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USE OF TRICHO-COMPOST AND TRICHO-LEACHATE FOR MANAGEMENT OF SOIL-BORNE PATHOGENS AND PRODUCTION OF HEALTHY CABBAGE SEEDLINGS

M. S. NAHAR[1], M. A. RAHMAN[2], M. G. KIBRIA[3]
A. N. M. REZAUL KARIM[4] AND S. A. MILLER[5]

Abstract

Tricho-compost, a *Trichoderma* based compost fertilizer, was developed by mixing a definite concentration of spore suspension of a *Trichoderma harzianum* strain with measured amounts of processed raw materials, such as cowdung, poultry refuse, water hyacinth, vegetable wastes, sawdust, maize bran, and molasses. Tricho-leachate, a liquid by-product of the Tricho-compost, was obtained during decomposition of Tricho-compost materials. These bio-products were tested both in the laboratory and in seedbed nurseries to evaluate their effectiveness against soil-borne pathogens for growing cabbage seedlings. Application of Tricho-compost and Tricho-leachate reduced the seedling mortalities of cabbage caused by *Sclerotium rolfsii* by about 98%. In laboratory tests, *Trichoderma harzianum*, after re-isolation from Tricho-compost and tricho-leachate, was also found to be highly effective to arrest the growth of *S. rolfsii*. *T. harzianum* destroyed the radial growth of *S. rolfsii* mycelium by 59.7% after five days, and effected total destruction of the mycelium in 10 days. In seedbed nurseries, soil applications of Tricho-compost and Tricho-leachate significantly increased the seedling germination rate and reduced the incidence of soil-borne diseases and infestation of root-knot nematodes. Field experiment showed that combined application of Tricho-compost and Tricho-leachate reduced the seedling mortalities by 40.9% to 64.5% in Gazipur and 53.3% to 62.1% in Bogra. Application of Tricho-leachate at 500 ml per sq. metre increased plant weight by about 55.6%, and reduced the seedling mortality by about 84.0% in Gazipur. Seedbed nurseries treated with Tricho-compost and Tricho-leachate had only *Pythium* spp as a soil-borne pathogen, whereas the control plot had as many as four soil-borne pathogens -*Pythium, Rhizoctonia, Sclerotium* and *Fusarium* spp. Use of richo-compost and Tricho-leachate also reduced the infestation of root-knot nematode by about 80.7% to 91.0%. The results clearly showed that use of Tricho-compost and Tricho-leachate is highly effective for production of healthy cabbage seedlings.

Keywords: Cabbage seedling, tricho-compost, tricho-leachate, root-knot nematode, *Sclerotium rolfsii*.

[1] Principal Scientific Officer, [2]Chief Scientific Officer, [3]Senior Scientific Officer, Horticulture Research Centre, Bangladesh Agricultural Research Institute (BARI), Gazipur 1701, Bangladesh; [4]Site Coordinator, IPM-CRSP/Virginia Tech., USA; and [5]Professor, Department of Plant Pathology, The Ohio State University, USA, (Corresponding author: E-mail: nahar321@yahoo.com)

Introduction

Composts have the potential to provide biological control of various disease pathogens, particularly the soil-borne pathogens through microbial actions (Hoitink and Fay, 1986, Inbar *et al.,* 1993).

Liquid bio-products commonly referred to as bio-fertilizers contain living micro-organisms that influence the soil ecosystems and produce supplementary substances for plant growth (Parr *et al.,* 2002). However, the species of the micro-organisms and their quantity may vary depending on the source of cultures and raw materials used for producing the product. Presently, different types of bio-products are available and their quality differs mainly due to the kinds of raw materials used, forms of utilization, and the sources of micro-organisms (DOAE, 2003; Higa and Parr, 1994). The bio-products that are available in liquid form have effective micro-organisms (EM) which include filamentous fungi, lactic acid bacteria, and other soil bacteria (Setboonsarng and Gilman, 1999). Compost extracts considered as bio-fertilizers have been found to enhance plant growth and to suppress pathogens (Gharib *et al*., 2008; Naidu *el al*., 2010).

Effects of the *Trichoderma* species in promoting seedling establishment, enhancement of plant growth and elicitation of plant defense reaction in some vegetable crops have been examined by a number of reaerchers (Celar and Valic 2005, Rabeerdran *et al.,* 2000; Inbar *et al.,* 1994; Lynch *et al.,* 1991; Hoyos-Carvajal *et al.,* 2009). However, due to lack of suitable carriers for large scale production, poultry refuse was tested and was found to be a suitable medium for growing *Trichoderma harzianum* (Anon., 2006). Additionally, combined application of poultry refuse and *Trichoderma harzianum* was found to be highly effective to control soil-borne diseases in seedbed nurseries (Anon., 2007). Since information on the effects of Tricho-compost and Tricho-leachate on seedling production and control of soil-borne diseases are not available, studies were conducted to assess the efficacy of the two bio-products in controlling soil-borne disease pathogens including root-knot nematode in the laboratory as well as in seedbed nurseries for producing healthy seedlings of cabbage.

Materials and Method

The isolate of *Trichoderma harzianum* used to prepare Tricho-compost and Tricho-leachate was tested for its effectiveness to control soil borne pathogens in the laboratory and in seed bed nurseries and the following experiments were conducted for this purpose.

Expt. 1. Tricho-compost and Tricho-leachate production by using *T. harzianum*

Tricho-compost was produced at Gabtoli of Bogra by using the procedure of house system as described by Ilias *et al.* (2005). Raw materials of Tricho-compost, such as cowdung, poultry refuse, water hyacinth, vegetable waste, sawdust, maize bran, and molasses were collected from nearby areas. Water-hyacinth was chopped into small pieces. All the raw materials were mixed in a definite proportion. The spore suspension of *Trichoderma harzianum* (3 x 10^7) was drenched onto the processed raw materials, and loaded them in layers in a Tricho-compost production house that measured 10ft in length x 5 ft in width x 4.5 ft in height. Tricho-leachate, the liquid by-product of the Tricho-compost, was collected during the decomposition of Tricho-compost materials. Chemical compositions of both Tricho-compost and Tricho-leachate were analyzed in the laboratory.

Expt. 2. Tests for effectiveness of Tricho-compost and Tricho-leachate against *Sclerotium rolfsii*.

The experiment was conducted in tin trays (75 cm x 50 cm x 20 cm) with four replications using a completely randomized design during August-September 2009. The treatments were (a) Soil incorporation of Tricho-compost @ 50 g/kg soil, (b) Soil incorporation of Tricho-leachate @ 50 ml/kg of soil, and (c) Soil incorporation of cowdung @ 200 g/kg soil (control treatment). Before application of the treatments, the soils in the tin trays were inoculated with *S. rolfsii* grown on rice husk @ 20 g/kg. All the treatments were added 15 days before seed sowing. After applications of the treatments, the soils of the tin trays were adequately moistened with water for providing enough moisture for seed germination as well as for activating the *Trichoderma* spores. The soils of the tin trays receiving Tricho-leachate applications were covered with polyethylene sheet. One hundred seeds of cabbage (variety BARI Cabbage-1) were sown in each tray. Data were recorded after 25 days of seedling germination, while the data on disease incidence were recorded regularly from initiation of seed germination.

Expt. 3. Re-isolation of T. harzianum from Tricho-compost and Tricho-leachate and tests of its effectiveness against Sclerotium rolfsii on PDA plate in the laboratory

Two mg each of Tricho-compost and Tricho-leachate was mixed with sterilized Richards solution (glucose media) and allowed to shake at 40 rpm for 10 days. After 10 days, the mycelia that grew in Richards solution were carefully separated, and a small amount was re-cultured on a PDA plate. After three days, the pure culture was repeatedly transferred 2-3 times to regain its original from.

The tests for effectiveness of *T. harzianum* derived from Tricho-compost and Tricho-leachate were studied against the soil-borne pathogen *Sclerotium rolfsii* by assessing the inhibition zones using 'Dual Culture Technique' (Rao, 2003). Two assessments were made following the dual culture technique. The first was to obtain the percentage inhibition of radial growth (PIRG) of *S. roflsii*; and the second one was the number of days taken for the *T. harzianum* isolates for totally destroying the *S. roflsii* colony. Sterilized PDA was poured into petri dishes at 20 mL per plate. Each plate was then seeded with 5mm diameter agar disc cut from the edge of an actively growing pure culture of *S. roflsii*, and placed at the circumference of a 8.5 cm diameter PDA culture plate. Similarly, a 5mm disc was taken from the edge of a 4-day old pure culture of each *T. harzianum* isolates and placed at the periphery (0.5cm) on the opposite side of the same petri dish. In case of the control plate, only *S. rolfsii* was placed in a similar manner without *T. harzianum* on a fresh petri dish. Plates were repeated at least four times under room temperatures of 28±2ºC. The results were recorded as the mean colony growth of the causal pathogen in presence of the antagonistic pathogen, and its comparative growth on the control plate. The outcome of two readings was incorporated into the formula (Skidmore and Dickinson, 1976) for calculating the percentage of inhibition of radial growth (PIRG) as below:

$$PIRG = \frac{R_1 - R_2}{R_1} X \ 100$$

where, PIRG = percentage inhibition of radial growth; R_1 = radial growth of *S. rolfsii* in absence of the antagonist (control); R_2 = radial growth of *S. rolfsii* in presence of the antagonist.

Expt. 4. Use of Tricho-compost and Tricho-leachate for producing cabbage seedlings

In order to control the soil-borne diseases in seedbed nurseries, two experiments were conducted, one at BARI farm, Gazipur and the other at OFRD farm in Sheujgari of Bogra during the summer season of 2010.

(a) Experiment at BARI Farm, Gazipur: Seedlings of cabbage (variety Summer Warrior) were raised in seedbed nursery at BARI farm (Gazipur) by using different doses of Tricho-compost and Tricho-leachate for management of the soil borne diseases. The following four treatments were used: T_1= Soil incorporation of Tricho-compost @150g + Tricho-leachate @100 mL per sq. metre; T_2= Soil incorporation of Tricho-compost @100g + Tricho-leachate @ 150 mL per sq. metre; T_3= soil incorporation of Tricho-leachate @ 500 mL per sq. metre; and T_4 (control) = Soil incorporation of cowdung 2 kg + TSP 25 g per sq. metre. The seedbed size was 3.5 sq. metre. Cabbage seeds were sown on 01 August 2010. The treatments were laid out in a completely randomized block design with three replications. Tricho-compost and Tricho-leachate were used 12 days before seed sowing. After applications of Tricho-compost and Tricho-leachate, the seedbeds were covered with polyethylene sheets. In the control treatment, cowdung and TSP were used one day before seed sowing. Two gms of cabbage seeds (about 1620-1650 seeds) were sown in each seedbed. The initial germination rate of the cabbage seeds was 96%. Fifty seedlings of one-month old were sampled for determining the fresh and dry weights of the seedlings.

(b) Experiment at OFRD farm in Sheujgari of Bogra: The experiment was conducted with the following three treatments in four replications using a completely randomized block design: T_1= Soil incorporation of Tricho-compost @150g + Tricho-leachate 100 mL per sq. metre; T_2= Soil incorporation of Tricho-compost @100g + Tricho-leachate @ 150 mL per sq. metre; and T_3= Soil incorporation of cowdung 2 kg + TSP 25 g per sq. metre. The cabbage seeds were sown on July 19, 2010. The cabbage variety, plot size and cultural practices were same as used at BARI farm in Gazipur. Data on disease incidence and seedling growth were monitored regularly and recorded at appropriate times.

Results and Discussion

Results

Expt. 1. Tricho-compost and Tricho-leachate production by using *T. harzianum*

Two and half tons of Tricho-compost were produced in 45 days after full decomposition of five tons of raw materials. At the same time as much as 200 litres of Tricho-leachate were collected as by-product of the Tricho-compost. Analyses of chemical compositions of both Tricho-compost and Tricho-leachate show that Tricho-compost contained as much as 20% organic carbon, and considerable amount of 11 different nutrient elements -N-1.2%, P- 0.61, K-0.77%, S-0.24%), Ca-1.71%, Mg-0.4%, B-0.01%, Cu-0.01%, Fe-0.12%, Mn-0.026%, and Zn-0.02%. Tricho-leachate, on the other hand, contained 2.05% organic carbon, and five different nutrient elements- N-0.01%, P-0.02%, K-0.42%, S-0.10%, and Z_n-0.003%. The results of the chemical analysis clearly show that Tricho-compost is rich in various nutrients. In addition, *Trichoderma harzianum*, which is present in Tricho-compost can effectively control various pathogens including root-knot nematode.

Expt. 2. Effectiveness of Tricho-compost and Tricho-leachate against S. rolfsii in the tray

Applications of both Tricho-compost and Tricho-leachate significantly reduced the pre- and post- emergence mortalities of cabbage seedlings caused by *S. rolfsii*; seedling mortalities were reduced by as much as 98%. The growth of the seedlings due to Tricho-leachate treatment as measured by seedling weight was lower than that in the control treatment (use of cowdung) indicating that the application rate of Tricho-leachate was probably inadequate (Table 1).

(a) Experiment at BARI Farm, Gazipur: Soil borne pathogens, such as *Pythium*, *Rhizoctonia*, and *Fusarium* were identified in the seedbed nurseries. *Pythium* was the only pathogen recorded in Tricho-compost and Trico-leachate treatments, while *Pythium* (4%), *Rhizoctonia* (5%) and *Fusarium* (3%) were recorded in the control treatment (Table 2).

Table 1. Effect of Tricho-compost and Tricho-leachate on seedling quality and seedling mortality of cabbage.

Treatments	Seedling weight (g)	Pre- and post-emergence death (%)	Seedling mortality reduced (%)
Tricho-compost @ 50 g/kg soil	3.7 a	01.0 b	98.3
Tricho-leachate @ 50 ml/kg soil	2.5 b	01.0 b	98.3
Control (Cowdung @ 200 g/kg soil)	3.7 a	57.0 a	-
CV (%)	13.1	14.7	-
LSD	0.7**	5.5***	-

***P<0.01* and ****P<0.001*

Expt. 3. Re-isolation of *T. harzianum* from Tricho-compost and Tricho-leachate and tests of its effectiveness agaist *S. rolfsii* on PDA plate in the laboratory

Both of the isolates of *T. harzianum*, collected from Tricho-compost, inhibited 59.7% growth of *S. rolfsii* after 144 hours (Fig. 1 & 2). However, for total destruction of the colony, it took 10 days for both the isolates. The results thus confirmed that *T. harzianum* present both in Tricho-compost and Tricho-leachate is viable and active for controlling a pathogen like *S. rolfsii.*

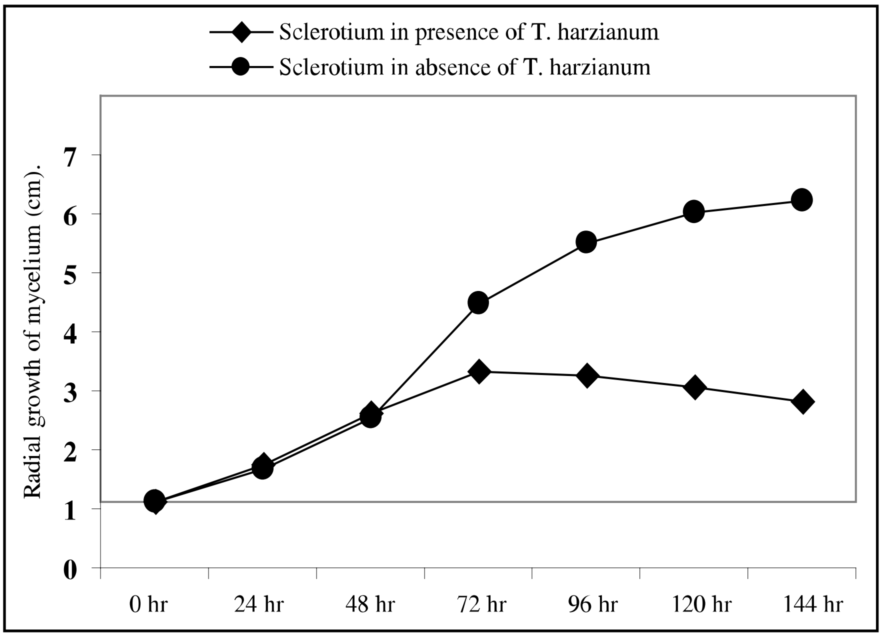

Fig. 1. Mycelial growth of *Sclerotium rolfsii* in absence and in presence of *T. harzianum* (Isolated from Tricho-compost).

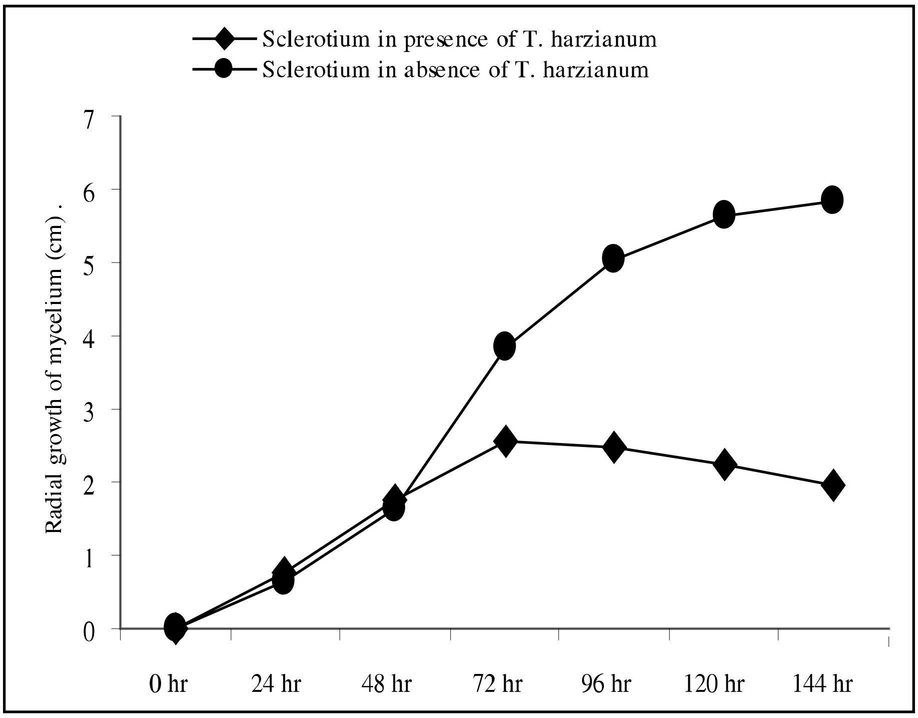

Fig. 2. Mycelial growth of *Sclerotium rolfsii* in absence and in presence of *T. harzianum* (Isolated from Tricho-leachate)

Expt. 3. Use of Tricho-compost and Tricho-leachate for production of cabbage seedling in seed bed nurseries.

The highest number of seedlings was recorded in T_3 (Tricho-leachate @ 500 mL /m^2), followed by T_1 (Tricho-compost @150g + Tricho-leachate @100 mL /m^2) and T_2 (Tricho-compost @100g + Tricho-leachate @150 mL /m^2) and the minimum was in T_4 (control treatment). Applications of Tricho-compost and Tricho-leachate increased the shoot length and plant weight (fresh and dry) of the cabbage seedlings significantly as compared to the control treatment, Tricho-leachate @ 500 mL per sq. metre and Tricho-leachate @100mL + Tricho-compost @ 150g per sq. metre effected as much as 55.6% increase in plant weight of cabbage seedlings. Seedling mortality was the lowest in T_3 followed by T_2 and T_1 treatments. The highest mortality was recorded in the control plot. Plant morality was reduced by 84.0% due to application of Tricho-leachate @ 500 mL per sq. metre soil, while the combined application of Tricho-compost and Tricho-leachate reduced the seedling mortalities by 40.9 to 64.5% over the control treatment (Table 3).

Table 2. Seedling mortality due to diseases in seed bed nursery at BARI, Gazipur.

Treatment applied/m^2	Seedling mortality due to diseases (%)			
	Pythium	*Rhizoctonia*	*Sclerotium*	*Fusarium*
BARI, Gazipur				
TC 150g + TL 150 mL	7	0	-	0
TC 100g + TL 100mL	4	0	-	0
TL 500 ml	2	0	-	0
CD 2 kg + TSP 25 g (C)	4	5	-	3
Shiujgari, Bogra				
TC 150 g + TL 150 mL	23	0	0	0
TC 100g+ TL 100mL	19	0	0	0
CD 2 kg + TSP 25 g (C)	20	15	8	7

TC= Tricho-compost; TL= Tricho-leachate, CD= Cowdung and C=Control

Table 3. Effect of Tricho-compost and Tricho-leachate on seedling germination and disease incidence in seed bed nursery at BARI farm, Gazipur.

Treat.	Seedling popu./m^2	Shoot length (cm)	Plant wt. (g)	Plant wt increased (%)	Dry wt. of plant (g)	Dry wt increased (%)	Plant mortality (%)	Mortality reduced (%)
T1	431.8 b	15.7 a	3.2 a`	55.6	1.7 a	58.3	7.5 b	40.9
T2	423.7 bc	14.8 a	2.6 b	27.1	1.7 a	54.6	4.5 c	64.5
T3	476.9 a	15.8 a	3.2 a	55.6	1.7 a	62.0	2.0 d	84.0
T4	403.6 c	11.5 b	2.1 c	-	1.1 b	-	12.7 a	-
CV (%)	3.1	6.5	0.9	-	3.3	-	19.0	-
LSD	26.5**	.131**	0.01***	-	0.11***	-	0.09**	-

***P<0.01* and ****P<0.001*

T_1= Tricho-compost @150g + Tricho-leachate 100 mL/m^2, T_2= Tricho-compost @100g + Tricho-leachate 150 mL /m^2, T_3=Tricho-leachate @ 500ml /m^2 and T_4=Cowdung 2 kg and TSP 25 g/ m^2

(b) Experiment at OFRD farm at Sheujgari, Bogra: *Pythium, Rhizoctonia, Sclerotium*, and *Fusarium* were identified in seed bed nursery at Bogra. Only *Pythium was* recorded in Tricho-compost and Tricho-leachate application plots, while *Pythium* (20%), *Rhizoctonia* (15%), *Sclerotium* (8%) and *Fusarium* (7%) were recorded in control plots (Table 2).

In general, Tricho-compost and Tricho-leachate increased seedling germination, shoot length and weight of cabbage seedlings. Both the fresh and dry weights of the seedlings were the highest in T_1 (Tricho-compost @150g +

Tricho-leachate @100mL), followed by T_2 (Tricho-compost @100g + Tricho-leachate @ 150 mL). Fresh weight increased by 106 % in T_1, and by 58 % in T_2 treatment. Combined applications of Tricho-compost and Tricho-leachate reduced the infection of soil- borne diseases and infestation of root-knot nematode significantly over the control treatment. The minimum plant mortality was observed in T_2 (19.3%) followed by T_1 (23.8%). Nematode infestation was reduced by as much as 91.0% in T_1, followed by 80.7% in T_2 (Table 4).

Table 4. Effect of Tricho-compost and -leachate on seedling germination and disease incidence in seed bed nursery at OFRD farm, Bogra.

Treat	Seedling popu./m^2	Shoot length (cm)	Plant weight (g)	Dry wt of plant (g)	Dry weight increased (%)	Seedling mortality (%)	Mortality reduced (%)	Gall index (0-10 scale)	RKN infes. reduced (%)
T1	511 a	16.00 a	2.78 a	1.67 a	111.0	23.78 b	53.31	0.16 b	91.00
T2	507 a	15.00 a	2.13 b	1.56 b	97.0	19.31 c	62.09	0.35 b	80.69
T3	442 b	12.38 b	1.35 c	0.79 c	-	50.93 a	-	1.81 a	-
CV (%)	3.99	7.18	1.68	3.54	-	7.06	-	25.0	-
LSD	33.6**	1.8**	0.06	0.08***	-	3.81***	-	0.33***	-

***$P<0.01$* and ***$P<0.001$*

T_1=Tricho-compost @150g + Tricho-leachate @100 mL/m^2, T_2= Tricho-compost @100g + Tricho-leachate @ 150 mL/m^2, and T_3 (control) =Cowdung 2 kg + TSP 25 g/m^2

Discussion

The results of the experiments carried out at BARI farm (Gazipur) and OFRD farm (Bogra) clearly showed that the applications of Tricho-compost and Tricho-leachate containing *T. harzianum* were highly effective in improving seed germination rates, promoting plant growth and vigour and reduced seedling mortalities caused by *Rhizoctonia*, *Sclerotium*, and *Fusarium* spp. The Tricho-products, however, could not control the attacks of *Pythium* spp., probably due to the failure of uncontrolled irrigation and drainage system providing favourable condition for proliferation of the pathogen.

Trichoderma is a genus of fungi present in nearly all types of soils. There are 89 species of *Trichoderma*. Among them, *Trichoderma harzianum* which usually proliferates at the rhizosphere of plants has numerous mechanisms to attack pathogenic fungi thus controlling various plant diseases, and promote plant health, growth, and vigour (Hajieghrari, 2010; Sultana *et al.*, 2012). It is a culturable fungi and has been observed to thrive well on organic materials. Trials conducted at BARI showed that *T. harzianum* accelerated the decomposition of organic materials in only 45 days (Anon., 2007). In the present study, application of organic compost produced by infusing *T. harzianum* increased plant population by improving seed germination rate, enhanced plant growth, and reduced plant mortalities and disease incidence of cabbage seedlings. Similar results were obtained by Haggag and Abosedera (2005) by applying peanut compost inoculated with *Trichoderma* spp. that reduced wilt disease incidence and pathogen population, and increased yield components. A number of researchers (Celar and Valic, 2005; Rabeerdran *et al*., 2000; Inbar *et al*., 1994; Lynch *et al.*, 1991; Hoyos-Carvajal *et al.,* 2009) reported that *Trichoderma* spp. promoted seedling establishment, enhanced plant growth and elicited plant defense reaction in different crops. The antagonistic effect of *T. hazianum* against soil borne diseases is also well documented (Elad *et al.*, 1980; Biswas and Sen, 2000; Chamswamg, 1992; Benitez *et al*. 2004). In the present study, the high seed germination rates obtained in Tricho-compost and Tricho–leachate treatments were due to the hyperparasitic action of *T. harzianum* that has been reported by Venkatasubbaiah *et al.* (1984). On the other hand, higher doses of *T. harzianum* have also been observed to inhibit seed germination in several crops (Venkatasubbaiah and Muthappa, 1981; Hajieghrari, 2010).

Cabbage seedling is highly susceptible to the attacks by *Rhizoctonia*, *Fusarium* and *Sclerotium* spp. in seedbed nurseries at high temperature and humid conditions (June- July). In the present study, applications of both Tricho-compost and Tricho-leachate have been found to control these pathogens effectively as *T. harzianum*, present in Ticho-compost and Tricho-leachate, is known to be antagonistic to these pathogens (Venkatasubbaiah *et al.,* 1984; Elad *et, al.* 1980).

Agricultural soils harbour many species of *Pythium* having varying degrees of pathogenicity; they are usually saprophytic in nature, but can also damage crops severely under favouarable conditions. Diseases infected by *Pythium* have symptoms of seed decay, pre- or post-emergence damping off and infection of the roots or stems of young plants. Yang *et al.* (2004) and Kean *et al.* (2010) observed that *Trichoderma* spp. controlled *Pythium* spp. incidence in many seedling crops. But, the results of the present study showed that the applications of Tricho-compost and Tricho-leachate containing *T. hrazianum* could not control or suppress the infection by *Pythium* spp. in cabbage. This may be due to the presence of excess moisture in the seedbed nursery that was conducive to the proliferation of *Pythium* spp. and this aspect needs further investigation.

The results of the present study confirm and agree with most of the findings of other workers in the past, and therefore, application of organic compost or products fortified with *T. harzianum* in agricultural soils will not only reduce many soil-borne disease pathogens but will also enhance seedling growth and plant health.

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