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Effect of Tricho-compost against Seedling Blight Disease of Wheat Caused by *Sclerotium rolfsii*

M. Iqbal Faruk*

Plant Pathology Division Bangladesh Agricultural Research Institute Joydebpur, Gazipur-1701, Bangladesh

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The efficacy of formulated *Trichoderma harzianum*-based Tricho-compost, seed treatment with Tricho-inocula, and chemical fungicide Provax 200 WP against foot and root rot diseases of wheat caused by *Sclerotium rolfsii* was tested in the pot house and in the research field of Plant Pathology Division, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh. Tricho-compost was prepared with a mixed substrate of cow dung, rice bran, and poultry refuse colonized by *T. harzianum*. Seedling mortality of wheat was significantly reduced by the Tricho-compost, Tricho-inocula, and Provax 200 WP both in the pot house as well as in the field experiments. The yield of wheat was sharply increased over the control due to the *T. harzianum* formulations and Provax 200 WP. Among the treatments, soil application of Tricho-compost was more efficient in reducing seedling mortality and accelerating plant growth with an increased yield of wheat with *S. rolfsii*-inoculated pot cultures and field experiments.

Keywords: **Trichocompost, *Trichoderma harzianum*, Tricho-inocula, *Sclerotium roflsii*, seedling blight, wheat**

Introduction

Wheat (*Triticum aestivum* L.) is considered as one of the most important cereal crops in the world and it became to the second most important cereal crop after rice in Bangladesh. The average yield of this crop is only 2.60 t/ha in Bangladesh [1]. The average yield of wheat in Bangladesh is lower in comparison to other countries. Diseases play an important role in lower yield of wheat in the country. Wheat is attacked by at least 20 different diseases in Bangladesh [2, 3], of which five are considered as major diseases, they are Bipolaris leaf blight-BpLB (*Bipolaris sorokiniana*), leaf rust (*Puccinia recondita*), seedling blight (*Bipolaris sorokiniana*), foot and root rot (*Sclerotium rolfsii* Tode) and black point (*B. sorokiniana, Alternaria alternata* (Fr) Keissler, *Curvularia lunata* (Wakker) Boedijn and a species of *Fusarium*. *S. rolfsii,* an omnivorous, soil-borne fungal pathogen, causes disease on a wide range of agricultural and horticultural crops.

Management of this pathogen through conventional method such as physical, cultural etc. is difficult. The use of chemicals has been found effective in controlling fungal diseases of plant, but chemical control using fungicides required large amount which made health hazardous to grower and pollution of environment and soil. Public concern with fungicide residues, as well as pathogen resistance to some pesticides, has increased the need to find alternative methods for protection of crops against the diseases [4, 5]. Thus, biological control using antagonistic microbes alone, or as supplements to minimize the use of chemical pesticides in a system of integrated plant disease management, has become more important in recent years [6]. Many studies have shown that biological control offers an environmentally friendly alternative method for soil-borne pathogens [7, 8]. Vari-

***Corresponding author**
Tel: +88-02-9294065, Fax: +88-02-49261415
E-mail: mifaruk2012@yahoo.com

ous fungal species have been used as biological agents that effectively control plant diseases, and about 90% of such bio-control agents are different strains of *T. harzianum*, *T. virens*, *T. viride* [9]. The effect of *T. harzianum* as bio-agent against *S. rolfsii* and *F. oxysporum* was reported by many investigators [10, 11]. *T. harzianum* has been identified as potential biocontrol agents for the management of various plant diseases including seedling diseases caused by several plant pathogenic fungi [12–14]. *Trichoderma* spp. have been reported to compete for nutrients and space, secrete antifungal compounds, parasitize fungal pathogens, and induce systemic resistance in the host plant [15, 16]. *T. harzianum* is commercially used as a preventive measure for several soil borne plant pathogenic fungi [11, 17]. The mass production of *Trichoderma* on solid substrates promotes the synthesis of enzymes which help in its bio-control mechanism [18].

The utilization of large number of agro-wastes as substrates, use of wide variety of materials, low capital involvement and potential higher volumetric productivity etc. are necessary to promote *Trichoderma*. Many researchers have been used cost effective substrates like wheat bran, rice bran, maize bran, sawdust [19]; rice straw, chickpea bran, grass pea bran, rice course powder, black gram bran [20]; cow dung, poultry manure, ground nut shell, black ash, coir waste, spent straw from mushroom bed, talc, vermiculite [21], sewage sludge compost [22]. So, mass production of *T. harzianum* on comparatively cheap, stable and easily available substrate is essential. Yet information on mass production of *T. harzianum* to control seedling disease of wheat is inadequate under Bangladesh condition. Therefore, the present study was aimed to find out a suitability of solid substrate for mass production of Tricho-compost with *T. harzianum* and also its inocula for seed treatment to reduce foot and root rot disease caused by *S. rolfsii* Sacc. of wheat.

Materials and Methods

The performance of Tricho-inocula (*T. harhianum*), Tricho-composts and Provax 200 WP in controlling foot and root rot disease of wheat caused by *Sclerotium rolfsii* was investigated both in pot culture for one season during 2012–13, and in the field experiments at three cropping seasons during 2013–14, 2014–15 and 2015–16. Previously, seventy two isolates of *T. harzianum* were obtained from different location of Bangladesh and their efficacy was tested against different soil borne pathogens including *S. rolfsii* in the laboratory. Few isolates of *T. harzianum* including TM7 were found more vigorous to suppress the soil borne pathogens including *S. rolfsii*. A pure culture of *T. harzianum* (TM7) was grown in potato dextrose agar (PDA) medium which was used to formulate in the substrates.

Tricho-compost preparation

Isolated *T. harzianum* (TM7) was initially multiplied on substrate containing a mixture of rice bran, wheat bran and mustard oilcake to obtain a formulated *T. harzianum*. The formulated *T. harzianum* was used for mass multiplication in two different mixtures of cow dung based compost materials. One of those composts contained cow dung and rice bran and the other contained a mixture of cow dung, rice bran and poultry manure and these composts were designated as Trico-compost-1 and Trico-compost-2, respectively. The formulated *T. harzianum* was added in between two layers of compost materials and kept for 45–50 days maintaining the moisture content approximately 60–70% for rapid multiplication of *T. harzianum* in the compost materials.

Pathogenic fungal inocula preparation: The pure cultures of the pathogenic fungi *S. rolfsii* was prepared on PDA medium. The inoculum of *S. rolfsii* was multiplied separately on a mixture of wheat bran, khesari bran and mustard oilcake (MOC).

Seed treatment

The *T. harzianum* was cultured in PDA and potato dextrose broth (PDB) media and the spores were harvested from 10 days old culture separately. The seeds of wheat (var. BARI Gom 26) were treated with the spore suspension of *T. harzianum* maintaining the approximate spore concentration of 1×10^8/ml. Similarly another set of seeds were also treated with Provax 200 WP (Carboxin + Thiram37.5%WS) at 2.5 g/kg seeds at the time of sowing.

Pot experiment

The pot experiment was carried out in the pot house of Plant Pathology Division, Bangladesh Agricultural

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Research Institute (BARI), Joydebpur, Gazipur during cropping season of 2012–2013. There were six treatments viz. (i) seed treatment with Provax 200 WP (ii) seed treatment with *Trichoderma* spore suspension-1(spores were harvested from PDA culture and spore concentration approx. 1×10^8/ml) (iii) seed treatment with *Trichoderma* spore suspension-2 (spores were harvested from PDB culture spore concentration approx. 1×10^8/ml) (iv) soil amendment with Tricho-compost-1 (v) soil amendment with Tricho-compost-2 and (vi) untreated control. The pot experiment was conducted in completely randomized design (CRD) with 5 replications. The sterilized pot soil was inoculated with the *S. rolfsii* colonized substrates at 20 g/kg soil. Inoculated soil was incubated for 10 days maintaining proper soil moisture then the soil was amended with Tricho-compost at 100 g/kg soil and kept for 7 days with proper soil moisture. One hundred seeds of wheat var. BARI Gom 26 were sown in each pot (size 20 cm) and allowed to emerge the seedlings under congenial environment in the pot house.

Field experiment

The field trials were conducted in the fields of Plant Pathology Division, BARI, Gazipur during 2013–14, 2014–15 and 2015–16 cropping years. The treatment combination were similar as used in the pot experiment such as (i) Seed treatment with Provax 200 WP (ii) Seed treatment with *Trichoderma* spore suspension-1 (iii) Seed treatment with *Trichoderma* spore suspension-2 (iv) Soil amendment with Tricho-compost-1 (v) Soil amendment with Tricho-compost-2 and (vi) Untreated control. The field experiments were laid out in randomized complete block design (RCBD) with 3 replications. The unit plot size was 2.5 m × 3 m. The field soil was inoculated with *S. rolfsii* colonized substrate consisting of khesari bran, wheat bran and MOC at 100 g/m^2 of soil and allowed the pathogen establishment in the soil for 10 days before seed sowing. The field soil was again treated with the Tricho-compost at 3t/ha and kept for 7 days. The seeds of wheat were sown at 120 kg ha^{-1} in the experimental plots maintaining row to row distance of 20 cm. Proper intercultural operations were done for better growth of wheat in the field. Additional plant protecting chemicals (insecticides or fungicides) were not applied in the field.

Determination of foot and root rot disease

The experimental plot was inspected routinely to observe the foot and root rot disease on plant. In case of complexity to identify the disease, symptoms-bearing plants were collected from the field using polythene bag and brought to the Plant Pathology Laboratory, BARI. From the infected plants, the fungi were isolated following tissue planting methods [23]. After incubation, the fungi that grew over PDA were purified by the hyphal tip culture method. The isolated fungi were identified as *S. rolfsii* according to reference mycology books and manuals [24, 25]. The pure cultures of the fungi were preserved in PDA slants at 4℃ in the refrigerator as stock culture for future use.

Data collection and analysis

Data on different parameters viz., germination, post-emergence seedling mortality, shoot length, root length, shoot weight, root weight, yield of wheat were taken. Data were analysis by using MSTATC program following ANOVA. Treatment means computed using least significant difference (LSD) test.

Results

The effect of *T. harzianum* used as seed treatment by its spore suspension (Tricho-inocula) and its mass culture in compost materials called Tricho-composts for the management of foot and root rot disease of wheat caused by *S. rolfsii* was tested in the plastic tray as well as in the field experiments during the period from 2012–13 to 2015–16. The results of one pot experiment and three field experiments were discussed individually.

Efficacy of Tricho-compost, *T. harzianum* inocula and Provax on seedling emergence, post-emergence seedling mortality and growth of wheat in pot

The results obtained for the plastic tray/soil experiment are given in Table 1. Data showed that foot and root rot disease incidence, seed germination and growth of plants were significantly influenced by the application of *T. harzianum* in soil (Table 1). The maximum seedling mortality (11.96%) was recorded in untreated control tray and comparatively lower seedling mortality was recorded from Tricho-composts, Tricho-inocula and Provax 200 WP treated trays. The reduction of seedling

Table 1. Effect of *T. harzianum* based Tricho-compost on reduction of seedling mortality and growth of wheat under plastic tray culture conditions during 2012–2013.

Treatments	Germination (%)	Seedling mortality (%)	Reduction of seedling mortality over control (%)	Shoot length (cm)	Fresh shoot weight (g/plant^{-1})	Dry shoot weight (g/plant^{-1})
Seed treatment with Provax	91.50	2.52	78.93	40.51 abc	2.69 bc	1.31 c
Seed treatment with Tricho-inocola-1	90.00	4.45	62.79	41.02 bc	2.41 c	1.11 d
Seed treatment with Tricho-inocola-2	82.25	6.07	49.25	39.37c	2.30 cd	1.06 d
Soil amendment with Tricho-compost-1	92.25	2.21	81.52	41.90 ab	3.01b	1.49 b
Soil amendment with Tricho-compost-2	91.00	2.49	79.18	42.91 a	3.46 a	1.66 a
Untreated control	82.00	11.96	-	36.84 d	1.88 d	0.98 d

Values in a column having same letter did not differ significantly ($p = 0.05$) by LSD.

mortality range from 49.25% to 81.52% by the application of different formulations of *T. harzianum* and Provax 200 WP compared to untreated control. The highest shoot length, fresh and dry shoot weight of wheat seedlings were recorded from Tricho-compost-2 treatment followed by Tricho-compost-1, seed treatment with Provax and Tricho-inocula. The lowest shoot length, fresh and dry shoot weight of wheat seedlings were recorded from untreated control.

Effect of Tricho-compost, *T. harzianum* inocula and Provax on the shoot growth and shoot weight of wheat in the field

Shoot height and shoot weight of wheat seedlings were significantly influenced by application of formulated *T. harzianum* and provax in all the years (Table 2). In 2013–14 cropping year, all the treatments gave significantly higher shoot height whereas the lowest shoot height was recorded from untreated control (Table 2). More or less similar results were also observed during 2015–16 cropping year. During 2013–14 cropping year, soil amendment with Tricho-compost-2 gave the highest shoot height followed by Tricho-compost-1, seed treatment with Provax 200 WP and *T. harzianum* inocula and the lowest shoot height was recorded from untreated control. More or less similar trend was also observed in the case of shoot weight during three consecutive cropping years where the highest shoot weight was recorded due to soil amendment with Tricho-compost-2 followed by Tricho-compost-1, seed treatment with Provax 200 WP and *T. harzianum* inocula and the lowest shoot weight was recorded from untreated control (Table 2).

Effect of Tricho-compost, *T. harzianum* inocula and Provax on the root growth and root weight of wheat

Root length and root weight of wheat were significantly increased by soil amendment with different formulation of *T. harzianum* and seed treatment with Provax 200 WP as compared to untreated control during 2013–14, 2014–15 and 2015–16 cropping years (Table 3). Among the treatments, soil amendments with Tricho-

Table 2. Effect of *T. harzianum* on shoot growth of wheat under *Sclerotium rolfsii* inoculated field soils during three consecutive years.

Treatments	Wheat shoot height during three consecutive years (cm)			Wheat shoot weight during three consecutive years (g/plant^{-1})		
	2013-14	2014-15	2015-16	2013-14	2014-15	2015-16
Seed treatment with Provax	50.93 a	51.70 bc	49.67 a	4.40 b	4.68 bc	6.30 bc
Seed treatment with Tricho-inocola-1	51.60 a	44.23 de	49.60 a	4.73 ab	5.27 abc	6.20 c
Seed treatment with Tricho-inocola-2	49.60 a	47 .17 cd	51.07 a	5.07 ab	6.39 abc	7.20 abc
Soil amendment with Tricho-compost-1	52.73 a	54.37 b	48.93 a	5.80 ab	6.20 ab	7.33 ab
Soil amendment with Tricho-compost-2	53.00 a	62.20 a	54.93 a	6.07 a	6.75 a	8.27 a
Untreated control	37.60 b	39.23 e	38.40 b	3.17 c	3.78 c	4.20 d

Values in a column having same letter did not differ significantly ($p = 0.05$) by LSD.

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Table 3. Effect of *T. harzianum* on root growth of wheat under *Sclerotium rolfsii* inoculated field soils during three consecutive years.

Treatments	Wheat root height during three consecutive years (cm)			Wheat root weight during three consecutive years (g/plant^{-1})		
	2013-14	2014-15	2015-16	2013-14	2014-15	2015-16
Seed treatment with Provax	7.47 ab	7.73 bc	8.07 a	1.27 ab	2.23 bc	1.67
Seed treatment with Tricho-inocola-1	7.20 ab	8.11 bc	8.07 a	1.00 ab	2.27 bc	1.67
Seed treatment with Tricho-inocola-2	8.40 ab	9.27 ab	8.47 a	0.97 ab	2.30 bc	1.33
Soil amendment with Tricho-compost-1	8.47 ab	10.10 a	9.20 a	1.17 ab	2.50 ab	1.73
Soil amendment with Tricho-compost-2	10.67 a	10.07 a	9.27 a	1.40 a	2.70 a	1.87
Untreated control	6.27 b	6.70 c	5.20 b	0.68 b	1.90 c	1.07

Values in a column having same letter did not differ significantly ($p = 0.05$) by LSD.

composts were found to be the better for enhancing root length and root weights of wheat over rest of the treatments. Besides, minimum root length and root weight of wheat was observed in untreated control during three consecutive cropping years.

Effect of Tricho-compost, *T. harzianum* inocula and Provax on the Post-Emergence seedling mortality of wheat

Seedling mortality of wheat was sharply reduced by the soil amendment with Tricho-composts, seed treatment with Tricho-inocula and Provax 200 WP (Table 4). The highest seedling mortality 23.00%, 41.00 and 19.67% in the first year, second year and third year, respectively was recorded in the untreated control plot. Lower seedling mortality range from 7.00–14.00% in first year, 11.67–20.67 in second year and 3.67–6.33% in the third year was recorded due to the soil amendment with Tricho-composts and seed treatment with Tricho-inocula and Provax 200 WP. The reduction of seedling mortality was ranged from 39.13–69.56% in first year, 49.59–71.54% in second year and 67.82–81.34% in third years due to various treatments as compared to untreated control.

Effect of Tricho-compost, *T. harzianum* inocula and Provax on the yield of wheat

The yield of wheat was significantly increased due to *T. harzianum* and Provax 200 WP (Table 5). The yield of

Table 4. Effect of *T. harzianum* on the reduction of wheat seedling mortality under *Sclerotium rolfsii* inoculated field soils during three consecutive years.

Treatments	Seedling mortality during three consecutive years (%)			Reduction of seedling mortality over control (%)		
	2013-14	2014-15	2015-16	2013-14	2014-15	2015-16
Seed treatment with Provax	7.00 c (15.36)	15.00 b (22.76)	5.00 b (12.81)	69.56	63.41	74.58
Seed treatment with Tricho-inocola-1	13.00 b (21.15)	20.67 b (26.99)	6.33 b (14.51)	43.48	49.59	67.82
Seed treatment with Tricho-inocola-2	14.00 b (21.95)	18.67 bc (25.52)	6.33 b (14.44)	39.13	54.46	67.82
Soil amendment with Tricho-compost-1	10.00 bc (18.46)	12.67 d (20.82)	3.67 b (11.02)	56.52	69.10	81.34
Soil amendment with Tricho-compost-2	9.00 bc (17.47)	11.67 d (19.87)	5.00 b (11.15)	60.87	71.54	74.58
Untreated Control	23.00 a (28.68)	41.00 a (39.79)	19.67 a (26.26)	-	-	-

Values in a column having same letter did not differ significantly ($p = 0.05$) by LSD; values within the parentheses were the Arcsine Transformed value.

Table 5. Effect of *T. harzianum* on the yield of wheat under *Sclerotium rolfsii* inoculated field soils during three consecutive years.

Treatments	Wheat yield during three consecutive years (t/ha^{-1})			Yield increased over control (%)		
	2013-14	2014-15	2015-16	2013-14	2014-15	2015-16
Seed treatment with Provax	3.67 bc	3.95 ab	4.09 bc	16.08	17.72	24.94
Seed treatment with Tricho-inocola-1	3.50 c	3.78 b	3.99 c	12.00	14.02	23.06
Seed treatment with Tricho-inocola-2	3.42 c	3.82 ab	3.83 c	9.94	14.92	19.84
Soil amendment with Tricho-compost-1	3.83 b	4.31 ab	4.53 ab	19.58	24.59	32.23
Soil amendment with Tricho-compost-2	4.42 a	4.37a	4.74 a	30.32	25.63	35.23
Untreated Control	3.08 d	3.25 c	3.07 d	-	-	-

Values in a column having same letter did not differ significantly ($p = 0.05$) by LSD.

wheat ranged from 3.42 to 4.42 t/ha^{-1} in the first year, 3.78 to 4.37 t/ha^{-1} in the second year and 3.83 to 4.74 t/ha^{-1} in the third year. Among the treatment, soil amendment with Tricho-compost-2 gave the highest yield by 4.42 t/ha^{-1} in the first year, 4.37 t/ha^{-1} in the second year and 4.74 t/ha^{-1} in the third year followed by soil amendment with Tricho-compost-1, seed treatment with Provax 200 WP, seed treatment with Tricho-inocula 2 and Tricho-inocula 1. The lowest yield of wheat was recorded from the untreated control plot by 3.08 t/ha^{-1}, 3.25 t/ha^{-1} and 3.07 t/ha^{-1} in the first year, second year and third year, respectively. Soil amendment with Tricho-compost-2 gave 30.32%, 25.63% and 35.23% higher yield in the 1st year, 2nd year and 3rd year, respectively compared to control where as it was 19.58%, 24.59% and 32.23% in the 1st year, 2nd year and 3rd year, respectively due to application of Tricho-compost-1. Seed treatments with Provax gave 16.08%, 17.72% and 24.94% in the 1st year, 2nd year and 3rd year, respectively higher yield of wheat than control where it was 12.00%, 14.02% and 23.06% in the 1st year, 2nd year and 3rd year, respectively higher by seed treatment with Tricho-inocula 1 compared to control. Therefore, considering the reduction of seedling mortality and enhancing the yield of wheat, the effect of Tricho-composts was seemed to be superior over Tricho-inocula and Provax-200WP.

Discussion

Soil borne plant pathogenic fungi especially *S. rolfsii* causing foot and root rot/seedling blight disease of many crops is a widespread problem and the use of chemicals is hardly successful. However, the high cost associated with the use of fungicides to control this disease is a limiting factor in the profitability of crop production. According to this study, biological control, especially the *Trichoderma* that are common saprophytic fungi found in almost all agricultural soil and rhizosphere micro flora, have been investigated as potential bio-control agents because of their ability to reduce the disease incidence caused by plant pathogenic fungi, particularly many common soil borne plant pathogens [26–31], although some have been occasionally recorded as plant pathogens [32]. The use of *Trichoderma* as a biological agent for the management plant diseases had long been known [33] but its potentiality in Bangladesh agriculture was yet been explored. Therefore, soil amendment with *Trichoderma* based Tricho-composts and seed treatment with *Trichoderma* inocula were evaluated against foot and root rot disease of wheat in the pot house as well as in the field of Plant Pathology Division at BARI, Gazipur, Bangladesh. Results from the studies proved that *T. harzianum* mass cultured on compost materials could be useful for the management of foot and root rot disease of wheat caused by soil borne pathogen *S. rolfsii*.

Synthetic media are costly for mass production of *T. harzianum*. Therefore organic substrates such as rice bran, wheat bran and their integration with mustard oilcake were used for mass production of *T. harzianum* and it is useful for large scale production of *T. harzianum* based compost for soil amendment. Rini and Sulochana [34] reported that locally available organic media viz., coir pith, cow dung, poultry manure and neem cake are the excellent sources of nutrition for antagonistic fungi like *T. harzianum* and *T. viride* Besides, cow dung and neem cake mixture was reported as a recommended practice for field multiplication of *Trichoderma* [35].

Seedling mortality of wheat due to foot and root rot disease caused by *S. rolfsii* was reduced by soil amend-

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ments with Tricho-compost and seed treatment with *Trichoderma* inocula and Provax 200 WP. The findings of Deshmukh and Raut [36], Xu *et al*. [37], Askew and Laing [38], Uzun [39] and Younis [40] indicated that *Trichoderma* isolates potentially reduced the disease caused by phtyopathogenic fungi such as *R. solani, F. oxysporum* and *S. rolfsii*.

The results of the present study clearly indicated that Tricho-compost having potential biological control agent *T. harzianum* enhanced plant growth with higher grain yield of wheat. Several researchers reported that application of *Trichoderma* resulted in higher shoot length, root length, and shoot weight of vegetable seedlings [41–43]. Soil and foliar application of *T. harzianum* reduced the population of soil-borne phytopathogens, especially *S. rolfsii*, *F. oxysporum*, *Rhizoctonia solani* and *S. sclerotiorum* [44, 45]. In the present study inhibition of seedling disease by *Trichoderma* was recorded. Different workers reported the antagonistic activity of *Trichoderma* isolates against different phtyopathogenic fungi such as R. *solani, F. oxysporum* and S. *rolfsii* [36–38]. Different mechanism are said to be involved i.e. competition, production of antibiotics, inhibiting fungal growth by producing volatile and non-volatile compounds as reported by Michrina *et al*. [46] and Calistru *et al*. [47]. Thus it was revealed from the study that soil amendment with Tricho-compost was most effective option for reducing seedling mortality and increasing plant growth as well as for higher yield of wheat. The other options were seed treatment with chemical fungicide Provax-200WP or *T. harzianum* inocula for reducing seedling mortality and higher yield of wheat. This finding is also supported by many researchers [48–50]. Therefore, it may be concluded that soil amendment with Tricho-compost is the best treatment followed by seed treatment with chemical fungicide Provax and seed treatment with *Trichoderma* inocula for reducing seedling disease caused by *S. rolfsii* and increasing plant growth and yield of wheat.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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