

Formulation of Eco-friendly Management Package Against Seedling Disease Caused by *Sclerotium rolfsii* of Lentil

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Abstract: The experiments were conducted in the fields of Plant Pathology Division, Bangladesh Agricultural Research Institute, Gazipur during 2014-15, 2015-16 and 2016-17 cropping years to observe the effect of formulated *Trichoderma harzianum* (Soil amendment with Tricho-compost and seed treatment with *T. harzianum* spore suspension) and organic soil amendment poultry refuse either singly or in combination with seed treatment with fungicide Provax 200 WP against soil-borne pathogens, *Sclerotium rolfsii* of lentil causing seedling disease. The partially decomposed poultry refuse was incorporated in the 2 weeks before seed sowing of lentil where Tricho-composts were incorporated in the soil 7 days before seed sowing. Seeds were treated with *Trichoderma* spore suspension and Provax 200 WP at the time of seed sowing. From this study it was revealed that soil amendment with Tricho-compost or integration poultry refuse with seed treatment by Provax 200 WP performed as the best treatments in reducing seedling mortality and increasing plant growth and yield of lentil which was significantly differed from the other treatments including control. Seed treatment with chemical fungicide provax showed better performance against the disease also seed treatments with *Trichoderma* spores suspension and soil amendment with poultry refuse which effect at per. Both of them reduced seedling mortality and increased plant growth and yield of lentil.

Keywords: *Trichoderma harzianum*, *Sclerotium rolfsii*, Lens Culinaris, Lentil, Seedling Disease

1. Introduction

Lentil (*Lens culinaris*) is the second most important pulse crop in terms of area (154,000 ha) and production (116,000 t), but it is the first consumed and cheap source of protein for human beings and animals in Bangladesh [1, 2]. It is an integral part of the daily diet as a direct source of protein for human beings in Bangladesh [2]. But the cultivated area and production of lentil is gradually decreasing from the last decades. The average yield of lentil per unit area in Bangladesh is low as compared to that of other lentil growing countries like Syria, Turkey, Canada, USA and Ethiopia [3]. Both biotic and abiotic factors are associated with low yield of lentil in Bangladesh. Among the biotic factors, diseases are considered as the major constraints which attack plants during seedling to maturity stages. Seedling diseases are more destructive and causing 30-40% yield loss in lentil [4, 5]. Seedling diseases caused by soil borne pathogens *Sclerotium rolfsii* and *Fusarium oxysporum* Schlecht

are more destructive pathogens of pulses in almost all legume-growing countries of the world and under congenial conditions resulting up to 100% seedling mortality culminating drastic reduction in grain [5, 6]. There is no effective resistant variety of lentils against this disease. On the other hand management of soil-borne pathogens by chemical fungicides hardly successful in the field and indiscriminate use of chemicals also causes environmental pollution and health hazards [7]. Due to environmental concerns, researchers have focused on finding eco-friendly diseases management alternatives to chemical pesticides for suppression of soil borne plant pathogens [8]. In this context, alternative approaches including crop rotation, use of soil amendment, solarization, bio-fumigation, biological soil disinfestations, grafting, and application of biocontrol agents or organic amendments, such as composts, are of considerable interest among scientists and agricultural producers [9-12]. Numerous studies have shown that biological control offers an environmentally friendly alternative to protect plants from soil-

borne pathogens [13, 14]. Various fungal species are used as biological agents that effectively control plant diseases especially soil borne diseases, and about 90% of such biocontrol agents are different species of *Trichoderma* genus such as *T. harzianum*, *T. virens*, *T. viride* [15]. *T. harzianum* is commercially used as preventive measure for several soil borne plant pathogenic fungi [16, 17]. For mass production of *Trichoderma*, many researchers have successfully used cost effective substrates like wheat bran, rice bran, maize bran, sawdust [18]; rice straw, chickpea bran, grass pea bran, rice coarse powder, black gram bran [19]; cow dung, poultry manure, ground nut shell, black ash, coir waste, spent straw from mushroom bed, talc, vermiculite [20], sewage sludge compost [21]. On the other hand the use of organic amendments such as animal manure, green manure, composts and peats has been proposed, both for conventional and biological systems of agriculture, to improve soil structure and fertility [22-24], and decrease the incidence of disease caused by soil borne pathogens [25, 26]. Therefore, the present study has been designed to formulate eco-friendly disease management packages by using different approaches viz.: *T. harzianum* based Tricho-composts, soil amendment with poultry manure or integration of poultry manure and seed treatment with chemical fungicides for the management of seedling disease of lentil caused by soil borne fungal pathogen *S. rolfsii* Sacc.

2. Materials and Methods

The effect of bio-control agent *T. harzianum* and organic soil amendment poultry manure in controlling seedling disease of lentil caused by *Sclerotium rolfsii* was investigated in the field of plant pathology Division of Bangladesh Agricultural Research Institute at three cropping seasons during 2014-15, 2015-16 and 2016-17. Previously, seventy two isolates of *T. harzianum* were isolated 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 TKC-3 were found more vigorous to suppress the soil borne pathogens including *S. rolfsii*.

2.1. Tricho-compost Preparation

A pure culture of *T. harzianum* (TKC-3) was grown in potato dextrose agar (PDA) medium which was used to formulate in the substrates containing a mixture of rice bran, wheat bran and mustard oilcake. 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. 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 in the compost materials. Based on compost materials used in composting these composts were designated as Tricho-compost-1 and Tricho-compost-2.

2.2. Pathogenic Fungal Inocula Preparation

The pure cultures of the pathogenic fungi *S. rolfsii* was prepared on potato dextrose agar (PDA) medium. The inoculum of *S. rolfsii* was multiplied on a mixture of wheat bran, khesari bran and mustard oilcake (MOC).

2.3. Seed Treatment

The *T. harzianum* was cultured in potato dextrose agar (PDA) medium and the spores were harvested from 10 days old culture. The seeds of lentil (var. BARI Mashur-5) were treated with the spore suspension of *T. harzianum* maintaining the approximate spore concentration of 1×10^8 /ml 6 hrs before seed sowing. After treated the seeds were air dry. Similarly another set of seeds were also treated with seed treating chemical Provax 200 WP @ 2.5 g/kg seeds at the time of seed sowing.

2.4. Field Experiment

The field trials were conducted in the fields of Plant Pathology Division, BARI, Gazipur during 2014-15, 2015-16 and 2016-17 cropping years. There were seven treatments such as (i) Seed treatment with Provax 200 WP @ 2.5 g kg^{-1} (ii) Seed treatment with *Trichoderma* spore suspension @ 1×10^8 spore ml^{-1} (iii) Soil amendment with Tricho-compost-1 @ 3 t ha^{-1} (iv) Soil amendment with Tricho-compost-2 @ 3 t ha^{-1} (v) Soil amendment with poultry refuse @ 5 t ha^{-1} (vi) Seed treatment with Provax 200 WP @ 2.5 g kg^{-1} + Soil amendment with poultry refuse @ 3 t ha^{-1} and (vii) Untreated control. The field experiment was laid out in randomized complete block design (RCBD) with 3 replications. The unit plot size was 3.5 m x 3 m. The field soil was inoculated with *S. rolfsii* colonized substrate consisting of khesari bran, wheat bran and mustard oilcake @ 100 g m^{-2} of soil and allowed the pathogen establishment in the soil for 7 days. Then the inoculated soil was again treated with the Tricho-composts and kept for 5 days for *Trichoderma* establishment. On the other hand the requisite quantity of partially decomposed poultry manure were incorporated in the soil 2 weeks before seed sowing and allowed to decompose properly. The seeds of lentil var. BARI Mashur-5 were sown in the experimental plots maintaining row to row distance of 40 cm. Proper intercultural operations were done for better growth of lentil in the field. No plant protecting chemicals (insecticides or fungicides) were applied in the field.

2.5. Determination of Seedling Disease

The experimental plots were inspected routinely to observe the foot and root rot of lentil disease initiation in the field. In case of any complexity to identify the disease, the infected plants were collected from the field and brought to the laboratory for further study. From the infected plants, the pathogens were isolated following tissue planting methods [27] (Baxter *et al.*, 1999). After incubation, the fungi that grew over potato dextrose agar (PDA) were purified by the hyphal tip culture method. The isolated fungus was identified as *S. rolfsii* according to reference mycology books and manuals [28 and 29]. The pure cultures of the fungi were preserved in PDA slants at 4°C in the refrigerator as stock culture for future use.

2.6. Data Collection and Analysis

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

3. Results

3.1. Seedling Emergence and Pre-emergence Mortality

In every year, soil amendment with Tricho-composts, poultry refuse, seed treatment with Tricho-inocula and Provax 200 WP and integration of poultry refuse with Provax 200 WP gave significantly higher seedling emergence of lentil compared to control (Table 1). In the 1st year, seedling emergence varied

from 92.33-95.67% among the treatments where control gave comparatively lower 74.67% seedling emergence of lentil (Table 1). Similarly, soil amendment with Tricho-composts, poultry refuse, seed treatment with Tricho-inocula, Provax 200 WP and integration poultry refuse with Provax 200 WP gave higher seedling emergence in the 2nd year and 3rd year trials ranged from 76.67-83.33% and 77.33 to 83.33%, respectively and the lowest seedling emergence of 48.33% in 2nd year and 49.33% in 3rd year was recorded in control treatment.

On the contrary, soil and seed treatment with the *Trichoderma* biocontrol agents, organic soil amendment poultry refuse and chemical fungicide Provax caused significant reduction in pre-emergence seedling mortality of lentil compared to control. The range of pre-emergence seedling mortality was 4.33- 7.67% in the 1st year, 16.67- 23.33% in second year and 16.67-22.67% in third year. The corresponding mortality under control was 25.33, 51.67 and 50.67% in first year, second year and third year, respectively (Table 1).

Table 1. Effect of Tricho-composts with *T. harzianum* and poultry refuse on the seedling emergence of lentil during three consecutive years.

Treatment	Average seedling emergence (%)			Pre-emergence seedling mortality (%)		
	2014-15	2015-16	2016-17	2014-15	2015-16	2016-17
Seed treatment with Provax	95.00 (78.10 a)	78.33 a (62.48)	79.00 ab (62.77)	5.00	21.67	21.00
Seed treatment with <i>Trichoderma</i> inocula	95.00 (78.10 a)	77.00 a (61.37)	78.00 b (62.04)	5.00	23.00	22.00
Soil amendments with Tricho-compost-1	95.67 (82.96 a)	79.00 a (62.90)	81.33 ab (64.45)	4.33	21.00	18.67
Soil amendments with Tricho-compost-2	95.67 (78.76 a)	77.00 a (61.37)	80.67 ab (63.96)	4.33	23.00	19.33
Soil amendments with poultry refuse	92.33 (77.54 a)	76.67 a (61.15)	77.33 b (61.58)	7.67	23.33	22.67
Poultry refuse + Seed treatment with Provax	96.00 (79.35 a)	83.33 a (65.95)	83.33 a (65.91)	4.00	16.67	16.67
Control	74.67 (60.01 b)	48.33 b (44.01)	49.33 c (44.62)	25.33	51.67	50.67
LSD	15.61	7.589	2.945	-	-	-

Values in a column having same letter did not differ significantly (P=0.05) by LSD; values within the parenthesis is the Arcsin Transformed value.

3.2. Post-emergence Mortality

Post-emergence seedling mortality due to foot and root rot of lentil was sharply reduced by soil amendment with Tricho-composts, poultry refuse, seed treatment with Tricho-inocula and Provax 200 WP and the integration poultry refuse and Provax 200 WP during three cropping years (Table 2). The highest seedling mortality 25.33%, 45.00% and 43.67% in the first year, second year and third year, respectively was recorded in the untreated control plot. Soil amendment with Tricho-composts, poultry refuse, integration of poultry refuse + seed treatment with Provax 200 WP and seed treatment with Tricho-inocula and Provax 200 WP gave lower seedling

mortality range from 2.67-11.00% in the first year, 12.33-17.67% in the second year and 10.00-19.33% in the third year. The reduction of seedling mortality was from 56.57-89.46% in first year, 60.73-72.60% in second year and 55.74-77.10% in third years due to various treatments as compared to untreated control. Integration of poultry refuse + Provax 200 WP gave the highest reduction of seedling mortality by 89.46% in the 1st year, 72.60% in the 2nd year and 77.10% in the 3rd year followed by soil amendment with Tricho-compost-2, Tricho-compost-1, poultry refuse and seed treatment with Provax 200 WP. Seed treatment with Tricho-inocula was the least effective treatment in reduction of seedling mortality compared to other treatments.

Table 2. Effect of Tricho-composts with *T. harzianum* and poultry refuse on the reduction of seedling disease of lentil during three consecutive years.

Treatment	Average post-emergence seedling mortality due to foot and root rot disease (%)			Reduction of post-emergence seedling mortality than control (%)		
	2014-15	2015-16	2016-17	2014-15	2015-16	2016-17
Seed treatment with Provax	6.33 (14.57 c)	15.00 b (22.60)	13.33 cd (21.39)	75.01	66.67	69.48
Seed treatment with <i>Trichoderma</i> inocula	11.00 (19.36 b)	17.67 b (24.82)	19.33 b (26.06)	56.57	60.73	55.74
Soil amendments with Tricho-compost-1	3.67 (10.76 d)	16.67 b (23.96)	14.67 bcd (22.48)	85.51	62.96	66.41
Soil amendments with Tricho-compost-2	2.67 (9.27 d)	12.67 b (20.67)	12.67 cd (20.84)	89.46	71.84	70.99
Soil amendments with poultry refuse	6.00 (14.39 c)	16.33 b (23.68)	16.67 bc (23.87)	76.31	63.71	61.83
Poultry refuse + Seed treatment with Provax	4.00 (11.48 d)	12.33 b (19.18)	10.00 d (18.42)	84.21	72.60	77.10
Control	25.33 (30.21 a)	45.00 a (42.12)	43.67 a (41.35)	-	-	-
LSD	2.56	6.354	3.979	-	-	-

Values in a column having same letter did not differ significantly (P=0.05) by LSD; values within the parenthesis is the Arcsin Transformed value.

3.3. Shoot Growth

Shoot growth such as shoot length and shoot weight of chickpea were significantly enhanced by different treatments in all the years (Table 3). The lowest shoot length 15.67 cm, 26.50 cm and 28.67 cm in the first year, second year and third year, respectively was recorded under control plot. In first year, integration of poultry refuse + seed treatment with Provax and soil amendment with Tricho-compost-2 gave the higher shoot length of 25.00 cm and 24.93 cm, respectively followed by soil amendment with Tricho-compost-1, poultry refuse and seed treatment with Provax 200 WP and Tricho-inocula. In the second year, integration of poultry refuse + seed treatment with Provax and soil amendment with Tricho-compost-1 and Tricho-compost-2 gave the higher shoot length of 36.40 cm, 35.73 cm and 35.15 cm, respectively followed by seed treatment with Provax 200 WP, soil amendment with poultry refuse and seed treatment with *Trichoderma* inocula where the shoot length was 32.67 cm, 32.27 cm and 31.60 cm, respectively (Table 3). During third year trial, integration of poultry refuse + seed treatment with Provax gave the

highest shoot length of 33.43 cm followed by soil amendment with Tricho-compost-2, Tricho-compost-1 and poultry refuse. Seed treatment with *Trichoderma* inocula was least effective treatment followed by seed treatment with Provax 200 WP. The lowest shoot height was recorded from untreated control in all the years.

Under control treatment the shoot weight of lentil was 1.63, 8.62 and 1.70 gplant⁻¹ in first, second and third year, respectively. Soil amendment and seed treatment with poultry refuse, Tricho-composts, *Trichoderma* inocula and chemical fungicide Provax increased the parameter to 2.20-3.27, 11.67-16.33 and 2.10-3.28 gplant⁻¹ in first, second and third year, respectively. Every year, the shoot weight of lentil was significantly increased due to different treatments compared to control. Among the treatments integration of poultry refuse + Provax 200 WP and soil amendment with Tricho-compost-2 gave the highest shoot weight in all the years followed by soil amendment Tricho-compost-1, poultry refuse alone and seed treatment with Provax 200 WP. The lowest shoot weight was recorded from control treatment in all the years (Table 3).

Table 3. Effect of Tricho-composts with *T. harzianum* and poultry refuse on the shoot growth of lentil during three consecutive years.

Treatment	Average shoot length (cm)			Average shoot weight (gplant ⁻¹)		
	2014-15	2015-16	2016-17	2014-15	2015-16	2016-17
Seed treatment with Provax	20.67 b	32.67 b	26.10 d	2.33 bc	12.67 b	2.23 c
Seed treatment with <i>Trichoderma</i> inocula	20.67 b	31.60 b	24.23 e	2.20 cd	11.67 c	2.10 c
Soil amendments with Tricho-compost-1	21.87 ab	35.73 a	29.63 c	2.47 bc	15.25 a	2.65 b
Soil amendments with Tricho-compost-2	24.93 a	35.15 a	31.81 b	2.93 ab	15.67 a	3.10 a
Soil amendments with poultry refuse	21.07 b	32.27 b	28.63 c	2.47 bc	12.67 b	2.73 b
Poultry refuse + Seed treatment with Provax	25.00 a	36.40 a	33.43 a	3.27 a	16.33 a	3.28 a
Control	13.22 c	23.13 c	19.27 f	1.63 d	8.62 d	1.70 d
LSD	3.058	3.639	1.505	0.59	1.913	0.308

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

3.4. Root Growth

The root length of lentil was significantly lower in the control by 6.40 cm, 6.10 cm and 7.23 cm in the 1st year, 2nd year and 3rd year, respectively compared to integration of poultry refuse + Provax 200 WP, soil amendments with poultry refuse, Tricho-composts and Provax treatments. The root length of lentil under different treatments was increased significantly compared to control which was ranged from

7.67-10.13 cm, 9.17-12.10 cm and 8.20-11.70 cm in the 1st year, 2nd year and 3rd year, respectively (Table 4). In case of root weight significantly higher root weight range from 530-600, 683-927 and 680-980 mgplant⁻¹ in the 1st year, 2nd year and 3rd year, respectively was recorded in the different treatments. The lowest root weight 420, 453 and 520 mgplant⁻¹ in the 1st year, 2nd year and 3rd year, respectively was recorded from control (Table 4).

Table 4. Effect of Tricho-composts with *T. harzianum* and poultry refuse on the root growth of lentil during three consecutive years.

Treatment	Average root length (cm)			Average root weight (mgplant ⁻¹)		
	2014-15	2015-16	2016-17	2014-15	2015-16	2016-17
Seed treatment with Provax	7.67 cd	9.17 b	8.83 de	530 a	683 b	700 d
Seed treatment with <i>Trichoderma</i> inocula	8.60 bc	9.60 b	8.20 e	540 a	750 b	680 d
Soil amendments with Tricho-compost-1	9.20 ab	10.22 b	9.87 bc	600 a	880 a	870 bc
Soil amendments with Tricho-compost-2	10.13 a	11.53 a	10.37 b	570 a	940 a	910 ab
Soil amendments with poultry refuse	9.13 ab	10.20 b	9.37 cd	570 a	687 b	820 c
Poultry refuse + Seed treatment with Provax	10.13 a	12.10 a	11.70 a	600 a	927 a	980 a
Control	6.40 d	6.10 c	7.23 f	420 b	453 c	520 e
LSD	1.355	0.718	0.813	0.079	0.128	0.079

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

3.5. Yield of Lentil

Every year, the yield of lentil was significantly increased by the integration of poultry refuse + Provax 200 WP, soil amendment Tricho-composts, poultry refuse, seed treatment with Provax 200 WP and *Trichoderma* inocula compared to control (Table 5). The lowest yield of lentil was recorded under control by 946, 952 and 1008 kg ha⁻¹ in the first year, second year and third year, respectively (Table 5). The yield was increased significantly ranging from 1238-1670, 1285-1689 and 1308-1705 kg ha⁻¹ in the first year, second year and third year, respectively due to different treatments. Among the treatments, soil amendment with poultry + seed treatment with Provax 200 gave the maximum yield by 1670, 1689 and 1705 kg ha⁻¹ in the 1st year, 2nd year and 3rd year, respectively followed by soil amendment with Tricho-compost-2, Tricho-compost-1, soil amendment with poultry refuse and seed treatment with Provax 200 where the yield was 1663, 1422, 1365 and 1368 kg ha⁻¹ in the 1st year, 1676, 1628, 1492 and

1418 kg ha⁻¹ in the 2nd year and 1605, 1508, 1524 and 1492 kg ha⁻¹ in the 3rd year, respectively. Seed treatment with *Trichoderma* inocula gave lower yield by 1238, 1285 and 1308 kg ha⁻¹ in the first year, second year and third year, respectively compared to other treatments but significantly higher than control. Integration of poultry refuse + Provax 200 gave the maximum 43.35%, 43.64% and 40.88% higher yield in the 1st year, 2nd year and 3rd year, respectively compared to control followed by Tricho-compost-2, Tricho-compost-1, soil amendment with poultry refuse and seed treatment with Provax 200 WP where the yield was 43.11%, 33.47%, 30.70% and 30.85% higher in the first year, 43.20%, 41.52%, 36.19% and 32.86% higher in the 2nd year, 37.20%, 33.16%, 33.85% and 32.44% higher in the 3rd year, respectively compared to control. But seed treatment with *Trichoderma* inocula gave only 23.58%, 25.91% and 22.94% higher yield in the 1st year, 2nd year and 3rd year, respectively compared to control.

Table 5. Effect of Tricho-composts with *T. harzianum* and poultry refuse on the yield of lentil during three consecutive years.

Treatment	Yield (kg ha ⁻¹)			Yield higher than control (%)		
	2014-15	2015-16	2016-17	2014-15	2015-16	2016-17
Seed treatment with Provax	1368 b	1418 b	1492 b	30.85	32.86	32.44
Seed treatment with <i>Trichoderma</i> inocula	1238 b	1285 c	1308 c	23.58	25.91	22.94
Soil amendments with Tricho-compost-1	1422 b	1628 a	1508 b	33.47	41.52	33.16
Soil amendments with Tricho-compost-2	1670 a	1689 a	1605 ab	43.35	43.64	37.20
Soil amendments with poultry refuse	1365 b	1492 b	1524 b	30.70	36.19	33.85
Poultry refuse + Seed treatment with Provax	1663 a	1676 a	1705 a	43.11	43.20	40.88
Control	946 c	952 d	1008 d	-	-	-
LSD	225.1	122.7	149.3	-	-	-

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

4. Discussion

Manure such as poultry manure and compost are organic sources of nutrients that have been shown to increase soil organic matter and improve soil quality [30]. Besides its nutrient components, compost contains high amounts of beneficial organisms that prevent and help controlling soil borne diseases. It has multiple mechanisms of disease suppression: increased plant vigor caused by nutrient availability, presence of large populations of beneficial microorganisms, and increased drainage [31]. On the other hand compost-inhabiting microorganisms such as *Trichoderma* spp. produce plant growth hormones and chemical compounds (e.g. siderophores, tannins, phenols) which are antagonistic to various soilborne pathogens. The soil borne plant pathogenic fungi *S. rolfii* and *Fusarium* causing seedling mortality and wilt diseases of many crops and are the widespread problem for crop production. The management of these diseases by using chemicals is hardly successful. In the present study, compost with antagonistic fungi *T. harzianum* called Tricho-composts, poultry refuse, seed treatment with *Trichoderma* spore suspension and Provax 200 WP as well as integration of poultry refuse with Provax 200 WP were evaluated against foot and root rot disease of lentil in the field during three consecutive years.

Results came out from the studies showed that integration of poultry refuse with Provax 200 WP and soil treatment with Tricho-compost suppressed foot and root rot disease caused by soil borne pathogens *S. rolfii*, increasing plant growth and yield of lentil. The use of organic amendments such as animal manure, green manure (the incorporation of crop residues into the soil), composts and peats has been improved soil structure and fertility [22-24], and decrease the incidence of disease caused by soil borne pathogens [25, 26]. Numerous studies have indicated that several established biocontrol agents, including strains from the genera *Bacillus*, *Pseudomonas* and *Trichoderma* can suppress vascular or soil borne fungal pathogens [32-35]. Fungi belonging to the genus *Trichoderma* and bacteria such as *Pseudomonas* spp, or *Bacillus subtilis*, are the most promising biocontrol agents [35]. On the one hand, they stimulate plant growth, while on the other they eliminate plant pathogens by their unique antimicrobial activities, including the production of antibiotics and toxins to compete with pathogenic organisms [36]. Uzun [37] and Younis [38] also reported that *Trichoderma* isolates potentially reduced the disease caused by phytopathogenic fungi such as *R. solani*, *F. oxysporum* and *S. rolfii*.

Combination of different methods of pest control is at the heart of integrated pest management, and may result in

either additive or synergistic effect. The expected benefit of this strategy is improved and sustainable control of pests and diseases. The goal of IPM methods is to employ measures that are more efficient, healthier, and more environmentally friendly in the long run, and to reduce the amount of pesticides used [39]. The results from the present study clearly indicated that integration of poultry refuse with chemical fungicide Provax 200 WP or Tricho-compost having biological control agent *T. harzianum* provided effective protection measure against seedling diseases of lentil and also caused plant growth promotion with higher grain yield of lentil. The use of biocontrol agents such as *Trichoderma* spp and organic soil amendment in combination with other control methods, e.g. chemical fungicides, steam disinfection and soil heating or solarization has provided an effective control of soil borne pathogens and have the potential to improve soil properties, plant health and yield [40-43]. Several workers also reported that the antagonistic activity of different *Trichoderma* isolates against various phytopathogenic fungi such as *R. solani*, *F. oxysporum* and *S. rolfsii* and enhanced plant growth parameter such as shoot height, root length, and shoot weight [44-49]. Ristaino [50] also reported that organic soil amendments are effective against soil borne pathogen and enhanced the yield of the crop.

Therefore, it may be concluded that integration of poultry manure as a soil amendment with chemical fungicide Provax 200 WP as a seed treatment or soil amendment with Tricho-composts (compost having biological control agent *T. harzianum*) is the best treatment for management of seedling disease and increasing plant growth and yield of lentil.

5. Conclusion

Seedling disease of lentil caused by soil borne pathogen *Sclerotium rolfsii* is one of the most common and prevalent disease which affects seed germination and seedling survival as well as yield in the field. In this study the formulated bio-control agent *Trichoderma harzianum* based Tricho-compost, *Trichoderma* spore suspension called *Trichoderma* inocula, organic soil amendment poultry refuse and chemical fungicide Provax 200 WP were tested against the disease in the field. The findings of the present investigations revealed that integration of poultry refuse as a soil amendment with chemical fungicide Provax 200 WP as a seed treatment or soil amendment with the formulated *T. harzianum* based Tricho-compost is the best treatment for management of seedling disease and increasing yield of lentil under *S. rolfsii* inoculated field experiments in Bangladesh. Soil amendment with only poultry refuses or seed treatment with chemical fungicide also better treatment against the disease and also increasing yield of lentil.

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Conflict of Interest Statement

The authors whose name is listed immediately below certify that they have NO affiliation with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

References

- [1] BBS. 2011. Bangladesh Bureau of Statistics. Ministry of Planning. Dhaka, Bangladesh. 126.
- [2] Sattar MA Podder AR, Chandra MC., Rahman M. 1996. The most promising BNF technology for green legume production in Bangladesh. BNF Association, Dhaka, BD. 28, Nov, 1994 pp. 15-20.
- [3] Hossain I, Khan MAI, Podder AK. 1999. Seed treatment with Rhizobium in controlling *Fusarium oxysporum* and *Sclerotium rolfsii* for biomass and seed production of lentil (*Lens culinaris* M.). Bangladesh J. Environ. Sci. 5: 61-64.
- [4] Fakir GA. 1983. Status of research on pulse disease at the BAU, Department of Plant Pathology BAU, Mymensingh.
- [5] Begum F. 2003. Integrated control of seedling mortality of lentil caused by *Sclerotium rolfsii*. MS thesis submitted to the Department of Plant Pathology, Bangladesh Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh.
- [6] Ahmed HU. 1985. Disease problems of pulse and oil seed crops in Bangladesh. A paper presented in the 1st National Phytopathology. Conf. BARI Gazipur.
- [7] Gerhardson B. 2002. Biological substitutes for pesticides. Trends Biotechnol. 20: 338-343.
- [8] Larkin RP, Roberts D., Gracia-Garza JA. 1998. Biological control of fungal diseases. In *Fungicidal activity, chemical and biological approaches*. (pp 141- 191). New York, NY: Wiley.
- [9] Kirkegaard JA, Sarwar M, Wong PTW, Mead A, Howe G, Newell M. 2000. Field studies on the biofumigation of take-all by Brassica break crops. *Australian Journal of Agricultural Research*, 51 (4), 445-456. doi: 10.1071/AR99106.
- [10] Ryckeboer J. 2001. *Biowaste and yard waste composts: Microbiological and hygienic aspects: Suppressiveness to plant diseases*. Katholieke Universiteit Leuven, Faculteit Landbouwkundige en Toegepaste, Biologische Wetenschappen, Laboratorium voor Fytopathologie en Plantenbescherming.

- [11] Bailey KL, Lazarovits G. 2003. Suppressing soilborne diseases with residue management and organic amendments. *Soil and Tillage Research*, 72 (2), 169-180.
- [12] Louws FJ, Rivard CL, Kubota C. 2010. Grafting fruiting vegetables to manage soilborne pathogens, foliar pathogens, arthropods and weeds. *Scientia Horticulturae*, 127 (2), 127-146.
- [13] Harman GE. 2011. Multifunctional fungal plant symbionts: new tools to enhance plant growth and Productivity. *New Phytologist Commentry, Forum* (3): 647-649.
- [14] Singh BN, Singh A, Singh SP, Singh HB. 2011. *Trichoderma harzianum*-mediated reprogramming of oxidative stress response in root apoplast of sunflower enhances defense against *Rhizoctonia solani*. *European Journal of Plant Pathology* 131 (1): 121-134.
- [15] Benitez T, Rincon AM, Limon MC, Codon AC. 2004. Biocontrol mechanisms of *Trichoderma* strains, a review article. *Intl. Microbiol.* 7: 249-260.
- [16] Harman GE. 2006. Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathology*, 96: 190-194.
- [17] Shalini KP, Lata Narayan, Kotasthane AS. 2006. Genetic relatedness among *Trichoderma* isolates inhibiting a pathogenic fungi *Rhizoctonia solani*, *African Journal of Biotechnology*, 5 (8): 580-584.
- [18] Das BC, Roy SK, Bora LC. 1997. Mass multiplication of *Trichoderma* species on different media. *J. Agril. Sci. Society of North East India*. 10 (1): 95-100.
- [19] Shamsuzzaman, Islam SMA, Hossain I. 2003. *Trichoderma* culture and germination of sweet gourd seed. *Bangladesh J. Seed Sci. and Tech.* 7 (1 and 2): 91-95.
- [20] Retinassababady C, Ramadoss N. 2000. Effect of different substrates on the growth and sporulation of *Trichoderma viride* native isolates. *Agril. Sci. Digest*. 20 (3): 150-152.
- [21] Cotxarrera L, Trillas-Gay MI, Steinberg C, Alabouvette C. 2002. Use of sewage sludge compost and *Trichoderma asperellum* isolates to suppress *Fusarium* wilt of tomato. *Soil Biology and Biochemistry*, 34, 467-476.
- [22] Magid J, Henriksen O, Thorup-Kristensen K, Mueller T. 2001. Disproportionately high N-mineralisation rates from green manures at low temperatures – implications for modelling and management in cool temperate agro-ecosystems. *Plant and Soil* 228: 73-82.
- [23] Conklin AE, Erich MS, Liebman M, Lambert D, Gallandt ER, Halteman WA. 2002. Effects of red clover (*Trifolium pratense*) green manure and compost soil amendments on wild mustard (*Brassica kaber*) growth and incidence of disease. *Plant and Soil* 238: 245-256.
- [24] Cavigelli MA, Thien SJ. 2003. Phosphorus bioavailability following incorporation of green manure crops. *Soil Science Society American Journal* 67: 1186-1194.
- [25] Litterick AM, Harrier L, Wallace P, Watson CA, Wood M. 2004. The role of uncomposted materials, composts, manures, and compost extracts in reducing pest and disease incidence and severity in sustainable temperate agricultural and horticultural crop production: A review. *Critical Reviews in Plant Sciences* 23: 453-479.
- [26] Noble R, Coventry E. 2005. Suppression of soil-borne plant diseases with composts: a review. *Biocontrol Science and Technology* 15: 3-20.
- [27] Baxter AP, Rong IH, Roux C, Van der Linde EJ. 1999. Collecting and Preserving Fungi-A Manual for Mycology. Plant Protection Research Institute. Private Bag X134, Pretoria, 0001 South Africa.
- [28] Barnett HL, Hunter BB. 1972. Illustrated Genera of Imperfect Fungi. 3rd Ed. Burges Co., Minneapolis, USA.
- [29] Booth C. 1971. The Genus *Fusarium*. Commonwealth Mycology Institute Kew, Surrey, England.
- [30] Wright RJ., (Ed.) 1998. *Agricultural uses of municipal, animal, and industrial byproducts*. USDA. Agricultural Research Service, Conservation Research Report, (44). Retrieved from http://agrienvarchive.ca/bioenergy/download/ag_use_ars.pdf
- [31] Mehta CM, Palni U, Franke-Whittle IH, Sharma AK. 2014. Compost: Its role, mechanism and impact on reducing soil-borne plant diseases. *Waste Management*, 34 (3), 607-22. pmid 24373678.
- [32] Ramette A, Frapolli M, Defago G, Moenne-Loccoz Y. 2003. Phylogeny of HCN synthase-encoding hcnBC genes in biocontrol fluorescent pseudomonads and its relationship with host plant species and HCN synthesis ability. *Molecular Plant-Microbe Interactions*, 16 (6), 525-35. pmid: 12795378.
- [33] Berg G, Kurze S, Buchner A, Wellington EM, Smalla K. 2000. Successful strategy for the selection of new strawberry-associated rhizobacteria antagonistic to *Verticillium* wilt. *Canadian Journal of Microbiology*, 46 (12), 1128-37. pmid: 11142403.
- [34] Mavrodi OV, Walter N, Elateek S, Taylor CG, Okubara PA. 2012. Suppression of *Rhizoctonia* and *Pythium* root rot of wheat by new strains of *Pseudomonas*. *Biological Control*, 62 (2), 93-102.
- [35] Bhattacharjee R, Dey U. 2014. An overview of fungal and bacterial biopesticides to control plant pathogens/diseases. *African Journal of Microbiology Research*, 8 (17), 1749-1762. doi 10.5897/AJMR2013.6356.
- [36] Mukry SN, Ahmad A, Khan SA. 2010. Screening and partial characterization of hemolysins from *Bacillus* sp.: Strain S128 & S144 are hemolysin B (HBL) producers. *Pakistan Journal of Botany*, 42 (1), 463-472.
- [37] Uzun I. 2004. Use of spent mushroom compost in sustainable fruit production. *Journal of Fruit and Ornamental Plant Research*. 12: 157-165.
- [38] Younis NA. 2005. Mycoparasitism of *Trichoderma harzianum* and *Trichoderma longibrachiatum* on *Fusarium oxysporum* f.sp. *phaseoli* the causal of bean wilt disease. *Bull. Faculty Agric. Cairo Univ.* 56: 201-219.
- [39] Katan J. 1999. The methyl bromide issue: Problems and potential solutions. *Journal of Plant Pathology*, 81, 153-159. doi 10.4454/jpp.v81i3.1071.
- [40] Omar I, O'Neill TM, Rossall S. 2006. Biological control of fusarium crown and root rot of tomato with antagonistic bacteria and integrated control when combined with the fungicide carbendazim. *Plant Pathology*, 55 (1), 92-99. doi: 10.1111/j.1365-3059.2005.01315.x.

- [41] Klein E, Katan J, Austerweil M, Gamliel A. 2007. Controlled laboratory system to study soil solarization and organic amendment effects on plant pathogens. *Phytopathology*, 97 (11), 1476-1483. pmid: 18943518.
- [42] Gamliel A, Katan J. 2009. Control of plant disease through soil solarization. In D. Walters (Ed.), *Disease Control in Crops*. (pp 196-220). Edinburgh, UK: Wiley-Blackwell Publishing Ltd.
- [43] Slusarski C, Ciesielska J, Malusa E, Meszka B, Sobiczewski P. 2012. Metam sodium, metam potassium and dazomet. In *Sustainable use of chemical fumigants for the control of soil-borne pathogens in the horticultural sector*. Skierniewice, Poland: Research Institute of Horticulture.
- [44] Deshmukh PP, Raut JG. 1992. Antagonism by *Trichoderma* spp. on five plant pathogenic fungi. *New Agriculturist*. 3 (2): 127-130.
- [45] Xu T, Zhong JP, Li DB. 1993. Antagonism of *T. harzianum* T82 and *Trichoderma* species NF9 against soil and seed borne pathogens. *Acta. Phytopathol. Ca. Scinica*, 23 (1) 63-67.
- [46] Askew DJ, Laing MD. 1994. The in-vitro screening of *Trichoderma* isolates for antagonism to *Rhizoctonia solani* and an evaluation of different environmental sites of *Trichoderma* as sources of aggressive strains. *Plant and Soil* 159 (2): 227-281.
- [47] Hossain I, Shamsuzzaman SM. 2003. Developing *Trichoderma* based bio-fungicide using agro-waste. *BAU Res. Progr.* 14: 49-50.
- [48] Hossain I., Naznin M. H. A. 2005. BAU biofungicide in controlling seedling disease of some summer vegetables. *BAU Res. Progr.* 15: 32-35.
- [49] Shaban WI, El-Bramawy MA. 2011. Impact of dual inoculation with *Rhizobium* and *Trichoderma* on damping off, root rot diseases and plant growth parameters of some legumes field crop under greenhouse conditions. *Int. Res. J. Agric. Sci. Soil Sci.* 1: 98-108.
- [50] Ristaino JB. 2002. Effect of synthetic and organic soil fertility amendments on southern blight, soil microbial communities, and yield of processing tomatoes. *Phytopathology* 92: 181-189.