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Antagonistic Potentiality of *Trichoderma harzianum* Towards Seed-Borne Fungal Pathogens of Winter Wheat cv. Protiva *In Vitro* and *In Vivo*

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The antagonistic effect of *Trichoderma harzianum* on a range of seed-borne fungal pathogens of wheat (*viz. Fusarium graminearum*, *Bipolaris sorokiniana*, *Aspergillus* spp., and *Penicillium* spp.) was assessed. The potential of *T. harzianum* as a biocontrol agent was tested *in vitro* and under field conditions. Coculture of the pathogens and *Trichoderma* under laboratory conditions clearly showed dominance of *T. harzianum*. Under natural conditions, biocontrol effects were also obtained against the test fungi. One month after sowing, field emergence (plant stand) was increased by 15.93% over that obtained with the control treatment, and seedling infection was reduced significantly. Leaf blight severity was decreased from 22 to 11 at the heading stage, 35 to 31 at the flowering stage, and 86 to 74 at the grain filling stage. At harvest, the number of tillers per plant was increased by 50%, the yield was increased by 31.58%, and the 1,000-seed weight was increased by 21%.

Keywords: Antagonist, *Trichoderma harzianum*, seed-borne disease, wheat

Seed-borne pathogenic fungi affecting plant health are a major and constant menace to food production worldwide. Such pathogens cause seed rot and reduce seedling emergence, diminishing crop yield [4, 26]. Wheat plants are particularly vulnerable to different seed-borne diseases. In Bangladesh, of the 120 diseases of wheat that have been identified, 8 are caused by seed-borne fungi [14]. Seed-borne infection by fungal pathogens is important not only because of its association with germination failure and/or disease in newly emerged seedlings or growing plants, but also because of the soil contamination that occurs owing to the permanent establishment of inocula [3]. *Drechslera sorokiniana* (Sacc), the causal agent of spot/blotch of wheat, is a predominant pathogen and is seed-borne [24]. This disease has become a major problem for wheat production in humid subtropical climates; in one study, the yield loss due to this disease ranged from 10–21% in 6 selected cultivars and advanced lines [23].

Over the past few decades, agricultural production has expanded and farmers have increasingly relied on chemical pesticides as a relatively dependable method of protecting plants against pathogens [8]. However, the increasing use of chemical pesticides negatively affects the environment and human health [15]. Concerns about their safety and environmental effects in water, soil, and food require that such chemical inputs be minimized.

Biological control has been proposed as a replacement for chemical control of plant diseases [16]. It represents a natural and ecological approach that reduces chemical inputs and their effects on the environment. Biological control also tends to have more specific effects, with only the target pathogenic organism(s) being adversely affected; other beneficial organisms and a diverse soil microbial community are left intact to provide for healthier plants and roots [27]. Thus, biological control can be safer for humans, crops, and the environment. It also has the potential to be more stable and longer-lasting than other controls, and is compatible with the concepts and goals of integrated pest management and sustainable agriculture. Therefore, incorporation of biological control is of primary significance.

The potential of *Trichoderma* spp. as biocontrol agents against plant disease was first recognized in the early 1930s; subsequently, the usefulness of a variety of biocontrol agents has been demonstrated [16]. However, the efficacy of *Trichoderma* spp. makes them some of the most well known and most used biological agents for the control of plant disease [18]. Several strains of *Trichoderma* are effective

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as biocontrol agents against various soil-borne fungi, such as *Fusarium* spp., *Sclerotium* spp., and *Rhizoctonia solani. T. harzianum* significantly reduces the growth of *S. rolfsii*. The antagonistic effect of *T. harzianum* on a range of phytopathogenic fungi, including *Alternaria solani*, *Botrytis fabae*, *Cladosporium cucumerinum*, *F. oxysporum*, and *F. tricinctum*, has been studied [5]. Coculture of the phytopathogens and *Trichodema* under laboratory conditions has clearly demonstrated the dominance of *Trichoderma* species [6]. *Trichoderma* produces a compound called trichodermin, which is responsible for its antagonistic properties. Thus, *T. harzianum* may be an eco-friendly biocontrol agent that minimally disrupts the natural soil ecology, enabling the preservation of many beneficial microorganisms. This biocontrol agent has the potential to protect seedlings against several diverse plant pathogenic fungi [1]. *T. harzianum* is the most effective mycoparasite that is able to penetrate the hyphae of *F. culmorum* and can be used successfully to control *Fusarium* foot rot in wheat seedlings [13]. *T. harzianum* produces antibiotics that are capable of inhibiting the mycelial growth of *F. culmorum* [25]. *Trichoderma* spp. are effective in reducing the severity of foliar disease on wheat plants compared with untreated plants [29].

Biological control agents have been reported as suitable for controlling seed-borne fungal diseases. In one study Knudsen *et al.* [20] reported that the number of tillers per row was increased by 53% after using seeds treated with a biocontrol agent. The yield of winter wheat was increased by 160% and the 1,000-grain weight by 4%. In another study, Sliesaravicius *et al.* [34] reported an increase in the number of healthy grains/ear as well as the yield of wheat after using biocontrol agents.

The efficacy of *Trichoderma* and other biocontrol agents has been demonstrated on a varitey of crop plants under field conditions suggesting that *T. harzianum* increases field emergence and yield, with a low incidence of disease severity in wheat [31]. It may play an important role in reducing the incidence of seedling disease and enabling a greater percentage of plant stands in the field [17]. *Trichoderma* biological control agents were reported to increase plant height in pigeon pea and bitter gourd [22]. *Trichoderma* spp. effectively reduced the severity of leaf blight in wheat [12]. In several field experiments, it has been observed that this biocontrol agent has a great influence on the yield and yield attributes of crop plants.

Despite the proven potential of *Trichoderma* as a biocontrol agent, there is still a need to further evaluate its efficiency under different conditions, climates, and available varieties of wheat and other similar crops in different geographical regions. Therefore, the potential of *T. harzianum* as a biocontrol agent against seed-borne diseases of wheat both *in vitro* and *in vivo* was evaluated in the present study. The effect of *T. harzianum* on the growth and yield of wheat under field conditions was also assessed.

Materials and Methods

Seed Samples

The wheat cultivar Protiva was used and seed samples were collected from the Bangladesh Wheat Research Institute, Shyampur, Rajshahi.

Isolation and Identification of Seed-Borne Fungi

Bipolaris sorokiniana, Fusarium graminearum, *Aspergillus* spp., and *Penicillium* spp. were isolated from wheat seed samples using the blotter method described by the International Seed Testing Association [19]. Isolated fungi were identified to the genus level with the help of standard mycological reference publications [5, 35]. The isolated fungi were cultured on potato dextrose agar (PDA) at 28 ± 2°C. Pure cultures of the fungi were preserved in PDA slants at 4°C as stock cultures for future use.

Isolation, Purification, Identification, Preservation, and *In Vitro* Screening of Isolates of *T. harzianum*

T. harzianum was isolated from different garbage sources and soils and was cultured on PDA. The antagonistic effect of *T. harzianum* on fungal pathogens was evaluated using a dual culture technique [37]. Ten isolates of *Trichoderma* were tested for their antagonistic effect. On the basis of this effect, isolate RUT-103 (IMI-392432) was used in this study. A pure culture of the selected *Trichoderma* species was made on PDA using the hyphal tip culture technique. Cultures were preserved at 4°C for further use.

Inhibition (%) Effect of *T. harzianum* Against *B. sorokiniana*, *F. graminearum*, *Aspergillus* spp., and *Penicillium* spp.

PDA was inoculated with a 6 mm block of *T. harzianum* isolates on one side of the plate and with *B. sorokiniana* 7 cm away on the other side. Control plates contained only *B. sorokiniana* at the center of the plate. All PDA plates were then incubated at 28°C and observed regularly to assess growth. The growth of *B. sorokiniana* was inhibited after it contacted *T. harzianum*. The percent inhibition of radial growth (PIRG) of *B. sorokiniana* was calculated as previously described [36]:

$$\text{Inhibition (\%)} = \frac{X - Y}{X} \times 100$$

where X = mycelial growth (radial) of the pathogen on the control plate, and Y = mycelial growth (radial) of the pathogen on the dual culture plate. The same procedure was followed for *F. graminearum*, *Aspergillus* spp., and *Penicillium* spp. for calculation of inhibition.

Inoculation of Wheat Seed with *T. harzianum* Followed by Isolated Test Fungi

A 5-day-old culture of *T. harzianum* (isolate RUT-103) was used to prepare a spore suspension containing 10^5 spores/ml. Spore suspensions of *B. sorokiniana, F. graminearum, Aspergillus* spp., and *Penicillium* spp. were also prepared. Four hundred seeds of each variety of wheat were first inoculated with a spore suspension of test fungi and the treated seeds were dried under a laminar hood. The treated seeds were then dipped in the *T. harzianum* spore suspension for 20 min.

Field Experiments

The treated seeds were sown in the field and the field experiment was carried out in a randomized block design with three replications for each treatment. The distance between blocks and between plots was 80 and 50 cm, respectively. Each unit plot size was 10 m^2. Thus, there were 18 plots for the study. Seeds were sown in lines maintaining a spacing of 20 and 5 cm between rows and seed-to-seed, respectively. Three foliar sprays of the *T. harzianum* spore suspension (10^5 spores/ml) were done at the tillering, heading, and grain filling stages along with seed treatment.

Data Collection and Analysis

Data regarding field emergence, number of infected seedlings per plot, tillers per plant, plant height, ear length, number of spikelets per ear, healthy grains per ear, infected grains per ear, 1,000-seed weight, and yield per plot were collected. Leaf blight severity was scored on a scale of 00–99 [32]. All analyses were conducted in duplicate with three replicates for each experiment. Data are expressed as the mean ± standard error. The results were analyzed using the SPSS statistical package (SPSS, Chicago, IL, USA). An analysis of variance (ANOVA) was used to evaluate the treatments. Duncan's Multiple Range Test was used to determine the significant difference at $p \leq 0.05$.

RESULTS

Antagonistic Effect of *T. harzianum* in Coculture

The *in vitro* antagonistic effect of *T. harzianum* on the radial growth of *F. graminearum*, *B. sorokiniana*, *Aspergillus* spp., and *Penicillium* spp. was determined after 6 and 8 days of incubation at 28 ± 1°C. In every case, *T. harzianum* showed good antagonistic effect against the tested fungi. The percent inhibition of radial growth (PIRG) of *F. graminearum* was 39.21 and 43.33 at 6 and 8 days, respectively. The PIRG of *B. sorokiniana* and *Aspergillus* spp. was 42.85 and 45.71 at 6 days, and 28.35 and 31.66 at 8 days, respectively. Similarly, the PIRG of *Penicillium* spp. was 53.48 and 55.18 at 6 and 8 days, respectively.

Effect of *T. harzianum* Either Alone or in Combination with Seed-Borne Fungi on Field Emergence, Number of Infected Seedlings and Leaf Blight Severity of Wheat Under Field Conditions

Plant stand under different treatments varied significantly from 46.00% to 53.33% (Table 1). Treatment of seeds with *T. harzianum* only gave the highest plant stand (53.33%) followed by the treatment combinations *T. harzianum* + *Penicillium* spp. and *T. harzianum* + *F. graminearum*, whereas the lowest plant stand was recorded in the control treatment (46.0%). In this experiment, *T. harzianum*-treated seeds displayed up to 15.93% higher plant stand over the control. Except for the treatment combinations *T. harzianum* + *F. graminearum* and *T. harzianum* + *B. sorokiniana*, all other treatments completely controlled seedling infection in the experimental plot. On the other hand, the untreated control plot displayed the highest rate of seedling infection. Leaf blight severity was also recorded on a scale of 00–99 at the heading, flowering, and grain filling stages. Treatment of seeds with *T. harzianum* either alone or in combination with seed-borne fungi, and later on foliar spray with *T. harzianum*, reduced leaf blight severity from 22 to 11 at the heading stage, 35 to 31 at the flowering stage, and 86 to 74 at the grain filling stage (Table 1).

Effect of *Trichoderma harzianum* in Combination with Seed-Borne Fungi on Number of Tillers/Plant, Plant Height, Ear Length, and Spikelets/Ear of Wheat Under Field Conditions

Under different treatments, the number of tillers/plant varied from 6.00 to 9.00. Most of the treatments significantly

Table 1. Antagonistic effect of *Trichoderma harzianum* either alone or in combination with seed-borne fungi on field emergence, seedling infection, and severity of leaf blight in wheat cv. Protiva under field conditions.

Treatments	Seed treatment			Seed treatment + foliar spray		
				Leaf blight severity (00–99)		
	Field emergence (Plant stand)	Increase in field emergence over control (%)	Seedling infection (%)	Heading stage	Flowering stage	Grain filling stage
T. harzianum + *F. graminearum*	50.00 ± 1.15ab	8.00	1.44 ± 0.11b	21	32	77
T. harzianum + *B. sorokiniana*	49.33 ± 0.33bc	7.23	0.66 ± 0.19c	22	34	77
T. harzianum + *Aspergillus* spp.	48.67 ± 1.54bc	5.80	0.00 ± 0.00d	12	32	75
T. harzianum + *Penicillium* spp.	51.33 ± 1.34ab	11.58	0.00 ± 0.00d	22	31	76
T. harzianum	53.33 ± 0.96a	15.93	0.00 ± 0.00d	11	31	74
Control	46.00 ± 1.32c	-	5.00 ± 0.28a	22	35	86
Significance level (P)	**		***			

***P<0.001, **P<0.01

Data are means ± standard error from a representative experiment. Each value represents the average of three replicates. Mean values with the same letters are not significantly different: Duncan's Multiple Range Test; P<0.05.

Table 2. Antagonistic effect of *Trichoderma harzianum* either alone or in combination with seed-borne fungi on number of tillers/plant, plant height, ear length, and spikelets/ear of wheat cv. Protiva under field conditions.

Treatment	Seed treatment + foliar spray				
	No. of tillers/plant	Increase in tillers/plant over control (%)	Plant height (cm)	Ear length (cm)	No. of spikelets per ear
T. harzianum + *F. graminearum*	8.00 ± 0.28ab	33.33	95.00 ± 2.59a	19.00 ± 0.57a	20.33 ± 1.07b
T. harzianum + *B. sorokiniana*	9.00 ± 0.76a	50.00	95.33 ± 2.88a	19.33 ± 0.38a	20.67 ± 0.84ab
T. harzianum + *Aspergillus* spp.	6.33 ± 0.38bc	5.50	92.00 ± 1.15a	19.66 ± 0.33a	21.67 ± 0.19ab
T. harzianum + *Penicillium* spp.	8.33 ± 0.33a	38.83	99.00 ± 2.30a	19.00 ± 0.57a	22.33 ± 0.38a
T. harzianum	9.00 ± 0.28a	50.00	84.00 ± 2.30b	18.99 ± 0.38a	22.00 ± 0.76ab
Control	6.00 ± 0.57c	-	83.00 ± 1.73b	17.33 ± 0.19b	18.00 ± 0.28c
Significance level (P)	*		**	*	**

**P<0.01, *P<0.05

Data are means ± standard error from a representative experiment. Each value represents the average of three replicates. Mean values with the same letters are not significantly different: Duncan's Multiple Range Test; P<0.05.

increased the number of tillers/plant. *T. harzianum* either alone or in combination with *B. sorokiniana* produced the highest number of tillers/plant (9.00) and a similar trend was also found in the case of *T. harzianum* + *Penicillium* spp. (Table 2). This result indicates an increase of up to 50% in the number of tillers/plant over the control. Plant height and ear length were also significantly influenced by seed treatment and foliar spray with *T. harzianum*. Plant height ranged from 83–99 cm, where the highest and the lowest plant heights were recorded with the *T. harzianum* + *Penicillium* spp. treatment and in the control, respectively. Ear length varied from 17.33 to 19.66 cm. According to Duncan's Multiple Range Test, all of the treatments showed a statistically identical result for increased ear length, but the control treatment produced the lowest result (Table 2). The treatments significantly differed in the number of spikelets/ear and grains/ear. Spikelets/ear varied from 18.00–22.33, where the highest number of spikelets/ear was recorded when seeds were treated with *T. harzianum* + *Penicillium* spp. and foliage sprayed with *T. harzianum.* Seed treatment and foliar spray with *T. harzianum* also resulted in 22 spikelets/ear.

Effect of *T. harzianum* Either Alone or in Combination with Seed-Borne Fungi on Number of Grains/Ear, Health Grains/Ear, Infected Grains/Ear, 1,000-seed Weight, and Yield of Wheat Under Field Conditions

The effect of *T. harzianum* either alone or in combination with seed-borne fungi on the number of grains/ear, healthy grains/ear, 1,000-seed weight, and yield/plot of wheat cv. Protiva was determined under field conditions. The summarized results are shown in Table 3. Owing to seed treatment and foliar spray, the number of grains/ear varied from 41.00 to 51.10. Except for the control, the rest of the treatments resulted in a significantly increased number of grains/ear and the treatment effects were statistically identical. The number of healthy and infected grains/ear ranged from 36.33 to 48.00 and 2.50 to 4.66, respectively. In this case, most of the treated plots produced the highest number of healthy and the lowest number of infected

Table 3. Antagonistic effect of *Trichoderma harzianum* either alone or in combination with seed-borne fungi on number of grains/ear, healthy grains/ear, infected grains/ear, and 1,000-seed weight of wheat cv. Protiva under field conditions.

Treatments	No. of grains/ear	No. of healthy grains/ear	No. of infected grains/ear (spotted)	1,000-Seed weight (g)
T. harzianum + *F. graminearum*	48.00 ± 1.73a	45.50 ± 0.76ab	2.50 ± 0.28b	44.00 ± 1.15ab
T. harzianum +*B. sorokiniana*	47.33 ± 1.15a	44.33 ± 0.96b	3.00 ± 0.14b	38.00 ± 1.73d
T. harzianum + *Aspergillus* spp.	47.33 ± 0.57a	44.67 ± 1.15b	2.66 ± 0.38b	40.00 ± 1.15bcd
T. harzianum + *Penicillium* spp.	51.10 ± 1.15a	48.00 ± 0.57a	3.10 ± 0.10b	42.00 ± 2.08abc
T. harzianum	50.33 ± 1.34a	47.33 ± 0.33ab	3.00 ± 0.57b	46.00 ± 1.04a
Control	41.00 ± 0.76b	36.33 ± 1.15c	4.66 ± 0.38a	37.00 ± 0.50d
Significance level (P)	***	***	**	**

***P<0.001, **P<0.01

Data are means ± standard error from a representative experiment. Each value represents the average of three replicates. Mean values with the same letters are not significantly different: Duncan's Multiple Range Test; P<0.05.

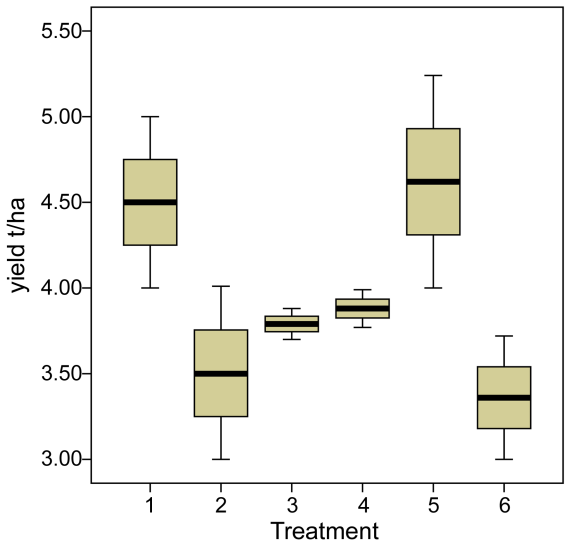

Fig. 1. Antagonistic effect of *Trichoderma harzianum* either alone or in combination with seed-borne fungi on yield of wheat cv. Protiva under field conditions.
Treatment 1 (T_1) = *T. harzianum* + *F. graminearum*, T_2 = *T. harzianum* + *B. sorokiniana,* T_3 = *T. harzianum* + *Aspergillus* spp., T_4 = *T. harzianum* + *Penicillium* spp., T_5 = *T. harzianum*, and T_6 = Control.

grains/ear. In every case, the control treatment produced the lowest number of healthy and highest number of infected grains/ear (Table 3). The 1,000-seed weight under different treatments varied significantly from 37.00 to 46.00 g, where the maximum was due to seed treatment and foliar spray with *T. harzianum*, followed by treatment with the combination of *T. harzianum* + *F. graminearum*, whereas the minimum (37 g) was recorded in the control treatment (Table 3).

To determine the antagonistic potential of *T. harzianum*, we recorded yield/plot under different treatments; it varied from 3.36 to 4.62 t/ha (Fig. 1). The highest grain yield was recorded after application with *T. harzianum* as a seed treatment and foliar spray, resulting in up to 37.50% higher grain yield over the control. Seed treatment with the combination of *T. harzianum* + *F. graminearum* and foliar spray with *T. harzianum* produced a 33.92% increase in grain yield over the control (Fig. 1).

DISCUSSION

Seed health testing of the collected wheat seed samples revealed that the seeds were associated with six different fungi: *B. sorokiniana, F. graminearum*, *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp., and *Alternaria* spp. The association of fungi with wheat seeds has been reported [33]. The mycoparasitic modes of action of *T. harzianum* against the seed-borne fungi *B. sorokiniana, F. graminearum, Aspergillus* spp., and *Penicillium* spp. were studied extensively in coculture assays *in vitro*. Coculture of the phytopathogens and *Trichoderma* under laboratory conditions clearly showed the dominance of the latter. The lytic action of the pathogen was clearly apparent and the inhibition of growth appeared to be directly related to its ability to hydrolyze the cell walls of the tested microorganisms, rather than through the inhibitory action of antibiotics or toxins [21]. It has been found that culture filtrates of *T. viride* and *T. harzianum* inhibit *F. moniliforme* [6]. Based on the results of the present investigation, *Trichoderma* spp. are potential candidates for biocontrol of some mycotoxin-producing fungi, but there exists some doubt as to their osmotolerance within air-dried seeds. In our study, we observed that *T. harzianum* inhibited the radial growth of *F. graminearum* by 43.33%. The interaction of the pathogen *F. moniliforme* and antagonistic *T. harzianum* isolates has been studied; *Trichoderma* isolates retarded the radial mycelial growth of *F. moniliforme* by 32.5% and 45% [13]. This result is in accordance with that of the present investigation, where a good inhibitory effect of *T. harzianum* against *B. sorokiniana* was observed on PDA (45.71%). In one study, a compound produced in agar culture by *T. koningii* inhibited the growth of pathogens including *B. sorokiniana, F. oxysporum, Gaeumannomyces graminis* var. *tritici*, *R. solani*, *Phytophthora cinnamomi*, and *Pythium middletonii* [11]. The collective previous and current data support the view that *Trichoderma* spp. can be used as a biological control agent.

In the present study, we used a cultivation-based approach (field study) to characterize the *in vivo* antagonistic potential of *T. harzianum* towards seed-borne pathogenic fungi. The highest percentage of plant stands was recorded after treatment of the wheat seeds alone with *T. harzianum* and the lowest was found in the untreated control. After seed treatment, up to a 15.93% increase in plant stand could be obtained over the control. *F. graminearum* is associated with the cereal damping-off complex, which reduces germination, seedling stand, and yield. Furthermore, *T. harzianum* exerted good control in seedling blight of wheat [10]. In a study where five isolates of *T. harzianum* were tested against *Fusarium* spp. of wheat, all the treatments gave a significant increase in field emergence and yield, with a low incidence of disease severity when compared with the untreated control [30, 31].

During the present investigation, the antagonistic effect of *T. harzianum* against the test fungi was studied regarding seedling infection in the field. No infected seedlings were found in the plots where seeds were sown after treatment with *T. harzianum* alone or in combination with *Aspergillus* spp. and *Penicillium* spp., whereas the highest seedling infection (5%) was recorded in the control plot. Similar results have been amply reported [17, 28]. Combined treatment with *T. harzianum* + *F. graminearum* and *T. harzianum* + *B. sorokiniana* also produced a minimum number of seedling infections per plot. In the present

investigation, leaf blight severity was recorded on a scale of 00–99 at the heading, flowering, and grain filling stages. After seed treatment with *T. harzianum*, either alone or in combination with seed-borne fungi, and foliar spray with *T. harzianum*, leaf blight severity was reduced from 22 to 11 at the heading stage, 35 to 31 at the flowering stage, and 86 to 74 at the grain filling stage. In most of the cases, seed treatment and foliar spray alone with *T. harzianum* showed a better result in reducing leaf blight severity. Greenhouse experiments conducted in 2000 and 2001 demonstrated that seven strains of *Trichoderma* spp. significantly reduced disease severity in wheat plants compared with untreated plants [29]. In another study, it was observed that application of *Trichoderma* spp. on wheat plants reduced disease severity significantly (16–23%) in comparison with the control treatment [2]. Another study yielded similar findings [34]. Significant results were found regarding ear length when wheat plants were sprayed with *T. harzianum* at 35, 45, and 60 days after sowing. Herein, we also recorded ear length and observed a variation from 17.33 to 19.66 cm. The highest ear length was recorded in a plot where seeds were sown after treatment with *T. harzianum* + *Aspergillus* spp., and it was statistically identical to the other treatments. The highest number of grains per ear was counted in the plot that received seed treatment in combination with *T. harzianum* + *Penicillium* spp. and foliar spray with *T. harzianum*, whereas the lowest number of grains per ear was recorded in the control. The number of healthy grains per ear was significantly increased by the different treatments, varying from 36.33 to 48.00 (Table 3). Another study determined that the biological agent Biojodis produced the highest number of healthy grains, as compared with the untreated plot [34]. After treatment either alone or in combination with *T. harzianum* followed by foliar spray, a 1,000-seed weight of up to 46 g was obtained from the plot receiving seed treatment and foliar spray with *T. harzianum*. Similar findings have been reported in two other studies [7, 9]. Seed treatment with *T. harzianum* either alone or in combination with seed-borne fungi and foliar spray with *T. harzianum* resulted in significant variation in grain yield per plot. In this study, we recorded an increase in grain yield of up to 37.50% over the control, similar to the increased grain yield of winter wheat obtained following the application of Biojodis [34]. In an experiment conducted under field conditions with biocontrol agents, the yield of winter wheat was increased by 160% and the 1,000-grain weight by 4% [20].

Wheat growers can be advised to use biocontrol agents for treating their seeds before sowing in the field and to spray wheat plants. Such an approach will reduce disease incidence and increase yield. For confirmation, this study needs to be repeated in different agroclimatic zones of Bangladesh using different selected wheat varieties.

References

1. Adekunle, A. T., T. Ikotun, D. A. Florini, and K. F. Cardwell. 2006. Field evaluation of selected formulations of *Trichoderma* species as seed treatment to control damping-off of cowpea caused by *Macrophomina phaseolina*. *Afr. J. Biotechnol.* **5:** 419–424.
2. Analía, P., M. Virginia, R. S. María, and S. Marina. 2003. Tan spot of wheat (*Triticum aestivum* L.) infection at different stages of crop development and inoculum type. *Crop Prot.* **22:** 157–169.
3. Arshad, J., B. Rukhsana, J. Amna, and A. Tehmina. 2005. Fungi associated with seeds of pulses collected from Lahore and their effect on seed germination. *Mycopathologia* **3:** 13–16.
4. Aulakh, K. S., S. Kaur, S. S. Chahal, and H. S. Randhawa. 1988. Seed-borne *Drechslera* species in some important crops. *Plant Dis. Res*. **3:** 156–171.
5. Booth, C. 1971. *The Genus Fusarium*. Commonwealth Mycological Institute, Kew, Surry, England.
6. Calistru, C., M. McLean, and P. Berjak. 1997. *In vitro* studies on the potential for biological control of *Aspergillus flavus* and *Fusarium moniliforme* by *Trichoderma* species: A study of the production of extracellular metabolites by *Trichoderma* species. *Mycopathologia* **137:** 115–124.
7. Chowdhury, S. P., M. M. Hasan, S. Alam, A. N. Chowdhury, and M. S. Alam. 2005. Biocontrol of seed-borne fungi in relation to plant growth characters and yield of pulse. *Bangladesh J. Crop Sci*. **16:** 189–196.
8. Compant, S., B. Duffy, J. Nowak, C. Clement, and E. Barka. 2005. Use of plant-growth promoting bacteria for biocontrol of plant disease: Principles, mechanisms of action and future prospects. *Appl. Environ. Microbiol*. **71:** 4951–4959.
9. DaLuz, W. C., G. G. Bergstron, and C. A. Stockwell. 1998. Seed-applied bioprotectants for control of seed-borne *Pyrenophora tritici-repentis* and agronomic enhancement of wheat. *Can. J. Plant Pathol.* **19:** 384–386.
10. Dal Bello, G. M., C. I. Monaco, and M. R Simon. 2002. Biological control of seedling blight of wheat caused by *Fusarium graminearum* with beneficial rhizosphere microorganisms. *World J. Microbiol. Biotechnol.* **18:** 627–636.
11. Dunlop, R. W., A. Simon, and K. Sivasithamparam. 1989. An antibiotic from *Trichoderma koningii* active against soilborne plant pathogens. *J. Nat. Prod*. **52:** 67–74.
12. Elad, Y. 2000. Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. *Crop Prot*. **19:** 709–714.
13. El-Hasan, A., F. Walker, and H. Buchenauer. 2008. *Trichoderma harzianum* and its metabolite 6-pentyl-alpha-pyrone suppress fusaric acid produced by *Fusarium moniliforme*. *J. Phytopathol*. **156:** 79–87.
14. Fakir, G. A. 1980. *An Annotated List of Seed Borne Disease in Bangladesh*. Agricultural Information Service, Dhaka, Bangladesh.
15. Gerhardson, B. 2002. Biological substitutes for pesticides. *Trends Biotechnol*. **20:** 338–343.
16. Harman, G. E. 2000. Myths and dogma of biocontrol: Changes in perceptions derived from research on *Trichoderma harzianum* T-22. *Plant Dis*. **84:** 377–393.

17. Hossain, I. and M. H. A. Naznin. 2005. BAU-Biofungicide in controlling seedling disease of some summer vegetables. *BAU Res. Prog.* **15:** 35–40.
18. Howell, C. R. 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant disease: The history and evaluation of current concepts. *Plant Dis*. **87:** 4–10.
19. International Seed Testing Association. 1996. International rules for seed testing. *Seed Sci. Technol*. **4:** 3–49.
20. Knudsen, I. M. B., J. Hockenhull, and D. F. Jensen. 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 Pathol*. **44:** 467–477.
21. Labudova, I. and L. Gogorova. 1998. Biological control of phytopathogenic fungi through lytic action of *Trichoderma* species. *FEMS Microbiol. Lett*. **52:** 193–198.
22. Lo, C. T. and C. Y. Lin. 2002. Screening strains of *Trichoderma* spp. for plant growth enhancement in Taiwan. *Plant Pathol. Bull*. **11:** 215–220.
23. Malakar, P. K., M. A. Reza, S. M. Alam, and M. A Shaheed. 2004. Bipolaris leaf blight: A major constraint to sustainable production of wheat grown under humid conditions. 4th International Crop Science Congress (ICSC). Brisbane, Australia, 26 September-1 October 2004.
24. Mandal, S., K. D. Srivastava, R. Aggarwal, and D. V. Singh. 1999. Mycoparasitic action of some fungi on spot blotch pathogen (*Drechslera sorokiniana*) of wheat. *Indian Phytopathol*. **52:** 39–43.
25. Michalikova, A. and A. Nichrina. 1997. Biological control of *Fusarium* root rot in wheat seedling by *T. harzianum*. *Biol. Bratislava* **52:** 592–598.
26. Nesterov, A. N. 1981. Black embryo grain as the source of root rot of spring wheat. *Seed Abstracts* **5:** 20–53.
27. O'Neill, T. M., A. Niv, Y. Elad, and D. Shtienberg. 1996. Biological control of *Botrytis cinerea* on tomato stem wounds with *Trichoderma harzianum. Eur. J. Plant Pathol*. **102:** 635–643.
28. Padder, B. A., P. N. Sharma, R. Kapil, A. Pathania, and O. M. Sharma. 2010. Evaluation of bioagents and biopesticides against *Colletotrichum lindemuthianum* and its integrated management in common bean. *Not. Sci. Biol.* **2:** 72–76.
29. Perello, A., C. Monaco, M. R. Simon, M. Sisterna, and G. Dal Bello. 2003. Biocontrol efficacy of *Trichoderma* isolates for tan spot of wheat in Argentina. *Crop Prot*. **22:** 1099–1106.
30. Poldma, P., S. Vabrit, A. Merivee, and K. Suigusaar. 2008. Influence of *Trichoderma viride*-inoculated growing substrate on the growth and yield of lettuce (*Lactuca sativa* l.). *Acta Hortic*. **779:** 85–90.
31. Roberti, R., L. De Vero, A. Pisi, and A. Cesari. 2001. Biological control of wheat foot rot by antagonistic fungi and their modes of action. *IOBC/WPRS Bulletin* **24:** 13–16.
32. Saari, E. E. and M. Prescott. 1975. A scale of appraising the foliar intensity of wheat disease. *Plant Dis. Rep*. **59:** 377–380.
33. Singh, J., S. Srivastiva, A. S. Shikha, and B. Bose. 2011. Studies on seed mycoflora of wheat (*Triticum aestivum* L.) treated with potassium nitrate and its effect on germination during storage. *Res. J. Seed Sci*. **4:** 148–156.
34. Sliesaravicius, A., J. Pekarskas, V. Rutkoviene, and K. Baranauskis. 2006. Grain yield and disease resistance of winter cereal varieties and application of biological agent in organic agriculture. *Agron. Res*. **4:** 371–378.
35. Subramanian, C. V. 1971. *Hyphomycetes: An Account of Indian Species, Except Cercosporae*. Indian Council of Agricultural Research, New Delhi.
36. Sundar, A. R., N. D. Das, and D. Krishnaveni. 1995. *In-vitro* antagonism of *Trichoderma* spp. against two fungal pathogens of castor. *Indian J. Plant Protect.* **23:** 152–155.
37. Wu, W. S. and J. K. Chou. 1995. Chemical and biological control of *Alternaria carthami* on zinnia. *Seed Sci. Technol*. **23:** 193–200.