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Suitability Evaluation of Affordable Organic Materials as Substrates to Multiply *Trichoderma* spp. in Soil

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ABSTRACT

Use of antagonistic ability of *Trichoderma* spp. to control plant disease is an alternative disease management practice that protects the environment from the hazardous effect of the chemicals. Low density of population of *Trichoderma* spp. in the normal field is a challenge to gain best effect of antagonist over soil pathogens. Seven organic substrate treatments; compost, cow dung, paddy husk, paddy straw, coir dust, *Gliricidia sepium* and control were tested for multiplication and long-term survival of *Trichoderma* spp. Three test conditions; substrates mixed with soil, substrates without mixing in soil and substrates sterilized were used in the experiment. In addition the bio-fungicide powder applied as the inoculum of *Trichoderma* spp. was subjected for confirmation. The Blister Blast was confirmed to contain of *Trichoderma* spp. inoculum. The concentration of *Trichoderma* spp. (spores/mL) was counted by Hemocytometer and growth pattern was recorded throughout the study. Among the conditions substrates mixed with soil and sterilized substrates had similar effect. A significant effect on growth of *Trichoderma* spp. was shown between treatments. Substrates compost and paddy husk recorded highest multiplication of *Trichoderma* spp. in all three conditions. Coir dust was not successful in multiplying *Trichoderma* spp. which recorded lower results than the control. The cost of application for compost stands greater than paddy husk. Therefore, paddy husk was the affordable organic substrate for farmers that could be applied in fields in order to multiply *Trichoderma* spp. in soil.

KEYWORDS: Antagonist, Bio-fungicide, Organic substrates, Spore concentration, *Trichoderma* spp.

INTRODUCTION

The fungal diseases of plants especially those caused by soil-borne pathogens are among the major threats to agricultural cultivations. It has been assessed that about 25% of the yield in western countries and nearly 50% in developing countries are lost due to plant diseases; one third of it is due to fungal pathogens (Rajput *et al.*, 2015).

The root infecting soil-borne fungi often produce root rot disease complexes that may result in the death of the plants. Since soil applied pesticides are costly and produce environmental hazards, crop resistance to soil pathogens is believed to be the ideal means of controlling plant diseases (Rajput *et al.*, 2015). Use of microbial antagonists in the control of plant disease is an alternative method for disease management that would also protect the environment from the hazardous effect of the chemicals (Bennet *et al.*, 2006).

Fungal biological control agents (Antagonist microorganisms) have several mechanisms of action that allow them to control pathogens, including mycoparasitism, production of antibiotics, enzymes, competition for nutrients and the induction of plant host defense.

Trichoderma spp. are a genus of asexual fungi found in the soils of all climatic zones in Sri Lanka. They are secondary opportunistic invader, with fast growing rates, strong spore producers, source of cell wall degrading enzymes (cellulases, chitinases, glucanases, *etc.*) and an important antibiotic producers. *Trichoderma* spp. as a potent fungal biocontrol agent against a range of plant pathogens such as *Rhizoctonia* spp., *Phytopthora* spp., *Pythium* spp., and *Fusarium* spp. has attracted considerable scientific attention (Rini and Sulochana, 2007). So far, *Trichoderma* spp. are the most studied fungal biological control agent and commercially marketed as bio pesticides, bio fertilizers and soil amendments (Harman *et al.*, 2004).

In farms although *Trichoderma* spp. are found naturally in the soil, a satisfactory population density is not met to perform antagonistic effect on pathogenic microbes. Also the survival of *Trichoderma* spp. under normal field conditions is about four to five weeks (Kelaniyangoda *et al.*, 2003). Therefore, the normal practice spraying *Trichoderma* spp. biomass on soil has to be coupled with addition of soil substrates in order to effectively control soil pathogens (Anon, 2016). According to past research different organic media like neem cake, coir pith, farm yard manure and decomposed coffee pulp have been suggested for its multiplication (Saju *et al.*, 2002). Yet high cost of these substrates has kept Sri Lankan farmers far from using them in fields. Compost, cow dung, paddy husk, paddy straw, coir dust and *Gliricidia sepium* are most common affordable organic materials to be found in local farm fields. These materials can be used to

easily obtain a considerable population of *Trichoderma* spp at farmers field after optimization of the conditions.

Therefore, this study was conducted to evaluate organic substrates; compost, cow dung, paddy husk, paddy straw, coir dust and *Gliricidia sepium* for multiplication and promote long-term survival of *Trichoderma* spp. in cultivation fields.

MATERIALS AND METHODS

Location

The study was conducted at the Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura during the period from December 2015 up to May 2016.

Confirmation of Trichoderma Suspension

The commercially available Bio fungicide powder (available at Sri Biotech Pvt. Ltd) was confirmed to contain *Trichoderma* spp. One gram of Blister Blast powder was weighed, added into beaker, volume up to 100 mL with autoclaved distilled water and mixed well. Then 100 µL of suspension was plated on PDA media under aseptic condition. After one week culture plates were observed under light microscope.

Test for the Best Substrate

In the experiment six organic substrates as mentioned in Table 1 were tested under three conditions; a) substrate mixed with soil in field b) without mixing in soil c) substrates sterilized *(in vitro)* to evaluate the multiplication of *Trichoderma* spp.

Substrates Mixed with Soil in Field (a)

Preparation of Field: For the field experiment an open field site expose to direct sunlight was selected. Field was ploughed manually to obtain fine tilth. Unnecessary particles and weeds were removed from the field. Then field was sterilized by using burning method. For that the ground was moistened thoroughly and layers of paddy straw, paddy husk were placed alternatively over soil as burning media. Fire was set opposite to the wind direction. After sterilization the burnt particles were removed and field was demarcated into 0.75×0.75 m sized of twenty one plots with three replicates for each treatment. The substrates (Table 1) were applied to the field in a CRD layout. Each substrate was incorporated in to the soil and forked to ensure better mixing with soil. After application of *Trichoderma* spp. frequent watering was done throughout the experimented period.

Substrates without Mixing in Soil (b)

The experiment was repeated with the substrates mentioned in Table 1 placed under the shade net without mixing with soil. Here, *Trichoderma* spp. was applied on substrates and were kept under shade net. The substrates were moistened frequently throughout the experimented period.

Substrates in Sterilized Conditions (c)

The experiment was repeated with the substrates mentioned in Table 1 under *in-vitro* conditions in laboratory. Here each substrate was autoclaved before application of *Trichoderma* spp. The treatments were then kept in-door under shade. The substrates were moistened frequently throughout the experiment.

Application of Trichoderma spp

Bio fungicide power was applied in 12 g/L dosage on to substrates at the start of the experiment (followed same for all tests) a, b and c. Application of the Blister Blast was not repeated during the experiment.

Table 1. Preparation details of substrates

Treatment	Preparation
T_1	Two and half kilo gram of well decayed compost
T_2	Two and half kilo gram of dried cow dung
T_3	Six hundred gram of paddy husk
T_4	Dried Straw was broken into small pieces and one inch of layer was added
T_5	Normally available coir dust
T_6	Partially dried and broken leaves without branches
T_7	No substrate

T_1- compost, T_2- cow dung, T_3- paddy husk, T_4- paddy straw, T_5- coir dust, T_6- Gliricidia sepium and T_7- control

Data Collection

In each condition the growth of *Trichoderma* spp. was counted in terms of number of spores per milliliter for each substrate after dilution and culturing them in laboratory. Spore concentration (spores/mL) was counted by hemocytometer after five days of incubation. This procedure was continued every week for each tested substrate up to four weeks

Dilution and Culturing

Ten gram of substrate was measured into conical flasks from every sampled plot. Ninety milliliters of distilled water was added to each and every flask and the flasks were shaken well at 160 rpm for 30 min. Dilution series of 1:100 was carried out samples (Michel and Hartman, 1992). A 0.1 mL of diluted solution was spread

plated on Potato Dextrose Agar medium (PDA) plates under aseptic conditions. Two replications were done for each sample.

Preparation of Spore Suspension and Counting of Spores

Ten milliliter of sterilized distilled water was added on fungal culture on petri dish and it was dissolved well without disturbing the media and diluted culture was filtered through muslin cloth into 500 mL beaker and this procedure was repeated two times. Spore suspension was volume up to 300 mL by adding sterilized distilled water.

The light microscope was adjusted for clear visibility of grids of the hemocytometer which was kept on stage. A drop from suspension was added on either side of gridded area of hemocytometer and covered by cover slip. Spores were counted through 40x magnification.

Statistical Analysis

Experiment layout was Complete Randomized Design (CRD). The data generated from experiment were statistically analyzed using the General Linear Model (GLM) procedure of Statistical Analysis System (SAS version 9.2). Means were compared using HSD test at the 5% significant level.

RESULTS AND DISCUSSION

Confirmation of Trichoderma Suspension

Trichoderma spp. was confirmed by observe conidiophore of fungus (Figure 1). Branched structure and green colored conidia was observed in the latter phase of the fungal growth which was specified by Kubicek and Harman (1998). According to results commercially available blister blast powder was confirmed containing *Trichoderma* spp.

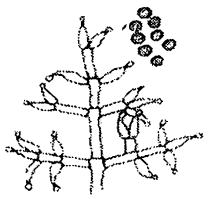

Figure 1. Conidiophore diagram of *Trichoderma* spp.

Test for the Best Substrates

Substrates Mixed with Soil in Field (a)

Each treatment showed a significant difference in mean number of spores as shown in Table 2. Significantly highest mean number of spore per mL was recorded in paddy husk (T_3) and lowest number was shown in coir dust (T_5; Table 2). It may be due to coir dust contain lack of nutrient and may not be fully decomposed and Sri Lankan made coir dust may contain higher amount of salt (Mason, 2003). However, coir dust (T_5) had not been able to show significant results than the control (T_7). The second best concentration was in compost (T_1). Paddy straw (T_4) when mixed with soil had multiplied *Trichoderma* spp. with a similar effect as paddy husk (T_3).

Substrates without Mixing Soil (b)

The maximum mean number of spores was reported in compost (T_1) which was significantly differed from control (T_7; Table 2). Compost may give a support to grow fungus by providing carbon, nitrogen, oxygen and moisture with adequate amount. The minimum number of spores was reported in coir dust (T_5) which was but not significant from control (T_7). All the treatment showed a higher growth of *Trichoderma* spp. compared to control. There can be seen an encouragement in multiplication of *Trichodrema* spp. when organic substrate is added other than only soil (Table 2).

Table 2. Growth performance of *Trichoderma* spp. under each treatment and condition

Treatment	*Trichoderma* spp. mean concentration (Spores/mL)		
	a	b	c
T_1	122^{a}	120^{a}	132^{a}
T_2	69^{bc}	66^{c}	89^{ab}
T_3	126^{a}	96^{b}	142^{a}
T_4	95^{ab}	55^{c}	100^{ab}
T_5	49^{c}	42^{d}	61^{b}
T_6	89^{abc}	62^{c}	101^{ab}
T_7	63^{c}	33^{d}	67^{b}

a- substrate mix with soil, b-without mixing soil, c-sterilized substrate without mix in soil. T_1- compost, T_2- cow dung, T_3- paddy husk, T_4- paddy straw, T_5- coir dust, T_6- Gliricidia sepium and T_7- control

Substrates in Sterilized Conditions (c)

As shown in Table 2, paddy husk (T_3) recorded highest number of spores and coir dust (T_5) treatment showed lowest (Table 2). The results of sterilized substrates had shown similar pattern to the results when substrates were mixed with soil. In both cases *Trichodrema* spp. growth had shown first and second highest respectively in paddy husk (T_3) and compost (T_1) while lowest was in coir dust (T_5).

Test for the Best Condition

Among the tested conditions a) substrate mixed with soil in field b) without mixing soil substrates sterilized (*in vitro*) had shown significant differences in growth of *Trichoderma* spp. (Table 3). Highest spore concentration was recorded in (c) sterilized conditions. The lowest growth was recorded in

condition (b) substrates without mixing in soil. (Table 3).

Table 3. Growth performance of *Trichoderma* spp. under each condition

Condition	Mean spore concentration (spores/mL)
a	88^{ab}
b	68^{b}
c	99^{a}

a- substrate mix with soil, b-without mixing soil, c-sterilized substrate without mix in soil

In a sterilized environment microbes do grow freely. There is no competition to hinder their growth. However, a similar growth of *Trichoderma* spp. in number to the growth in sterilized substrates were observed when substrates were mixed with soil compared to the substrate without mixing in soil. Therefore, according to the test, farmers can obtain better growth of *Trichoderma* spp. when substrates are mixed in soil.

Application of *Trichoderma* spp. on substrate without mixing in soil does not create the best environment for survival and multiplication of *Trichoderma* spp. Optimum growth conditions were achieved when substrates mixed with soil.

Weekly Performance of Trichodrema spp.

Trichodrema spp. in Compost (T_1), paddy husk (T_3), paddy straw (T_4) and control (T_7) showed an increasing growth pattern up to four weeks after inoculation (Figure 2). Cow dung (T_2), coir dust (T_5) and *Gliricidia sepium* (T_6) indicates a decline in multiplication of *Trichodrema* spp. after second and third week. The results suggests that paddy husk (T_3) is the most favorable organic material for the rapid multiplication of *Trichodrema* spp. followed by Compost stands (Figure 2).

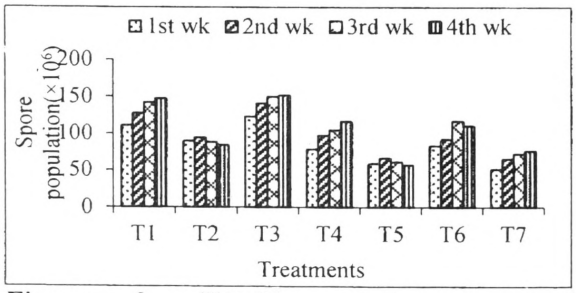

Figure 2. Weekly performance of *Trichoderma* spp. for each substrate. *T_1- compost, T_2- cow dung, T_3- paddy husk, T_4- paddy straw, T_5- coir dust, T_6- Gliricidia sepium and T_7- control*

Cost of Application of Each Substrates

According to cost of substrates (Table 4), compost is the highest cost and *Gliricidia sepium* is the lowest. The cost for paddy husk and paddy straw stands affordable for any farmer in Sri Lanka.

As shown in Table 2, application of *Trichoderma* spp. on sterilized substrate was the best condition to achieve the highest rate of multiplication. Maximum mean number of spores was recorded in paddy husk in that condition as shown in Table 2. Although compost was recorded as a best substrate in condition b (Table 2). The cost for compost is higher than others. In condition b, paddy husk was recorded as second best substrate and cost also lower than compost (Table 4). So, according to the growth performance of fungus and cost for substrate, paddy husk can be recommended as the best low cost organic soil amendment to encourage multiplication of *Trichoderma* spp.

Table 4. Cost of application of substrates

Substrate	Quantity (kg/m^2)	Rate (Rs/kg)	Total cost (Rs/m^2)
Compost	2.5	10.00	25.00
Cow dung	2.5	5.00	7.50
Paddy husk	0.6	2.00	1.20
Paddy straw	0.6	2.50	1.50
Coir dust	2.0	4.00	8.00
Gliricidia sepium	0.8	Free of charge	-

CONCLUSIONS

The results of the study suggest that provision of substrates is important for the multiplication of *Trichoderma* spp. in soil. It is interesting to note the fact that similar growth in a sterilized environment can be obtained when mixed substrate with soil. Adding paddy husk or compost to soil gives higher multiplication of *Trichoderma* spp. Applying paddy husk into the fields is affordable for farmers in Sri Lanka. However, considering the cost of application for farmers application of paddy husk into the field is recommended. Therefore, it is recommended to incorporate paddy husk and mixed with soil followed by *Trichoderma* spp. spray before crop establishment in the field.

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