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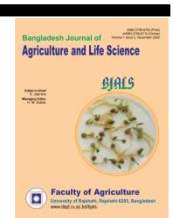

Improvement of growth performance and management of foot and root rot disease of lentil with *Trichoderma* sp. and *Rhizobium* sp.

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ABSTRACT

Microbial inoculants *Trichoderma* sp. and *Rhizobium* sp. were used to investigate their effects in controlling foot and root rot disease of lentil caused by *Rhizoctonia solani*, *Sclerotium rolfsii* and *Fusarium oxysporum* through seed and soil inoculation. In vitro study showed that *T. harzianum* significantly (P< 0.05) inhibited the growth of *R. solani*, *S. rolfsii* and *F. oxysporum.* From the results it reveals that combined seed treatment with *T. harzianum*-TH and *Rhizobium* sp. significantly (P< 0.05) increased the seed germination and decreased seed borne infection of the fungi. The treatments also showed significant (P< 0.05) effect in reducing disease incidence in the field over the control. The highest seedling emergence and the lowest seedling mortality were recorded by the combined application of *T. harzianum*-TH and *Rhizobium* sp. Like-wise the plant shoot length, root length, stem diameter and fresh weight were significantly influenced by the same treatment. The yield and yield contributing characters viz. plant height number of branches plant-1, effective pods plant-1, non-effective pods plant-1, total pods plant-1, normal seeds plant-1, deformed seeds plant-1, total seeds plant-1, 1000-seed weight (g) and grain yield (t ha-1) were also considerably influenced by the combined application of *T. harzianum*-TH and *Rhizobium* sp. From the results it may be concluded that soil inoculation with *T. harzianum*-TH followed by seed treatment with *T. harzianum*-TH + *Rhizobium* sp. was the best combination for combating foot and root rot disease and for enhancing the growth and yield of lentil. However, further investigations should be done to apply the inoculants to control the foot and root rot disease of lentil in farmer level.

Introduction

Lentil (*Lens culinaris*) is the most important major pulse crop in Bangladesh in respect of acreage of production. It is cultivated in about 1.6 million ha of land and its production is about 168837 MT (BBS, 2017). It is also occupied a unique position in the world agriculture. Lentil is an excellent protein source and provides vitamin A, B, fiber, potassium, and iron (Kochhar, 2009). One cup of cooked lentil contains 230 calories, barely a trace of fat and 40 g of total carbohydrates (Sandi and Busch, 2017). It also plays an effective role in human and animal nutrition and improvement of soil fertility (Sarker and Kumar, 2011). The yield and production of lentil in our country

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is comparatively lower than other lentil growing countries in the world. Various causes are accompanied with lower production of lentil in the country among which diseases are the most important one. Among the diseases, foot and root rot of lentil caused by *R. solani*, *S. rolfsii* and *F. oxysporum* are most common and destructive at seedling stage (Dey et al., 1993). Seedling infection at early-stage results in very poor plant stand which ultimately produces very low yield. Control of the soil-borne pathogens *F. oxysporum* and *S. rolfsii* with chemicals is impractical and costly. On the contrary, miscellaneous and long-term use of chemicals has harmful effects on human health, microbial ecosystem and environment (Gerhardson, 2002). In this regard, biological control is advisable for eco-friendly approach for plant protection. The antagonistic fungi *Trichoderma* spp. and *Rhizobium* sp. are correlative and have combined effect in controlling some soil borne pathogens (Hannan et al., 2012). *F. oxysporum* found to have greater susceptibility to biocontrol agents Mycostop and Micostat F in reducing both inoculum density and incidence of disease, however, the commercial formulations may improve Fusarium wilt management as well as increase yields of lentil (Campanella and Miceli, 2021). Considering the above facts, the present research was undertaken to control foot and root rot disease and as well as to improve the growth and yield performances of lentil by applying seed and soil inoculation with *Trichoderma* sp. and *Rhizobium* sp. in order to eco-friendly approaches.

Materials and Methods

Experimental details

The present study was carried out at the Plant Pathology Laboratory and Agronomy Experimental Field, Department of Agronomy and Agricultural Extension, University of Rajshahi, Rajshahi. The experiments were laid out following Randomized Complete Block Design with 3 replications. There were 21 plots of 4 square meters. One lentil variety (BARI Mosur-6) which was collected from Bangladesh Agricultural Development Corporation (BADC), Rajshahi and seven treatments including control were used in the experiment. The treatments were as T1= *Trichoderma harzianum*–BD, T2= *Trichoderma harzianum* -TH, T3= *Rhizobium* sp., T4= *Trichoderma harzianum* -BD + *Rhizobium* sp., T5= *Trichoderma harzianum* -TH + *Rhizobium* sp., T6=Chemical (Provax 200 WP) and T0= Control. Compost based *Trichoderma* spp. was applied to the plots (except control and chemical) before sowing of seeds as per treatment specifications.

Collection of Trichoderma strains and *Rhizobium* sp. and preparation of inoculums

Two Trichoderma strains *T. harzianum*- BD which was collected from BAU, Mymensingh and *T. harzianum*-TH which was collected from Thailand kindly provided by Dr. Jate Sathornkich, Researcher, Horticulture Innovation Lab, Regional Center at Kasetsart University, Thailand (Fig. 1 a and b) and *Rhizobium* sp. was collected from Bangladesh Institute of Nuclear Agriculture (BINA) Laboratory, Mymensingh, Bangladesh (Fig.1 c). *T. harzianum* strains were sub-cultured in the Plant Pathology Laboratory, Department of Agronomy and Agricultural Extension, University of Rajshahi. Previously prepared PDA plates were inoculated with the Trichoderma strains and then the plates were incubated for 25±2ºC. After 7 days of incubation the plates were covered with green conidia.

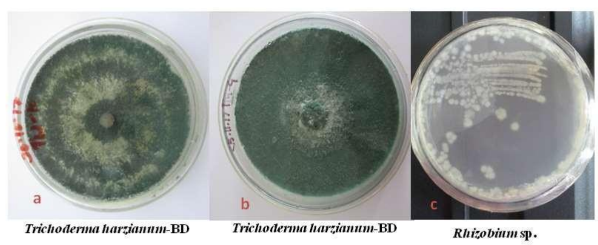

Fig. 1. Photographs showing pure culture plate of a) *T. harzianum*-BD, b) *T. harzianum*-TH and c) *Rhizobium* sp.

The conidia were harvested and finally the spore concentration was adjusted to 1×10^7cfu/mL with distilled water. Peat based Rhizobium inoculum as it was collected from BINA, Mymensingh, Bangladesh used for seed treatment @ 2.5 g/Kg seeds.

Seed treatment

Required quantities of seeds were taken in a beaker and the spore solution ($1x10^7$cfu/mL) of *T. harzianum* BD and *T. harzianum* TH was added separately on it and mixed properly. The treated seeds were dried under laminar air hood at room temperature (25°C). In the similar way the seeds of lentil were also treated with *Rhizobium* sp. inoculum @ 25g/kg (peat soil-based formulation). For combined inoculation, the Trichoderma inoculated seeds were again mixed with *Rhizobium* sp. Provax 200 WP was used for chemical seed treatment @ 0.25% of seed weight. Every case control (no inoculation or chemical treatment) was maintained for comparison of treatments effects.

Table 1. Effect of *Trichoderma* sp. and *Rhizobium* sp. on seed germination and seed borne fungi of BARI Mosur -6

Treatments	% of germination	Germination (%) increase (+) or Decrease (-) over control	% Seed borne fungi (*Aspergillus* sp.)
T_0	**78.67^b**	-	**2.67**
T_1	88.00^a	+10.60	1.33
T_2	88.00^a	+10.60	1.33
T_3	90.67^a	+13.23	0.00
T_4	90.67^a	+13.23	0.00
T_5	92.00^a	+14.49	0.00
T_6	88.00^a	+13.23	0.00
Level of significance	*	-	NS
LSD	6.565	-	-

In column, figure bearing similar letter(s) or without letter are identical and those dissimilar letters differed significantly as per DMRT.*=Significant at 5% level of probability, NS=Not significant, LSD=Least Significant Difference, T_0=Control, T_1=*T. harzianum*-BD, T_2=*T. harzianum*-TH, T_3=*Rhizobium*, T_4=*T. harzianum*-BD + *Rhizobium*, T_5=*T. harzianum*-TH + *Rhizobium*, T_6=Chemical, BD=Bangladesh and TH=Thailand.

Effect of *Trichoderma* sp. and *Rhizobium* sp. on seed germination and detection of seed borne fungi

Blotter method was used for germination test and for detection of seed borne fungi. Germination test was performed following the rules of International Seed Testing Association (ISTA, 1996). Twenty-five seeds of lentil were placed onto two layered moisten filter paper and incubated for 7 days in growth chamber at 250C. The observation started after 3 days of incubation and continued for 7 days for germination count (Fig. 2). Observation was made regularly in order to record infection of different seed borne fungi on seeds. The fungi grown on seed then transferred to Potato Dextrose Agar (PDA) medium in order to get pure culture. The fungi were identified based on their color, spore morphology and mycelia following standard mycological books and manuals (Booth, 1971; Barnett and Hunter, 1972; Singh, 1982).

Soil inoculation with Trichoderma based compost and sowing of seeds

The prepared and harvested Trichoderma inoculums were mixed with distilled water to prepare standard solution (1x10^{7}cfu/mL) and then sprayed the conidial solution to the previously prepared compost. Finally, the Trichoderma inoculums mixed compost was added to the soil of the experimental plots based on treatment specifications (except control and chemical). The treated seeds were sown in each plot by line sowing method. About 695 seeds were sown in each plot following 20×10 cm spacing with 5 cm depth.

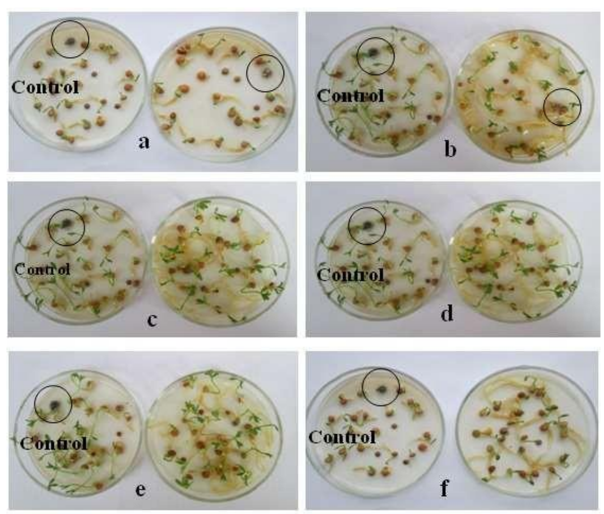

Fig. 2. Effect of *Trichoderma* spp. and *Rhizobium* sp. on seed germination of BARI Mosur-6. a) Control vs *T. harzianum* –BD, b) Control vs *T. harzianum* –TH, c) Control vs *Rhizobium* sp., d) Control vs *T. harzianum* –BD+ *Rhizobium* sp., e) Control vs *T. harzianum* –TH+ *Rhizobium* sp. and f) Control vs chemical.

Table 2. Effect of seed and soil inoculation with *Trichoderma* sp. and *Rhizobium* sp. on foot and root rot disease incidence of BARI Mosur-6

Treatments	**% of foot and root rot disease incidence in the field at different days after sowing (DAS)**					
	15 DAS	22 DAS	29 DAS	36 DAS	43 DAS	50 DAS
T_0	9.10^{a}	13.38^{a}	15.18^{a}	8.96^{a}	4.07^{a}	0.68^{a}
T_1	4.15^{b}	7.03^{b}	9.32^{b}	5.02^{b}	3.01^{ab}	0.20^{b}
T_2	2.95^{bc}	4.52^{cd}	6.47^{cd}	3.20^{c}	1.63^{bc}	0.12^{b}
T_3	3.46^{bc}	5.92^{bc}	7.82^{bc}	4.16^{bc}	2.39^{bc}	0.12^{b}
T_4	3.75^{b}	6.15^{b}	9.05^{b}	4.95^{b}	2.89^{ab}	0.18^{b}
T_5	2.07^{c}	4.09^{d}	6.11^{cd}	2.87^{c}	1.73^{bc}	0.20^{b}
T_6	2.04^{c}	3.92^{d}	5.63^{d}	2.82^{c}	1.09^{c}	0.06^{b}
Level of significance	**	**	**	**	**	*
LSD	1.65	1.60	1.98	1.55	1.39	0.30

In column, figure bearing similar letter(s) or without letter are identical and those dissimilar letters differed significantly as per DMRT. * =Significant at 5% level of probability, **=Significant at 1% level of probability, LSD=Least Significant Difference, T_0=Control, T_1=*T. harzianum*-BD, T_2=*T. harzianum*-TH, T_3=*Rhizobium*, T_4=*T. harzianum*-BD + *Rhizobium*, T_5=*T. harzianum*-TH + *Rhizobium*, T_6=Chemical, BD=Bangladesh and TH=Thailand.

Table 3. Effect of seed and soil inoculation with *Trichoderma* sp. and *Rhizobium* sp. on plant stand of BARI Mosur-6

Treatment	% of plant stand in field	plant stand in field (%) increase (+) or decrease (-) over control
T_0	65.49^{e}	-
T_1	70.07^{d}	+6.53
T_2	71.51^{d}	+13.79
T_3	75.97^{c}	+16.47
T_4	81.15^{b}	+19.29
T_5	86.15^{a}	+23.98
T_6	78.41^{bc}	+8.41
Level of significance	**	-
LSD	3.288	-

In column, figure bearing similar letter(s) or without letter are identical and those dissimilar letters differed significantly as per DMRT. **=Significant at 1% level of probability, LSD=Least Significant Difference, T_0=Control, T_1=*T. harzianum*-BD, T_2=*T. harzianum*- TH, T_3=*Rhizobium*, T_4=*T. harzianum*-BD + *Rhizobium*, T_5=*T. harzianum*-TH + *Rhizobium*, T_6=Chemical, BD=Bangladesh and TH=Thailand.

Table 4. Effect of seed and soil inoculation with *Trichoderma* spp. and *Rhizobium* sp. on some growth characters of BARI Mosur-6 at different days after sowing (DAS)

Treatment	**40 DAS**				**60 DAS**				**80 DAS**			
	Shoot length (cm)	**Root** Length (cm)	**Stem** Diameter (mm)	**Fresh** Weight (g)	**Shoot** length (cm)	**Root** Length (cm)	**Stem** Diameter (mm)	**Fresh** Weight (g)	**Shoot** length (cm)	**Root** Length (cm)	**Stem** Diameter (mm)	**Fresh** Weight (g)
T_0	10.70^{c}	3.10^{b}	3.00^{b}	0.710^{c}	20.42^{c}	5.03^{b}	5.33	2.11^{e}	29.80^{c}	5.76^{c}	6.00^{c}	6.15^{b}
T_1	13.13^{bc}	4.33^{ab}	4.00^{ab}	0.800^{bc}	22.43^{bc}	5.93^{b}	6.00	2.49^{de}	31.73^{bc}	6.63^{bc}	7.00^{bc}	7.20^{ab}
T_2	16.23^{ab}	5.93^{a}	5.00^{a}	1.20^{a}	25.20^{ab}	7.33^{ab}	7.00	3.82^{a}	37.13^{ab}	8.50^{ab}	9.00^{ab}	9.60^{a}
T_3	15.16^{ab}	4.96^{a}	5.00^{a}	0.850^{bc}	23.07^{abc}	6.13^{ab}	6.33	2.95^{cd}	34.63^{abc}	6.70^{bc}	7.00^{bc}	7.00^{ab}
T_4	15.30^{ab}	5.20^{a}	4.50^{a}	0.900^{bc}	24.10^{ab}	6.80^{ab}	6.00	3.12^{bc}	35.80^{abc}	7.50^{ab}	7.00^{bc}	8.80^{ab}
T_5	16.87^{a}	6.00^{a}	4.00^{ab}	1.20^{a}	25.93^{a}	7.83^{a}	7.00	4.01^{a}	39.27^{a}	9.73^{a}	11.00^{a}	10.00^{a}
T_6	15.83^{ab}	5.26^{a}	4.50^{a}	0.967^{ab}	24.83^{ab}	7.00^{ab}	5.66	3.73^{ab}	36.90^{abc}	7.83^{ab}	8.00^{bc}	8.80^{ab}
Level of significance	**	*	*	**	**	*	NS	**	**	*	**	*
LSD	3.284	1.679	1.455	0.2437	2.962	1.727	-	0.6362	7.315	2.353	2.902	3.241

In column, figure bearing similar letter(s) or without letter are identical and those dissimilar letters differed significantly as per DMRT. * =Significant at 5% level of probability, ** =Significant at 1% level of probability, NS= Not significant, LSD= Least Significant Difference, DAS= Days after sowing, T_0=Control, T_1=*T. harzianum*-BD, T_2=*T. harzianum*-TH, T_3=*Rhizobium*, T_4=*T. harzianum*-BD + *Rhizobium*, T_5=*T. harzianum*-TH + *Rhizobium*, T_6=Chemical. BD=Bangladesh and TH=Thailand.

Isolation and identification of *Rhizoctonia solani, Sclerotium rolfsii* and *Fusarium oxysporum* from infected root of lentil plant

The experimental plots were inspected routinely to observe the foot and root rot disease on plant. Infected plants were collected from the experimental field and brought to the Plant Pathology Laboratory, Department of Agronomy and Agricultural Extension, Rajshahi University, Rajshahi. The diseased samples were then washed thoroughly with tap water to remove sand and soil particles. The disease samples were cut into small pieces (1.0cm) along with healthy and dead tissues and surface sterilized with 70% alcohol followed by three times washing with double sterilized distilled water and placed on sterile filter paper to remove excess water adhering to the pieces. Five of such pieces were plated onto Potato Dextrose Agar (PDA) medium. The plates were incubated at room temperature (25oC) for 7 days in alternating cycles of 12 hours darkness and 12 hours light for growth of fungal structures on infected plant tissues. After incubation, the fungi that grew over plant tissues were transferred to PDA plates. The fungus which produced spore/conidia, in that case single spore culture method was followed to obtain pure culture and in other cases fungal tip culture method was followed. The isolated fungi were identified based on morphology, growth characters, colony color, conidia, sclerotia etc. following standard mycological books and manuals (Booth, 1971; Barnett and Hunter, 1972; Singh, 1982) (Fig. 3 a, b and c).

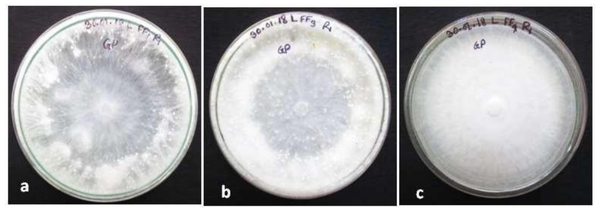

Fig. 3. Foot and root rot causing fungi isolated from infected root of lentil plant, a) *Rhizoctonia solani*, (b) *Sclerotium rolfsii* and (c) *Fusarium oxysporum* on PDA medium.

Table 5. Effect of seed and soil inoculation with *Trichoderma* spp. and *Rhizobium* sp. on yield contributing characters of BARI Mosur-6

Treatment	Plant height (cm)	Number of branches plant^{-1}	Number of effective pod plant^{-1}	Number of non-effective pod plant^{-1}	Number of total pod plant^{-1}	Number of normal seed plant^{-1}	Number of deformed seed plant^{-1}	Number of total seed plant^{-1}	1000 Seed Weight (g)
T_0	45.33^{b}	23.00^{d}	118.0^{e}	12.00	130.0^{e}	202.30^{e}	20.33^{a}	222.70^{e}	18.00^{b}
T_1	47.00^{ab}	28.00^{c}	151.3^{d}	10.33	160.0^{d}	206.70^{e}	18.00^{ab}	225.70^{e}	22.00^{ab}
T_2	49.33^{a}	38.67^{a}	182.3^{b}	7.33	159.7^{d}	286.00^{b}	15.66^{abc}	296.00^{b}	24.00^{ab}
T_3	50.00^{a}	26.67^{cd}	149.3^{d}	8.66	176.7^{c}	210.00^{e}	12.00^{bc}	224.70^{e}	24.00^{ab}
T_4	48.67^{ab}	28.67^{c}	152.0^{d}	9.00	161.0^{d}	239.00^{d}	13.00^{bc}	252.00^{d}	22.33^{ab}
T_5	49.67^{a}	35.67^{ab}	196.0^{a}	7.66	203.7^{a}	300.00^{a}	10.00^{c}	310.00^{a}	24.67^{a}
T_6	46.67^{ab}	33.00^{b}	168.0^{c}	9.00	189.7^{b}	258.00^{c}	13.33^{bc}	270.00^{c}	22.00^{ab}
Level of significance	*	**	**	NS	**	**	*	**	*
LSD	3.847	3.910	7.399	-	9.401	7.794	6.547	12.37	6.278

In column, figure bearing similar letter(s) or without letter are identical and those dissimilar letters differed significantly as per DMRT, * =Significant at 5% level of probability, **=Significant at 1% level of probability, NS=Not significant, LSD=Least Significant Difference, T_0=Control, T_1=*T. harzianum*-BD, T_2=*T. harzianum*-TH, T_3=*Rhizobium*, T_4=*T. harzianum*-BD + *Rhizobium*, T_5=*T. harzianum*- TH + *Rhizobium*, T_6=Chemical, BD=Bangladesh and TH=Thailand.

Inhibition of mycelial growth of foot and root rot fungi by *Trichoderma* sp. in dual culture technique

This study was carried out following dual culture technique (Raihan et al., 2016). on Potato Dextrose Agar (PDA). PDA plates were inoculated with 6 mm block of antagonist (*Trichoderma* sp.) at one side and foot and root rot fungi on the other side. The plates were incubated at room temperature (25°C) for 7 days. After incubation, the distance between inoculums blocks were measured in mm. At the same time control plates were maintained only for pathogen. The pathogens when come in contact with *Trichoderma* sp., the growth of the pathogens was inhibited (Fig. 4). The percent inhibition of the

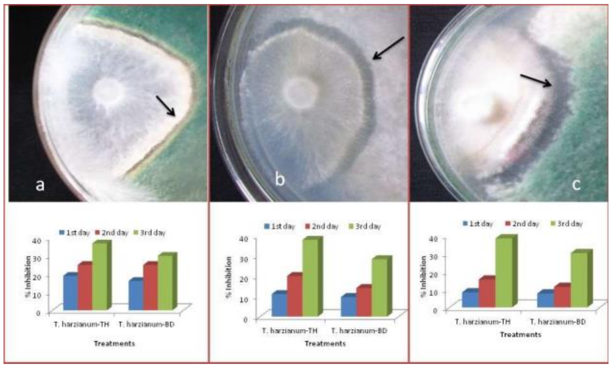

Fig. 4. Growth inhibition caused by *Trichoderma* sp. against (a) R. solani, (b) S. rolfsii and (c) F. oxysporum on PDA medium. Arrow head (black) indicates the inhibition zone employed by *Trichoderma* sp.

pathogenic fungus was calculated by the following formula:

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

Where,

X= Mycelial growth of foot and root rot fungus alone without antagonist (control).

Y= Mycelial growth of foot and root rot fungus along with antagonist.

Determination of disease incidence in the field

The incidence of foot and root rot disease was recorded at 7 days interval viz. 15, 22, 29, 36, 43 and 50 DAS. The seedlings which were found yellow and rotted at the base considered as infected (Fig. 5).

Percentage of plant infection was estimated by following formula:

% of plant infection

$$= \frac{\text{Number of infected plant}}{\text{Total number of plants}} \times 100$$

Recording data on plant growth and yield characters:

The following data was collected as field emergence, shoot length, root length, stem diameter and fresh weight, plant height, number of branches plant-1, effective pods plant-1, non- effective pods plant-1, total pods plant-1, normalseeds plant-1, deformed seeds plant-1, total seeds per plant-1, 1000-seed weight (g) and grain yield (t ha-1). For each treatment, five plants were randomly chosen to collect data on the above-mentioned characters. Grain yield was measured by harvesting the crops grown in one square meter of each plot. After that, the samples were threshed, dried, and weighed with a balance, and the results were stated in t ha-1.

Data analysis

The data recorded were compiled and tabulated for statistically analysis. The collected data were analyzed statistically using the statistical package MSTAT-C program. The mean differences were adjudged by Duncan's Multiple Range Test (Gomez and Gomez, 1984).

Results and Discussion

Effect of single or combined inoculation of *Trichoderma* sp. and *Rhizobium* sp. on seed germination and seed borne fungi of BARI Mosur-6

The single or combined effect of *Trichoderma* sp. and *Rhizobium* sp. on seed germination of lentil (BARI Mosur 6) was evaluated by blotter method in the laboratory which is shown in the Table 1 and Fig. 2. *Trichoderma* sp. alone or in combination with *Rhizobium* sp. showed significant impacts on seed germination of lentil. From the result it is revealed that the highest germination percentage (92.00%) was obtained from the combination of *T. harzianum*-TH+ *Rhizobium* sp. which was statistically identical with other treatments while the lowest germination percentage (78.67%) was recorded from control. This result also supported the findings of Kashem et al. (2014) where they reported that macerated extract of *F. solani* and *T. harzianum* showed the highest seed germination (100%). The seed infection was found to be higher (2.67%) with *Aspergillus* sp. in the control treatment, whereas no infected seedlings were found due to combined treatment with *T. harzianum*-TH+ *Rhizobium* sp. which was statistically identical with *Rhizobium* sp., *T. harzianum*-BD+ *Rhizobium* sp. and chemical treatment. T. harzianum-BD and *T. harzianum*-TH singly also exhibited only 1.33% of seed infection. The present result also supports the findings of Hoque et al. (2015) where minimum number of dead seed (12.00) and seed infection (2.00) due to seed treatment with Rhizobium leguminosarum were occurred.

Inhibition of mycelial growth of *R. solani, S. rolfsii* and *F. oxysporum* by *T. hazianum*

The growth inhibition employed by *T. harzianum* BD and *T. harzianum* TH against *R. solani*, *S. rolfsii* and *F. oxysporum* was measured in dual culture plate and the results are shown in Fig. 4 a, b and c. The highest inhibition zone against *R. solani, S. rolfsii* and *F. oxysporum* was recorded as 36.84%, 37.88% and 38.33% by *T. harzianum*-TH at 3rd day of incubation. Bhuiyan et al. (2012) screened some isolates of *Trichoderma* spp. and found significantly variable antagonism ranging from 65.01% to 83.06% reduction of radial growth of *S. rolfsii* and among the screened antagonists the isolate TH-18 of *T. harzianum* showed the highest inhibition (83.06%). Mandhare and Suryawanshi (2008) also reported that *T. harzianum* and *T. viride* are most efficient in inhibiting the growth of *F. oxysporum* by 60% and 58% respectively while, *T. viride* and *T. virens inhibited* the growth of *S. rolfsii* by 68% and 67% and the growth of *R. bataticola* was inhibited to the extent of 76% by *T. viride* and *T. harzianum.* This finding is in line with our observations.

Fig. 5. Photograph showing the infected (red circle) lentil plants in the field.

Effect of seed and soil inoculation with *Trichoderma* sp. and *Rhizobium* sp. on disease incidence of BARI Mosur-6

From the results it was observed that disease incidence was found to confine within lower limit in those plots which received *Trichoderma* sp. and *Rhizobium* sp. treatment (Table 2 and Fig. 5). At very early stage, the disease incidence was minimum (15 DAS) but after certain period it was increased gradually (at 22 DAS, 29 DAS and 36 DAS). Again, at maturity, the disease incidence was decreased (at 50 DAS). The lowest incidence at 15, 22, 29, 36, 43 and 50 DAS was recorded as 2.047%, 3.920%, 5.633%, 2.823%, 1.097% and 0.06%, respectively in the plots which received by chemical treatment. Like-wise the combined treatment of *T. harzianum*-TH + *Rhizobium* sp. also provided best result which was statistically similar with chemical treatment. Conversely, the highest disease incidence viz. 9.107%, 13.38%, 15.18%, 8.967%, 4.070% and 0.683% was recorded from control treatment respectively. The other treatments such as *T. harzianum*-TH, *T. harzianum*-BD, *Rhizobium* sp. and *T. harzianum*- BD + Rhizobium also provided 0.20%, 0.12%, 0.12% and 0.18% disease incidence (at 50 DAT) over the control group in controlling foot and root rot disease of lentil. Hannan et al. (2013) reported that BINA bio-fertilizer (peat soil-based *R. leguminosarum*) and BAU bio-fungicide (black gram bran-based *T. harzianum*) alone or in combination exhibited compatible effects in controlling the pathogenic fungi *F. oxysporum* and *S. rolfsii*. Khalequzzaman (2016) also reported the lowest foot and root rot disease of pulses due to seed treatment with *T. harzianum* compost (1:5). The present result supports the findings of Rao et al. (1999), they reported that under artificial pathogen pressure in the pot experiment, bacterio- rization of lentil with GPR3, GRP6 and PEN4 reduced the foot and root rot disease.

Effect of *Trichoderma* sp. and *Rhizobium* sp. on plant stand and growth characters of BARI Mosur-6

The plant stands and some growth characters viz. shoot and root length; stem diameter and fresh weight of lentil were studied and tabulated (Table 3, 4). The single or combined effect of *Trichoderma* sp. and *Rhizobium* sp. was recorded on plant stand in the field which is shown in the Table 3. The plant stand of lentil was found to be increased up to 23.98% over the control treatment due to soil inoculation with *T. harzianum*-TH followed by seed treatment with *T. harzianum*-THand *Rhizobium* sp. Kashem et al. (2014) reported that macerated extract of *F. solani* and *T. harzianum* showed the highest seedling emergence (95.73%). The plant stand of lentil was found to be increased up to 16.86% over control in the pots which received the treatment *T. harzianum*-TH + *Rhizobium* sp. Shoot length, root length, stem diameter and fresh weight was recorded at 40, 60 and 80 DAS and the resultshown in the Table 4. At 40 DAS, the highest shoot length (16.87cm), root length (6.00 cm) and fresh weight (1.20 g) was obtained with the application of *T. harzianum*-TH + *Rhizobium* sp. while the lowest result was recorded from control. But the treatment *T. harzianum* -TH was produced highest stem diameter. Like-wise at 60 DAS, the highest shoot length (25.93 cm), root length (7.83 cm) and fresh weight (4.01 g) was obtained with the application of *T. harzianum*-TH + *Rhizobium* sp. as seed treatment followed by soil inoculation with *T. harzianum*-TH. Nonetheless, the lowest result was recorded from control. But the result of stem diameter was not- significant. Similarly, at 80 DAS, *T. harzianum*-TH+ *Rhizobium* sp. showed the highest shoot length (39.27cm), root length (9.73 cm), stem diameter (11.00 mm) and fresh weight (10.00 g) in comparison with control Faruk and Rahman (2016) reported that Tricho-compost (Tricho-compost was prepared with a mixed substrate of cow dung, rice bran and poultry refuse colonized by *T. harzianum*) was found more efficient in the acceleration of plant growth. Hoque et al. (2015) also reported that seed treatment with the bio- control agents showed significant increase in shoot length and root length. The result is in accordance with Sharma et al. (2011), they demonstrated that *Trichoderma* sp. increase root development and proliferation of secondary roots of lentil. In another experiment, Sharma et al. (2011) observed that *Trichoderma* spp. can increase seedling fresh weight of pulses. The, increased biomass production may be due to the increased growth of lentil plant, that was occurred by Trichoderma and Rhizobium.

Effect of *Trichoderma* sp. and *Rhizobium* sp. on the yield contributing characters of BARI Mosur-6

The single or combined effects of *Trichoderma* sp. and *Rhizobium* sp. on the yield contributing characters were observed at the time of harvesting which is given in the Table 5. From the table, it is revealed that the highest plant height (50.00 cm) was produced by the single treatment with *Rhizobium* sp. and the lowest result (45.33 cm) was obtained by control. Lo and Lin (2002) observed that *Trichoderma* spp. significantly increased the plant height of bitter gourd as compared to control. The highest number of branches per plant (38.67) was produced by the *T. harzianum*-TH and the lowest number of branches per plant (23) was recorded by control. Kashem et al. (2014) also obtained the similar result due to application of *T. harzianum*. Gamalero et al. (2009) from their study reported that the Trichoderma strains are plant growth promoting microbes which enhanced plant growth and number of branches per plant. Maximum number of effective pods plant-1(196.0), total pods plant- 1(203.0), normal seeds plant-1 (300.0) total seeds plant-1 (310.0) and 1000-seed weight (24.67g) was produced by the treatment *T. harzianum*-TH + *Rhizobium* sp. Sharma et al. (2011) demonstrated that *Trichoderma* spp. can increase effective pod and crop yield. Knudsen et al. (1995) also reported that the *Trichoderma* spp. increases the number of effective pods plant-1. Sawant et al. (2003) observed that the product of biological control agents (*T. harzianum)* can increase germination, plant growth, pod number and increase yield. Tripathi et al. (2011) reported that *Trichoderma* spp. were found effective against *F. oxysporum*, *R. solani* and *S. rolfsii* and increased number of normal seeds plant-1 in beans. Singh et al. (2014) reported that the pathogenic fungi are soil-borne in nature, hence, seed treatment with botanicals and bio-control agents might be effective to increase total seed and yield of lentil. Knudsen et al. (1995) reported that the *Trichoderma* spp. increases the 1000-grain weight of lentil. This result is in accordance with our observation. Withfew exceptions, most of the cases the yield contribution parameters were positively enhanced due seed treatment with *T. harzianum*-TH + *Rhizobium* sp. along with soil inoculation by *T. harzianum*-TH. In case of number of deformed seeds plant-1, highest number of deformed seeds plant-1 was recorded from the T0 (control) while the treatment T5 (*T. harzianum*-TH + *Rhizobium* sp.) produced the lowest number of deformed seeds plant-1.

Effect of *Trichoderma* sp. and *Rhizobium* sp. on the grain yield (t ha-1) of BARI Mosur-6

Grain yield was varied significantly due to the treatment application which has been shown in the Fig. 6. From the figure, it was found that the combined treatment with *T. harzianum*-TH + *Rhizobium* sp. was produced the highest grainyield (2.35 t ha-1) while the lowest grain yield (1.53 t ha-1) was obtained from control. The grainyield was found to be increased up to 35.02% from the treated plots with same treatment.

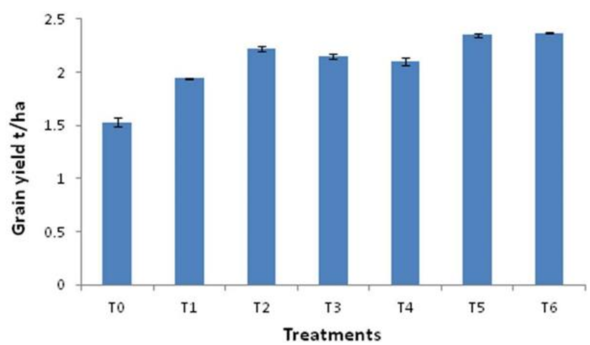

Fig. 6. Effect of *Trichoderma* sp. and *Rhizobium* sp. on yield of BARI Mosur-6. T_0 = Control, T_1 = *T. harzianum*-BD, T_2 = *T. harzianum*-TH, T3 = Rhizobium, T_4= *T. harzianum*-BD + Rhizobium, T_5 = *T. harzianum*-TH + Rhizobium, T_6 = Chemical, BD=Bangladesh and TH = Thailand. Capped bar indicates standard errors of three replications.

which was statistically similar with chemical treatment. Next to this, the highest grain yield (2.22 t ha-1) was obtained from *T. harzianum*-Th which indicated 31.21% increased grain yield over the control. Chowdhury et al. (2005) conducted anexperiment with biological agent *Trichoderma* spp. and they observed that seed treatment with *T. harzianum* either alone or in combination Rhizobium gave the highest grain yield and it was indicated up to 40.98% increased grain yield over control. Hannan et al. (2013) also observed that the BINA bio-fertilizer and BAU bio-fungicide are compatible and have combined effects incontrolling the pathogenic fungi *F. oxysporum* and *S. rolfsii*, which cause the root rot of lentil and eventually increased the growth and yield of lentil.In our study we also observed that soil inoculationwith *T. harzianum*-TH followed by seed treatment with *T. harzianum*-TH and *Rhizobium* sp. produced up to 35.02% increased seed yield over control treatment.

Conclusion

The finding of the research has pointed out that soil inoculation with *T. harzianum*-TH followed by seed treatment with *T. harzianum*-TH and *Rhizobium* sp. would be the most suitable combination for almost all cases and it could be used to control foot and root rot disease and successful cultivation of lentil. In some case chemical also showed good result over the control but use of chemical is impractical and costly. Again, miscellaneous and long-term use of chemicals has harmful effect on human health, microbial ecosystem and environment. Considering the eco-friendly nature of *T. harzianum*-TH and *Rhizobium* sp., it can be suggested to use them for controlling foot and root rot disease of lentil. However, the research needs to be carried out under field condition for consecutive years in different pulse growing areasof Bangladesh to draw a definite conclusion.

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Authors' contributions

Conceptualization, MMH and SK; Methodology, MMH and SK; Investigation, SK and MMH; Data collection and analysis, SK, AA and SS; Draft preparation, SK, AA and SS; Review and editing, MMH.

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