EVALUATION OF ORGANIC AND VERMI-COMPOST BASED *TRICHODERMA HARZIANUM* FORMULATIONS AGAINST *SCLEROTIUM ROLFSII* CAUSING FOOT AND ROOT ROT DISEASE OF LENTIL

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ABSTRACT

Faruk, M. I., Islam, M. M., Akhter, M.S., Rahman, M. S., Islam, R. and Jahan, K. 2022. Evaluation of organic and vermi-compost based *Trichoderma harzianum* formulations against *Sclerotium rolfsii* causing foot and root rot disease of lentil. Bangladesh J. Plant Pathol. 38(1&2): 1-8.

Lentil (*Lens culinaris*), an important pulse crop suffers from the devastating foot and root rot disease caused by soil borne pathogens *Sclerotium rolsfii* and *Fusarium oxysporum*. The efficacy of *Trichoderma harzianum* containing Tricho-organic-compost and Tricho-vermin-compost and seed treating fungicide Provax 200 WP was evaluated under field conditions during three consecutive years to control foot and root rot disease (*S. rolfsii* and *F. oxysporum*) of lentil. Soil amendment with Tricho-organic-compost and Tricho-vermi-compost gave substantial reduction of the foot and root rot disease of lentil. In addition to disease control, they enhanced plant growth and increased grain yield of lentil. The Tricho-organic-compost and Tricho-vermi-compost were found to be suitable alternatives to chemical fungicides for the management of foot and root rot disease of lentil. Seed treatment with chemical fungicide Provax 200 WP also reduced the foot and root rot disease of lentil.

Key word: Lentil, *Lens culinaris, Trichoderma harzianum*, *Sclerotium rolfsii*, Tricho-organic-compost, Tricho-vermin-compost

INTRODUCTION

Lentil (*Lens culinaris*) is the important pulse crop in terms of area (156,000 ha) and production (177,000 t), and it is an integral part of the daily diet and cheap source of protein for human being in Bangladesh (BBS 2019). The cultivated area of lentil is gradually decreasing and average yield of lentil per unit area is low as compared to other lentil growing countries like Syria, Turkey, Canada, USA and Ethiopia (Hossain *et al.* 1999). There are various reasons including biotic factors 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 (Begum 2003). Seedling diseases caused by soil borne pathogens *Sclerotium rolfsii* and *Fusarium oxysporum* Schlecht are more destructive to almost all legumes grown in different countries of the world and under congenial conditions resulting up to 100% seedling mortality culminating drastic reduction in grain (Begum 2003). Effective resistant varieties of lentils against this disease are not available. Management of soil-borne pathogens by chemical fungicides is rarely successful in the field and indiscriminate use of chemicals also causes environmental pollution and health hazards. In this context, alternative approaches including crop rotation, use of soil amendment, solarization, bio-fumigation, biological soil disinfestations, and application of biocontrol agents or organic amendments, such as composts, are of considerable interest among scientists and agricultural producers (Louws *et al*. 2010). Numerous studies have shown that biological control offers an environmentally friendly alternative to protect plants from soil-borne pathogens (Harman 2011). Various fungal species are used as biological agents and among them about 90% of such biocontrol agents are different species of

Trichoderma fungus such as *T. harzianum*, *T. virens*, *T. viride*. *T. harzianum* is commercially used as preventive measure for several soil borne plant pathogenic fungi (Harman 2006). The biocontrol activity of *Trichoderma* is of immense importance not only to agriculture and its crops but also to the environment as it does not accumulate in the food chain and thus does no harm to the plants, animals and humans. A major limitation of biocontrol by *Trichoderma* strains is the large scale production of inoculum. Many researchers have successfully used cost effective substrates like wheat bran, rice bran, maize bran, sawdust, rice straw, chickpea bran, grass pea bran, cow dung, poultry manure, ground nut shell, black ash, coir waste, talc, and vermiculite for large scale production of *Trichoderma* (Shamsuzzaman *et al*. 2003). Organic amendments such as animal manure, green manure, composts and peats decreased the incidence of disease caused by soil borne pathogens (Noble and Coventry 2005). Therefore, the present study has been designed to evaluate available organic compost and vermin-compost for mass production *T. harzianum* for the management of seedling disease of lentil caused by soil borne fungal pathogen *S. rolfsii* Sacc and *F. oxysporum*.

MATERIALS AND METHODS

The effect of formulated *T. harzianum* using two different composts viz. organic compost and vermi-compost designated as Tricho-organic-compost and Tricho-vermi-compost in controlling seedling disease of lentil caused by *S. rolfsii* and *F. oxysporum* was investigated in the field of Plant Pathology Division of Bangladesh Agricultural Research Institute during three consecutive cropping seasons. Out of seventy-two isolates of *T. harzianum* tested against different soil borne pathogens including *S. rolfsii*, few isolates of *T. harzianum* including TMP-3 were found more vigorous to suppress the soil borne pathogens *S. rolfsii* and *F. oxysporum*.

In-vitro preparation of Tricho-organic-compost and Tricho-vermi-compost

The pure culture of *T. harzianum* (TKC-3) was grown in potato dextrose agar (PDA) medium. The culture 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 composts viz. organic compost and vermin-compost. The formulated *T. harzianum* was properly mixed with organic compost and vermin-compost and kept under the shed for 7-10 days for multiplication of *Trichoderma* in the mixture. Based on the materials used in composting these composts were designated as Tricho-organic-compost and Tricho-vermi-compost.

In-vitro preparation of pathogenic fungal inocula

The foot and root rot disease infected plant samples of lentil were collected from the field and brought to the laboratory for isolation and identification of the pathogen following tissue planting methods (Baxter *et al*. 1999). The isolated fungi were identified as *Sclerotium rolfsii* and *Fusarium oxysporum* according to reference mycology books and manuals (Barnett and Hunter 1972, Booth 1971). The fungi that grew on potato dextrose agar (PDA) were purified by the hyphal tip culture method. The pure cultures of the pathogenic fungi *S. rolfsii* and *F. oxysporum* were multiplied on a mixture of wheat bran, khesari bran and mustard oilcake (MOC).

Field evaluation of Tricho-composts against seedling disease of lentil

The experiment was conducted in the field of Plant Pathology Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur using randomized complete block design with three replications, where the unit plot size was 2m x 3m. There were 6 treatments viz. (i) Seed treatment with Provax 200 WP @ 2.5 g/kg seed, (ii) Soil amendment with organic compost @ 3 t/ha, (iii) Soil amendment with Tricho-organic compost @ 3 t/ha, (iv) Soil amendment with vermi-compost @ 3 t/ha, (v) Soil amendment with Tricho-vermi-compost @ 3 t/ha, and (vi) Untreated control. The field soil was inoculated with *S. rolfsii* and *F. oxysporum* inocula colonized in the substrate consisting of khesari bran, wheat bran and mustard oilcake @ 100g/m^2 of soil and allowed the pathogen to establish in the soil for 7 days. Then the inoculated soil was again treated with organic compost, vermin-compost, Tricho-organic-compost and Tricho-vermi-compost applied in the soil @ 3 t/ha and properly mixed with the soils and kept for 5 days for *Trichoderma* establishment in the soil. Before sowing the seeds were treated with

Provax @ 2.5 g/kg seeds. The seeds of lentil variety BARI Mashur-5 were sown in the experimental plots maintaining 40cm row to row distance. Proper intercultural operations were done for better growth of lentil in the field. No other plant protecting chemicals such as insecticides or fungicides were applied in the field. The experimental plots were routinely inspected to observe the initiation of foot and root rot disease of lentil The infected plant samples were collected from the field and brought to the laboratory for isolation and identification of the pathogen following tissue planting methods (Baxter *et al*. 1999). Data were collected on seedling mortality, shoot height, shoot weight, root length, root weight and seed yield of lentil. The data collection on seedling disease incidence was started with the time of disease appearance and it was continued until 40 days of seedling age. Plant growth parameters viz. shoot height, shoot weight, root length, root weight were recorded at 35-40 days of seedling age. The percent data were converted into arcsine transformation values before statistical analysis. Data were analyzed statistically by using the MSTATC program. The treatment effects were compared by applying the least significant different (LSD) test at P=0.05 level.

RESULTS AND DISCUSSION

Seedling emergence and pre-emergence mortality of lentil

Soil amendment with Tricho-vermi-compost, Tricho-organic-compost, vermi-compost, organic-compost and seed treatment with Provax 200 WP gave significantly higher seedling emergence of lentil compared to control (Table 1). In the 1st year, seedling emergence varied from 79.67-82.67% among the treatments whereas control gave comparatively lower 59% seedling emergence of lentil (Table 1). Similarly, Tricho-vermi-compost, Tricho-organic-compost, vermi-compost, organic compost and seed treatment with Provax 200 WP gave significantly higher seedling emergence ranging from 71.67-85% in 2nd year and 77.33- 83.33% in 3rd year and the corresponding lowest seedling emergence of 46.67% and 49.33% was recorded from the control plot. On the contrary, soil amendment with Tricho-vermi-compost, Tricho-organic-compost, vermi-compost, organic compost and seed treatment with Provax 200 WP caused significant reduction in pre-emergence seedling mortality of lentil compared to control. The range of pre-emergence seedling mortality was 17.33- 20.33% in the 1st year, 15-28.33% in second year and 16.67-22.67% in third year and the corresponding mortality under control plot was 41.00, 53.33 and 50.67%, respectively (Table 1).

Post-emergence seedling mortality of lentil

Post-emergence seedling mortality of lentil due to foot and root rot disease was sharply reduced by soil amendment with Tricho-vermi-compost, Tricho-organic-compost, vermi-compost, organic compost and seed treatment with Provax 200 WP during three cropping years (Table 2). The highest seedling mortality of 44.67%, 55% and 41.67% was recorded from the control plots during the three consecutive years. Soil amendment with Tricho-vermi-compost, Tricho-organic-compost, vermi-compost, organic compost and seed treatment with Provax 200 WP gave significantly lower seedling mortality ranging from 10.33-17% in first year, 11-22.33% in second year and 14.33-23.33% in third year trial. The reduction of seedling mortality was 61.94-76.87% in first year, 59.40-80% in second year and 44.01-65.61% in third years due to various treatments as compared to untreated control. Soil amendment with Tricho-vermi-compost gave the highest reduction of seedling mortality of 76.87%, 80%, and 65.61% during three consecutive years. Besides, soil amendment with Tricho-organic-compost, seed treatment with Provax 200 WP, soil amendment with vermin-compost and organic-compost also gave significant reduction of seedling mortality of lentil every year.

Shoot growth of lentil

Shoot growth of lentil was significantly enhanced by different treatments in all the years (Table 3). The lowest shoot length 23.20 cm, 23.13 cm and 27.13 cm in three consecutive years was observed in the control plots. In first year, soil amendment with Tricho-organic-compost and Tricho-vermi-compost gave higher shoot length of 39.13 cm and 38.77 cm, respectively followed by soil amendment with vermin-compost, organic compost and seed treatment with Provax 200 WP where the shoot length was 35.10 cm, 34.73 cm and 32.27 cm, respectively.

Table 1. Effect of *Trichoderma* based Tricho-organic-compost and Tricho-vermi-compost on shoot growth of lentil

Treatments	Shoot length (cm)			Shoot weight (gmplant^{-1})		
	2017-18	2018-19	2019-20	2017-18	2018-19	2019-20
Seed treatment with Provax	32.27 b	32.67 b	35.87 ab	11.77 c	11.67 c	14.65 b
Soil amendments with organic compost	34.73 b	31.60 b	33.53 c	12.63 b	11.67 c	11.24 c
Soil amendments with Tricho-organic-compost	39.13 a	35.73 a	37.20 a	15.25 a	15.67 a	16.27 a
Soil amendments with vermi-compost	35.10 b	32.27 b	34.87 bc	12.80 b	12.67 b	12.47 c
Soil amendments with Tricho-vermi-compost	38.77 a	36.40 a	37.80 a	15.67 a	16.33 a	16.35 a
Control	23.20 c	23.13 c	27.13 d	8.62 d	8.62 d	8.35 d
LSD (P=0.05)	2.74	3.639	2.096	0.835	1.913	1.509

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

Table 2. Effect of *Trichoderma* based Tricho-organic-compost and Tricho-vermi-compost on root growth of lentil

Treatments	Root length (cm)			Root weight (mgplant^{-1})		
	2017-18	2018-19	2019-20	2017-18	2018-19	2019-20
Seed treatment with Provax	9.30 b	9.17 b	9.80 c	850 b	683 b	686.7 b
Soil amendments with organic compost	10.00 b	9.60 b	10.13 bc	880 b	750 b	706.7 b
Soil amendments with Tricho-organic-compost	12.20 a	11.53 a	11.33 a	1050 a	940 a	800.0 a
Soil amendments with vermi-compost	9.73 b	10.20 b	10.07 c	850 b	687 b	726.7 b
Soil amendments with Tricho-vermi-compost	12.47 a	12.10 a	11.13 ab	1100 a	927 a	820.0 a
Control	6.50 c	6.10 c	4.47 d	520 c	453 c	500.0 c
LSD (P=0.05)	0.688	0.718	1.029	0.081	0.128	49.71

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

In the second year, soil amendment with Tricho-vermi-compost and Tricho-organic-compost gave the higher shoot length of 36.40 cm and 35.73 cm, respectively followed by seed treatment with Provax 200 WP, soil amendment with vermin-compost and organic compost where the shoot length was 32.67 cm, 32.27cm and 31.60 cm, respectively (Table 3).

During third year trial, soil amendment with Tricho-vermi-compost and Tricho-organic-compost gave the higher shoot length followed by seed treatment with Provax 200 WP. In the 3rd year trial, soil amendment with organic compost was least effective treatment followed by soil amendment with organic compost. The lowest shoot height was recorded from untreated

control in all the years. The shoot weight of lentil in control plot was 8.26, 8.62 and 8.35 gmplant^{-1}, respectively. Soil amendment with Tricho-vermi-compost, Tricho-organic-compost, vermin-compost, organic compost and seed treatment with Provax 200 WP increased the shoot weight of 11.77-15.67, 11.67-16.33 and 11.24-16.35 gmplant^{-1}, respectively during three years study. Every year, the shoot weight of lentil was significantly increased due to different treatments compared to control. Among the treatments soil amendments with Tricho-vermi-compost and Tricho-organic-compost gave the higher shoot weight in all the years followed by soil amendment with vermin-compost, organic compost and seed treatment with Provax 200 WP. The lowest shoot weight was recorded from control treatment in all the years (Table 3).

Root growth of lentil

The root length of lentil was significantly lower in the control by 6.50 cm, 6.10 cm and 4.47 cm in the 1st year, 2nd year and 3rd year, respectively. The root length of lentil under different treatments was increased significantly compared to control which was ranged from 9.30-12.47 cm, 9.17-12.10 cm and 9.80-11.33 cm in the 1st year, 2nd year and 3rd year, respectively (Table 4). Similarly root weight of lentil was significantly higher ranging from 850-1100, 683-927, and 686.7-820 mgplant^{-1} in the corresponding years due to different treatments. The lowest root weight of lentil was recorded from the control plots (Table 4).

Yield of lentil

The seed yield of lentil was significantly increased by soil amendment with Tricho-vermi-compost, Tricho-organic-compost, vermin-compost, organic compost and seed treatment with Provax 200 WP compared to control (Table 5). The lowest yield of lentil was recorded under control treatment by 1001, 1000 and 1017 kgha^{-1} during three years trial (Table 5). The yield was increased significantly ranging from 1554-1812, 1511-1850 and 1539-1779 kgha^{-1} in the first year, second year and third year, respectively due to different treatments. Among the treatments, soil amendment with Tricho-vermi-compost and Tricho-organic-compost, gave the maximum yield by 1812 and 1783 kgha^{-1} in the 1st year, 1797 and 1850 kgha^{-1} in the 2nd year and 1779 and 1761 kgha^{-1} in the 3rd year, respectively followed by soil amendment with organic compost, vermin-compost and seed treatment with Provax 200 WP.

Table 3. Effect of *Trichoderma* based Tricho-organic-compost and Tricho-vermi-compost on seed germination and pre-emergence seedling mortality of lentil

Treatments	Seed Germination (%)			Pre-emergence seedling mortality (%)		
	2017-18	2018-19	2019-20	2017-18	2018-19	2019-20
Seed treatment with Provax	80.33 a (63.69)	78.33 a (62.29)	83.33 a (65.91)	19.67	21.67	16.67
Soil amendments with organic compost	79.67 a (63.41)	71.67 a (57.87)	77.33 b (61.58)	20.33	28.33	22.67
Soil amendments with Tricho-organic-compost	80.67 a (64.03)	83.33 a (66.14)	80.67 ab (63.96)	19.33	16.67	19.33
Soil amendments with vermi-compost	80.00 a (63.45)	75.00 a (60.07)	78.00 b (62.04)	20.00	25.00	22.00
Soil amendments with Tricho-vermi-compost	82.67 a (65.45)	85.00 a (67.40)	81.33 ab (64.45)	17.33	15.00	18.67
Control	59.00 b (50.20)	46.67 b (42.99)	49.33 c (44.62)	41.00	53.33	50.67
LSD (P=0.05)	4.195	9.582	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.

Table 4. Effect of *Trichoderma* based Tricho-organic-compost and Tricho-vermi-compost on the seedling mortality of lentil

Treatments	Post emergence seedling mortality (%)			Reduction of post emergence seedling mortality (%)		
	2017-18	2018-19	2019-20	2017-18	2018-19	2019-20
Seed treatment with Provax	14.00 bc (21.88)	16.00 cd (23.49)	16.00 c (23.56)	68.66	70.91	61.60
Soil amendments with organic compost	17.00 b (24.31)	18.00 bc (25.07)	23.33 b (28.86)	61.94	67.27	44.01
Soil amendments with Tricho-organic-compost	12.33 c (20.51)	12.00 de (20.22)	16.00 c (23.49)	72.40	78.18	61.60
Soil amendments with vermi-compost	14.33 bc (22.24)	22.33 b (28.18)	17.67 c (24.73)	67.92	59.40	57.59
Soil amendments with Tricho-vermi-compost	10.33 c (18.74)	11.00 e (19.32)	14.33 c (22.24)	76.87	80.00	65.61
Control	44.67 a (41.94)	55.00 a (47.88)	41.67 a (40.20)	-	-	-
LSD (P=0.05)	-	3.639	3.343	-	-	-

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.

Soil amendment with Tricho-vermi-compost gave 44.76% higher yield compared to control followed by soil amendment with Tricho-organic-compost, organic compost, vermin-compost and seed treatment with Provax 200 WP. In the 2nd year, soil amendment with Tricho-organic-compost gave 45.95% higher yield compared to control followed by soil amendment with Tricho-vermi-compost, organic compost, seed treatment with Provax 200 WP and soil amendment with vermin-compost. Similarly in 3rd year soil amendment with Tricho-vermi-compost gave the maximum 42.83% higher yield compared to control followed by soil amendment with Tricho-organic-compost, seed treatment with Provax 200 WP, vermin-compost and soil amendment with organic compost. The soil borne plant pathogenic fungi *S. rolfsii* and *Fusarium* causing seedling mortality and wilt diseases of many crops were the widespread problem for crop production. Two composts viz. organic-compost and vermi-compost were used for mass culturing of bio-control agent *T. harzianum* and its effects on foot and root rot disease caused by soil borne plant pathogenic fungi *S. rolfsii* and *Fusarium* of lentil was evaluated. The Tricho-organic-compost and Tricho-vermi-compost played significant role on germination of seeds, post-emergence death of plants, shoot and root length, shoot and root weight as well as yield of lentil. Post-emergence seedling mortality of lentil due to foot rot and root rot disease caused by *S. rolfsii* was found to be reduced by the soil amendment with Tricho-organic-compost and Tricho-vermi-compost.

The results were confirmed by other workers in their separate studies on suitability of compost based formulated *Trichoderma harzianum* in management soil borne pathogens (Najar *et. al*. 2016). The use of organic amendments such as animal manure, green manure, composts and peats decreased the incidence of disease caused by soil borne pathogens (Noble and Coventry 2005). Fungi belonging to the genus *Trichoderma* and bacteria such as *Pseudomonas* spp., or *Bacillus subtilis,* were the most promising biocontrol agents (Bhattacharjee and Dey 2014). They stimulate plant growth and eliminate plant pathogens by their unique antimicrobial activities, including the production of antibiotics and toxins to compete with pathogenic organisms (Mukry *et al*. 2010). The strong antagonistic activity of different *Trichoderma* isolates against the pathogenic fungi *R. solani, F. oxysporum* and *S. rolfsii* was reported and that enhanced plant growth such as shoot height, root length, shoot weight and crop yield (Shaban and El-Bramawy 2011).

Table 5. Effect of Trichoderma based Tricho-organic-compost and Tricho-vermi-compost on grain yield of lentil

Treatments	Yield (kgha^{-1})			Yield increased over control (%)		
	2017-18	2018-19	2019-20	2017-18	2018-19	2019-20
Seed treatment with Provax	1554 b	1574 b	1623 ab	35.58	36.47	37.34
Soil amendments with organic compost	1633 b	1589 b	1539 b	38.70	37.07	33.92
Soil amendments with Tricho-organic-compost	1783 a	1850 a	1761 a	43.86	45.95	42.25
Soil amendments with vermi-compost	1605 b	1511 b	1563 b	37.63	33.82	34.93
Soil amendments with Tricho-vermi-compost	1812 a	1797 a	1779 a	44.76	44.35	42.83
Control	1001c	1000 c	1017 c	-	-	-
LSD (P=0.05)	123.0	149.7	182.6	-	-	-

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

CONCLUSION

The soil amendment with organic and vermi composts-based formulation of antagonistic fungi *Trichoderma harzianum* viz. Tricho-organic-compost and Tricho-vermi-compost were seemed to be the alternative to chemical fungicides for management of foot and root rot disease and thereby increasing grain yield of lentil.

ACKNOWLEDGEMENT

The authors thankfully acknowledge Bangladesh Agricultural Research Institute, Gazipur to provide financial support and logistic support. Thanks go to Mr. Md. Abdur Razzak and Mr. Zamil Akter (Scientific Assistant) for their sincere assistance in this research work.

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