incidence (10%) and severity (4.42%), and increased the yield of wheat in field conditions at more than double compared with the *Fusarium* inoculated control and at the level of non inoculated control (3.85 vs. 1.69 and 3.88 t ha⁻¹ respectively).

Key words: biological control, *Fusarium graminearum*, *Trichoderma harzianum*, *Trichoderma virens* wheat.

INTRODUCTION

Wheat root rots are caused by several species of fungi, including *Pythium* spp., *Gaeumannomyces graminis* var *tritici*, *Cochliobolus sativus*, *F. graminearum*, *F. culmorum* and *Rhizoctonia solani*. Among them, *F. graminearum* is the most common pathogen causing wheat and barley root rots in Mazandaran province of Iran (Foroutan et al., 2007). The disease is characterized by a decay of the crown and basal stem tissues, premature blight and white heads. Diseased plants rarely show symptoms until after heading, by which time the foot rot is sufficiently advanced to prevent water transport.

It is a seed and soil borne disease, which causes significant losses on wheat crop. Conidia, mycelia, chlamydospores and infested plant debris can provide the primary inoculums of the pathogen.

Apart of quantity losses, some strains of the pathogen produce mycotoxins, which are hazardous to human and animals (Nelson et al., 1993; Parry et al., 1995).

Control of the disease is difficult. Soil fumigation is effective, but it is too costly. Limitations in the development of resistant

cultivars also exist. Traditional methods such as long term crop rotation, clean tillage, and organic amendments of soil have been useful for soil borne disease control, but these practices are being replaced with short term rotation, monoculture, and intensive cropping to increase productivity (Cook, 1968; Cook and Bruehl, 1968). Therefore, finding additional methods for disease control is essential.

Some fungi, including *Trichoderma* and *Gliocladium* species, have shown promise in biological control of the pathogens. The antagonistic activity of *Trichoderma* species against plant pathogens has been studied extensively (Hjeljord et al., 2001; Krause et al., 2001).

A number of commercial formulations, based on *T. harzianum* and *T. virens* are available for the control of soil borne and foliar diseases in a range of horticultural crops (Harman et al., 1996; Lumsden et al., 1992; Samuels, 1996), but there is little information on the efficacy of *Trichoderma* isolates on *Fusarium* root rot of wheat.

The objective of this investigation was to evaluate the potential of some isolates of *Trichoderma harzianum* and *T. virens* for the biological control of wheat *Fusarium* root rot in Mazandaran.

MATERIAL AND METHODS

Isolation of pathogen and antagonist strains

The pathogen and antagonist strains used in this study, were isolated from the diseased wheat plants and dry soil samples separately, which were collected from different fields of Mazandaran, including Kohkheil, Pahnab and Larim (Jouibar), Serajmohaleh, Tirtash, Yanehsar (Galogah), Rostamkola, Hoseinabad, Zirvan (Behshahar), Bai-e-kola, Estakhrposht, Nozarabad (Neka), Dashtnaz, Farahabad, Makran (Sari), Gharakheil, Arateh, Chmazcoti (Ghaemshahar), during 1999-2000 cropping season.

Fusarium graminearum was isolated from collected wheat, infected by root and foot rot, on PDA.

For isolation of the *Trichoderma* strains, soil samples of the rhizosphere area were dried by keeping them at room temperature for 8 days. Then dried samples were serially diluted in sterile distilled water (Wijesundera et al., 1991). After dilution, 100 µL aliquots of 10⁻⁴ to 10⁻⁶ dilutions were separately plated out on selective media of McFadden and Sutton (McFadden and Sutton, 1975).

In the primary test, 58 *F. graminearum* and 289 *Trichoderma* strains were isolated from the infected collected wheat plants and soil samples. Pathogenicity of the *F. graminearum* isolates was proved on Tajan wheat cultivar, and the most virulent strain of *F. graminearum* B-63 was selected for further studies.

All the antagonists and pathogen isolates were maintained on Potato Dextrose Agar (PDA) and incubated at 25°C.

Effect of *Trichoderma* strains on mycelial growth of *F. graminearum* B-63

Dual culture and cellophane overlays were used to determine the effect of *Trichoderma* isolates on pathogen (Dennis and Webster, 1971). All antagonist pathogen combinations were examined on 15 mL of PDA in 90 mm Petri dish.

For dual culture, a 5 mm diameter mycelial plug was taken from the actively

growing 3 day old colonies of *Trichoderma* or pathogen isolates and placed 5 cm apart on the agar. In the case of cellophane overlay technique, 9 cm diameter cellophane membranes (Australia Cellophane, Victoria) were boiled in distilled water, interleaved with filter papers and autoclaved before being placed on the agar medium. For control, a plug sterile PDA medium was used instead of antagonist. After 48 h, the cellophane membrane and adhering fungus or agar plug were removed (Etebarian et al., 2000).

The culture plates were incubated at 25°C in the dark. After 7 days, inhibition zones were measured compared with the controls and percentage inhibition of growth was calculated. The pathogen colony diameter was the average of two measurements and the area was calculated. The percent growth inhibition was calculated using the formula:

$$\% \text{ GI} = \frac{a - b}{a} \times 100$$

where:

%GI = percent growth inhibition,

a = *F. graminearum* uninhibited colony area or control;

b = inhibited colony area.

For antifungal activity of the *Trichoderma* strains, a 5 mm diameter mycelium inoculated plug of *F. graminearum*, without growth, was transferred to PDA. Randomised completed design with 3 replications was used for this study. Identification of the pathogen and antagonists strains was achieved on the basis of the morphological characterization of the colonies, measurement of hyphal diameter, conidiophores and conidia dimensions (Nelson et al., 1933; Rifai, 1969; Bissett, 1991). For the pathogen, sections of autoclaved sterile wheat straw were also placed on the PDA inoculated with *F. graminearum*, in order to produce *Gibberella zea* perithecium (Nelson et al., 1983).

F. graminearum B-63, which showed the highest virulence in pathogenicity test (as the causal agent), *T. harzianu* G-22 (from Gharakheil), *T. harzianu* S-35 (from Serajmohaleh), *T. harzianu* J-43 (from Jouibar), *T. harzianu* B-122 (from Bai-e-kola), *T. viride* H-63 (from Hoseinabad), and

T. viride B-85 (from Bai-e-kola), which produced the most developed inhibition zone against the pathogen (as antagonists), were selected for further study.

Biological control of *F. graminearum* B-63 on wheat in greenhouse

The selected antagonists were tested for their ability to reduce the incidence and severity of root and foot rot in wheat.

For this purpose, *F. graminearum* B-63 and *Trichoderma* isolates were grown on PDA for 1 week. Inoculums of *F. graminearum* B-63 was multiplied by transferring the pieces of 5 cm diameter culture to 250 Erlenmeyer flasks containing 100 g sand, 5 g maize meal and 20 ml of sterile distilled water, and inoculums of *Trichoderma* strains were multiplied by transferring the pieces of 5 cm diameter culture to 250 Erlenmeyer flasks containing 100 ml of moist wheat bran. Then the inoculated substrates were incubated at room temperature for 3 weeks, till all substrates were covered by *F. graminearum* B-63 and *Trichoderma* isolates.

Both multiplied inoculums of the pathogen and antagonists were mixed with the autoclaved potted soil (field soil) at the rate of 5 gram.kg⁻¹ soil. *F. graminearum* B-63 was used one day before sowing and *Trichoderma* strains were applied just the day of seeding.

Seeds of Tajan wheat cultivar were surface-disinfected by soaking in 1% sodium hypochlorite for 2 min, rinsed three times in sterile distilled water and sown in 20 cm diameter plastic pots containing the treated soils. There were 3 replicate pots per treatment, arranged in completed randomised design. Treatments were:

F. graminearum B-63 + *T. harzianum* T 22,
F. graminearum B-63 + *T. harzianum* T 35,
F. graminearum B-63 + *T. harzianum* T 43,
F. graminearum B-63 + *T. harzianum* T 122,
F. graminearum B-63 + *T. virens* T 63,
F. graminearum B-63 + *T. virens* T 85,
 Inoculated Control with *F. graminearum* B-63 (diseased control) and Uninoculated control without *F. graminearum* B-63 (healthy control).

Plants were maintained in the greenhouse of Gharakheil Crop Research Station of Mazandaran, Iran.

Biological control of *F. graminearum* B-63 on wheat in field trials

For confirmation of the greenhouse tests, the selected antagonists were re-examined in field trials. All treatments were the same as mentioned for the greenhouse, but the experimental design was randomised complete block.

Analysis of variance and Duncan's Multiple Range Test were used to determine differences among treatments (Little and Hills, 1978).

RESULTS

Effect of *Trichoderma* strains on mycelial growth of *F. graminearum* in vitro

All tested *Trichoderma* strains inhibited mycelial growth of *F. graminearum* B-63 in dual culture. There were significant differences among the *Trichoderma* strains. Growth inhibition of *F. graminearum* B-63 was reduced by *T. harzianu* 22, *T. harzianu* 35, *T. harzianu* 43, *T. harzianu* 122, *T. viride* 63, *T. viride* 85 by 45.76, 57.13, 58.8, 28.5, 55.13 and 29.16% respectively (Table 1 and Figure 1).

Cell free metabolites of *T. harzianu* 22, *T. harzianu* 35, *T. harzianu* 43, *T. harzianu* 122, *T. viride* 63, *T. viride* 85 reduced the growth *F. graminearum* B-63 at the rates of 37, 46, 48.12, 21.12, 45.56 and 23.12% respectively (Figure 2).

Antifungal activity of volatile metabolites on growth inhibition of *F. graminearum* B-63 varied among the *Trichoderma* strains. *T. harzianu* 22, *T. harzianu* 35, *T. harzianu* 43, *T. harzianu* 122, *T. viride* 63, *T. viride* 85 reduced the growth *F. graminearum* B-63 at the rates of 29, 39, 41, 18, 37 and 20% respectively. Percentage reduction of *F. graminearum* B-63 growth with the *T. harzianu* 43 was significantly larger than that of the other *Trichoderma* strains (Figure 3).

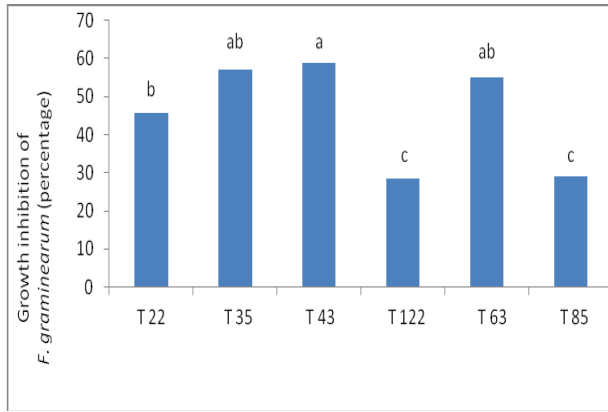


Figure 1. Effect of *Trichoderma* strains on growth inhibition of *F. graminearum* B-63 (dual culture)
Treatments with the same letters do not differ significantly ($P \leq 0.5$) according to the Duncan's multiple range test. The vertical bars represent standard deviation with three replicates.

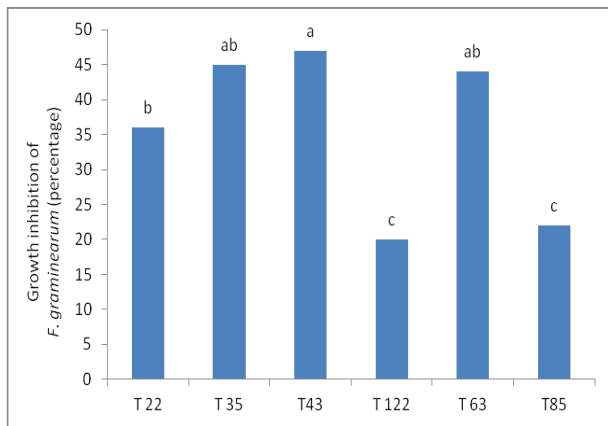


Figure 2. Effect of cell free metabolites of *Trichoderma* strains on growth inhibition of *F. graminearum* B-63 (cellophane method)
Treatments with the same letters do not differ significantly ($P \leq 0.5$) according to the Duncan's multiple range test. The vertical bars represent standard deviation with three replicates.

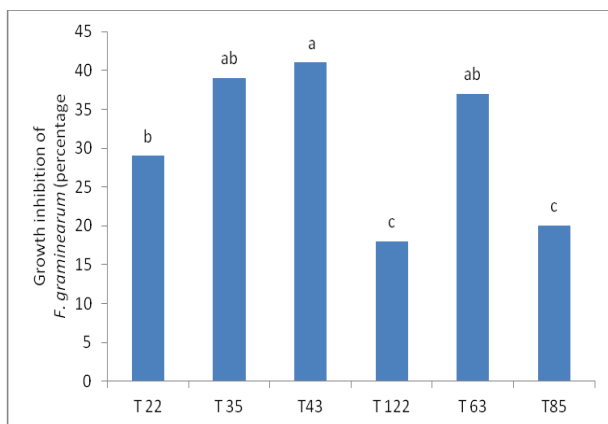


Figure 3. Antifungal activity of the *Trichoderma* strains volatile metabolites on growth inhibition of *F. graminearum* B-63.
Treatments with the same letters do not differ significantly ($P \leq 0.5$) according to the Duncan's multiple range test. The vertical bars represent standard deviation with three replicates.

Table 1. Effect of *Trichoderma* strains on growth inhibition of *F. graminearum* B-63

<i>Trichoderma</i> strains	Dual culture	Cell free metabolites	Volatile metabolites
<i>T. harzianu</i> 22	45.76 ^b	37.00 ^b	28.00 ^b
<i>T. harzianu</i> 35	57.13 ^{ab}	46.00 ^{ab}	39.00 ^{ab}
<i>T. harzianu</i> 43	58.80 ^a	48.12 ^a	43.22 ^a
<i>T. harzianu</i> 122	28.50 ^c	21.12 ^c	21.54 ^c
<i>T. viride</i> 63	55.13 ^{ab}	45.56 ^{ab}	38.50 ^{ab}
<i>T. viride</i> 85	29.16 ^c	23.12 ^c	23.20 ^c

Treatments with the same letters do not differ significantly ($P \leq 0.05$) according to the Duncan's multiple range test. The vertical bars represent standard deviation with three replicates.

Biological control of *F. graminearum* B-63 on wheat in greenhouse tests

Disease incidence and severity in plants inoculated with the pathogen and *T. harzianu* 43 were significantly less than the control inoculated only with pathogen (Control1) and than other treatments (Figure 4).

Thousand grains weight in wheat plants inoculated with the pathogen and *T. harzianu* 43 was significantly higher than in the control inoculated only with pathogen (Control) and than other treatments (Figure 5).

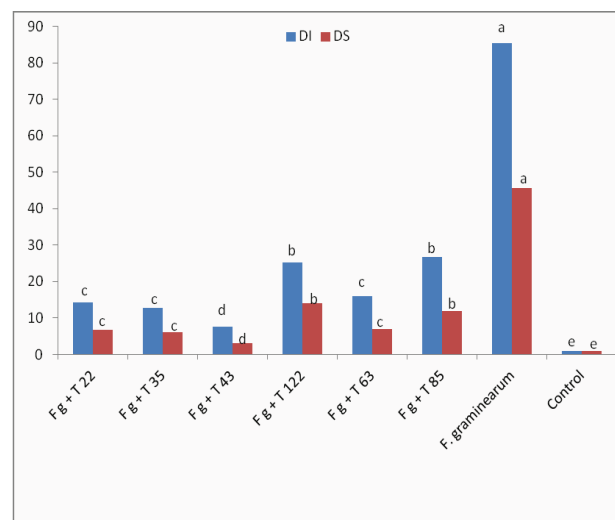


Figure 4. Effect of *Trichoderma* strains on disease incidence and severity in greenhouse tests.
Treatments with the same letters do not differ significantly ($P \leq 0.5$) according to the Duncan's multiple range test. The vertical bars represent standard deviation with three replicates.

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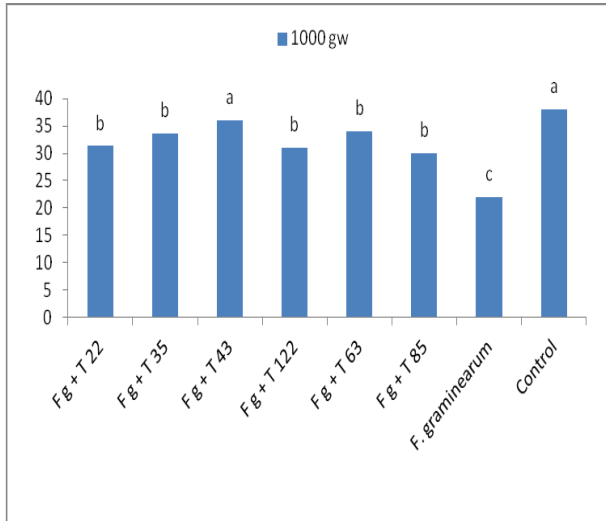


Figure 5. Effect of *Trichoderma* strains on 1000 grains weight in greenhouse tests

Treatments with the same letters do not differ significantly ($P \leq 0.5$) according to the Duncan's multiple range test. The vertical bars represent standard deviation with three replicates.

Biological control of *F. graminearum* B-63 on wheat in field trial

Disease incidence and severity in plants inoculated with the pathogen and *T. harzianu* 43 was significantly less than the pathogen inoculated control and other treatments (Table 2, Figure 6).

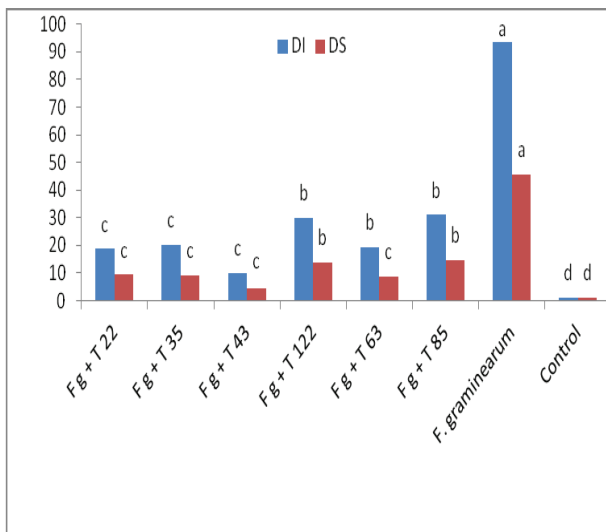


Figure 6. Effect of *Trichoderma* strains on disease incidence (DI) and severity (DS) in the field trial

Treatments with the same letters do not differ significantly ($P \leq 0.5$) according to the Duncan's multiple range test. The vertical bars represent standard deviation with three replicates.

Table 2. Effect of *Trichoderma* strains on disease incidence and severity in greenhouse test

Treatment	Disease incidence (%)	Disease severity (%)
<i>F. graminearum</i> B-63 + <i>T. harzianu</i> 22	14.33 ^c	7.66 ^c
<i>F. graminearum</i> B-63 + <i>T. harzianu</i> 35	12.66 ^c	6.00 ^c
<i>F. graminearum</i> B-63 + <i>T. harzianu</i> 43	7.66 ^d	3.00 ^d
<i>F. graminearum</i> B-63 + <i>T. harzianu</i> 122	25.33 ^b	14.00 ^b
<i>F. graminearum</i> B-63 + <i>T. viride</i> 63	16.00 ^c	7.00 ^c
<i>F. graminearum</i> B-63 + <i>T. viride</i> 85	26.66 ^b	12.00 ^b
Control 1 (inoculated with <i>F. graminearum</i> B-63 only)	85.33 ^a	45.66 ^a
Control 2 (non inoculated)	0 ^e	0 ^e

Treatments with the same letters do not differ significantly ($P \leq 0.05$) according to the Duncan's multiple range test.

The yield of wheat in plants inoculated with the pathogen and *T. harzianu* 43 was significantly higher than the control and other treatments (Table 3).

Table 3. Effect of *Trichoderma* strains on 1000 grain weight in greenhouse conditions and on yield in field trial

Treatment	1000 grain weight (g)	Yield (t ha ⁻¹)
<i>F. graminearum</i> B-63 + <i>T. harzianu</i> 22	31.33 ^b	3.80 ^a
<i>F. graminearum</i> B-63 + <i>T. harzianu</i> 35	33.66 ^b	3.63 ^b
<i>F. graminearum</i> B-63 + <i>T. harzianu</i> 43	36.00 ^a	3.85 ^a
<i>F. graminearum</i> B-63 + <i>T. harzianu</i> 122	31.00 ^b	3.43 ^c
<i>F. graminearum</i> B-63 + <i>T. viride</i> 63	34.00 ^b	3.53 ^{bc}
<i>F. graminearum</i> B-63 + <i>T. viride</i> 85	30.00 ^b	3.15 ^d
Control1 (inoculated with <i>F. graminearum</i> B-63 only)	22.00 ^c	1.69 ^e
Control 2 (non inoculated)	38.00 ^a	3.88 ^a

DISCUSSION

In dual culture all *Trichoderma* strains inhibited the growth of *F. graminearum* B-63. Zones of inhibition were observed between the colonies of the pathogen and *Trichoderma* strains. The inhibition zone could be due to the effect of diffusible inhibitory substances produced by the *Trichoderma* strains, which suppressed the growth of *F. graminearum* B-63. The presence and size of the zone of inhibition have been used as evidence of the production of antibiotics by the *Trichoderma* strains (Jackson et al., 1991; Crawford et al., 1993).

Cell-free metabolites produced by the strains of *Trichoderma* also reduced the colony area of *F. graminearum* B-63, even though the cellophane overlay technique has been used mainly for investigating non-volatile metabolites of *Trichoderma* (Jackson et al., 1991; Dennis and Webster, 1971).

Antibiotic substances from *Trichoderma* strains were not extracted and determined in this study, but some antibiotics such as tubercidin, candicidin, phospholactomycin, phenasin and 4-diacetylphloroglucinol or 2,4-diacetylphloroglucinol, produced by some antagonists like *Pseudomonas fluorescens*, *Streptomyces* spp. and *Trichoderma* spp. have been reported by several researchers (Hwang et al., 1994; Lechevalier et al., 1953; Mazzolla et al., 1992; Shanahan et al., 1992).

The results of volatile metabolite activity indicated that percentage reduction in the growth of *F. graminearum* B-63 with *Trichoderma* strains, was closely in accordance to the results of Fiddaman and Rossal (1993) on *Bacillus* volatile antagonizing *Pythium ultimum* and *Rhizoctonia solani*. Landy and his coworkers (1948) also reported two groups of antibiotics including antibacterial and antifungal antibiotics that were produced by *B. subtilis*.

Inoculation of wheat in the greenhouse conditions and field trials showed that treatments with *Trichoderma* strains could decrease the foot and root rot incidence and severity and increase the 1000 grain weight and total yield of tested wheat. Effectiveness of inoculation of wheat plants with

antagonists in reduction of diseases and increase of yield was reported by several investigators. Bochow and Fritzche (1991) reported that inoculation of plants with *Streptomyces* in greenhouse reduced the severity of *Phthophthora infestans*. This reduction was due to the induction of host resistance by the *Streptomyces* strain. Effectiveness of inoculation of wheat plants with bacterial antagonists in reduction of head blight due to *F. graminearum* B-63 and increase of yield were also reported (El-Abyad et al., 1993; Etebarian et al., 2003; Jones and Samac, 1996; Liu et al., 1995; Luz, 2000; Norozian et al., 2006; Okhovat et al., 1996).

In an experiment in Mazandaran province, isolates of *Bacillus subtilis* and *Pseudomonas fluorescens* significantly reduced the percentage of scabby spikelets under greenhouse conditions and severity of disease in field trials as well (Foroutan et al., 2005).

Several microbial agents like *P. fluorescens* are commercially available as Aspire for biological control (Janisiewicz and Kartsten, 2002). Some strains of *Streptomyces* spp. are commercially available as microbial pesticides registered on some crops for biological control of some diseases (Jones and Samac, 1966).

However, *Trichoderma* strains tested in this study should be investigated extensively for food safety before commercialisation.

Acknowledgments

I would like to express my deep sincere of gratitude to Agricultural and Natural Resources Research Center of Mazandaran for the support during this project and for making available all the facilities.

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