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Production technology of coir pith cake formulation of *Trichoderma harzianum*

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Abstract

Low cost formulations and delivery system are necessary for wide adoption of bio-control of plant diseases using microbial antagonists. Though several plantation crops wastes/by-products including coir pith are used for multiplication of *Trichoderma* species, no attempt has been made so far to develop a low cost commercial product with long shelf life and high population of antagonist. A technology has been developed to prepare a commercial product *viz*., Trichoderma coir pith cake (TCPC) using 'maida' flour and *Trichoderma harzianum* biomass. It has been found that dried TCPC packed in polythene bags can be stored at room temperature (26-30 °C) for 10 months with high level of *T. harzianum* population. Through a simple activation process of moistening the dried TCPC and incubating under room temperature for 2 days, luxurious growth of *Trichoderma* covering the entire product was obtained and thus the population could be increased to a very high level. Mean *T. harzianum* population in dry TCPC after 10 months of storage was found to be $5.0x10^6$ CFU g^{-1} which has increased to $14.4x10^6$ CFU g^{-1} on activation. Though the population after one year could be increased from $1.6x10^6$ CFU g^{-1} in dry TCPC to $5.2x10^6$ in activated, it is ideal to recommend a shelf life period of 10 months while commercializing the product. The new, simple and low cost technology developed, thus, clearly indicates that coir pith, a waste from coconut industry can be made into value added and environment friendly commercial product for management of plant diseases. Several such products can be commercially prepared for different microbial bioagents following this production technology.

Keywords: Trichoderma, coir pith, 'maida' flour, activation, colony forming units, biological control

Introduction

Trichoderma spp. have gained wide popularity as bio-control agents due to their ability to control many fungal pathogens on a variety of crop plants under field and green house conditions (Whipps, 1996; Harman, 2000; Whipps and Lumsden, 2001) as well as their growth promotion effect on host plants (Baker, 1988). The importance of developing bio-control agents and their commercial products for management of plant diseases is increasing day by day due to the obvious reasons that, dependency on bio-control agents for plant and soil health management became very much imperative in several situations like development of resistance to pathogen, when no other disease control measures are currently available, conventional pesticides cannot be used due to residual toxicity or when organic farming is practiced. Several commercial formulations, especially, *Trichoderma* prepared using inert carriers for plant disease control are costly and have short shelf life. Various plantation crop wastes/by-products such as coir pith, coffee husk, tea waste (Kousalya and Jeyarajan, 1990; Ponmurugan and Baby, 2005), arecanut leaf sheath and dried husk (Niveditha, 2010), cocoa pod husk and bean shell (Ambilikuttyamma, 2011) were found to be suitable for multiplication of *Trichoderma* spp.

The development of economically feasible and efficient bio-control product using locally

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available agricultural by-products/wastes like coir pith, for the application of appropriate microorganisms to the ecosystems is an important component of bio-control programme as well as for the effective utilization of agricultural wastes. Considering these facts, attempts were made to develop a coir pith cake dry formulation of *Trichoderma harzianum* with comparatively long shelf life and high population.

Materials and Methods

Trichoderma harzianum (TH1) in potato dextrose agar medium maintained in the fungal culture collection of Crop Protection Division at Central Plantation Crops Research Institute (CPCRI), Kasaragod was used for the study. Biomass was prepared using potato jaggery broth. It was prepared by following the standard method of preparation of potato dextrose broth except for the use of 20 g jaggery (solid) instead of dextrose. The broth was distributed into empty bottles with flat sides (375 ml capacity) @ 100 ml per bottle and plugged tightly with cotton. This was autoclaved at 15 p.s.i. for 20 minutes, cooled to room temperature and inoculated with 5 mm culture disc of three days old *T. harzianum* grown on potato dextrose agar medium. The inoculated bottles were incubated for 7 days at room temperature in slanting position so as to get the highest surface area of the medium for fungal growth. Seven days after inoculation, the spent medium with fungal biomass was blended in an electric mixer for 1-2 minutes to homogenize the contents. This fungal biomass slurry was used for the preparation of coir pith cake formulation.

After extracting coir (fibre) from coconut husk, the waste material *viz*., the coir pith consisting of dust and bits of fibres available in large quantity is dumped on the road side and factory premises in mounds. Coir pith rejected after removing fibre from husk without retting was collected from a coir industry near to the place of study. Lengthy fibres were removed from the coir pith. It was moistened with water to a moisture level of 70-75 per cent. The substrate was taken in a polypropylene bag (45x30 cm) @ 500 g bag^{-1} and the filled bag was kept in another bag of same size and both were sealed with electric sealing machine. The coir pith filled bags were autoclaved at 20 p.s.i for 30 minutes and allowed to cool at room temperature.

Inner white portion of wheat extract (available in market as maida flour) was used as a nutrient-cum-binding material for Trichoderma coir pith cake preparation. For this, maida flour was boiled in water to get a sticky thick paste. Different quantities of maida flour and water were used to get a thick paste like consistency after boiling. Based on a pilot study, the quantity of maida, coir pith and fungal biomass slurry required for making the product, Trichoderma coir pith cake (TCPC) was determined. The sterilized coir pith, *T. harzianum* biomass slurry and maida paste were thoroughly mixed in a plastic tray. This mixture was used for making the product.

To prepare the formulation as a solid cake, 30 g mixture was compressed manually using a kitchen press, very common in Indian kitchen used for making different food items. It consists of a metallic (iron) round hollow outer case (dia: 4.9 cm; height: 6.7 cm) with both ends opened and with handles on top two sides. A metallic, round, solid cylinder (dia 4.5 cm; height: 7.7 cm) with handles on top two sides is inserted into the outer case without leaving any space in between the two. Such an apparatus with provision for making more cakes at a time is being made locally. To prepare a single solid cake, 30 g mixture was kept inside the outer case of the kitchen press kept on a plastic sheet. Then it was compressed using the inner cylinder to get solid cake. The cakes thus prepared were dried in an oven at 38-40 °C for 4 days (provided ventilation for first two days for the moisture to escape). The moisture content of the cake after drying for 4 days was determined. The colony forming units of *T. harzianum* in the cake was determined after 4 days of drying by dilution plate technique (Pramer and Schmidt, 1956).

The dried Trichoderma coir pith cakes (TCPC) were packed in polythene bags and stored at room temperature (26-30 °C). The shelf life of *T. harzianum* population in the cake was determined at monthly interval for a period of one year. Cakes were also sun dried under glass roof during summer months and stored for one year. Simultaneously

another set of cake was dried in an oven at 38-40 °C for 4 days. The shelf life of sun dried and oven dried cakes after 6, 10 and 12 months was estimated. The sun dried and oven dried cakes were activated by moistening with 18-20 ml of water/cake and incubating in a tray covered with a perforated plastic sheet at room temperature for 2 days. The CFUs in cakes stored for 6, 10 and 12 months were determined before and after activation. The treatments were arranged in a completely randomized block design with 3-5 replications. The data were analyzed statistically.

Results and Discussion

When potato jaggery broth was used as the medium, *T. harzianum* showed very good growth and covered the entire surface of the medium with dark green sporulating mycelial mat during an incubation period of 7 days. This fungal biomass was used for the preparation of *Trichoderma* coir pith cake. The pilot study conducted to standardize the quantities of different constituents required for the preparation of coir pith cake revealed that 70 g maida flour boiled in 280 ml of water, 500 g sterilized coir pith and fungal biomass slurry (in 100 ml liquid medium) are ideal for the preparation of the product. The pH of the mixture was 4.7 to 5 with a moisture content of 80-83 per cent. Thirty gram of this mixture was used to prepare one cake (TCPC). After drying at 38-40 °C for 4 days in an oven, the mean weight of a single cake was 4.95 g with a moisture content of 6.5 per cent.

The mean population of *T. harzianum* in TCPC immediately after drying was found to be 132.33 x 10^6 colony forming units (CFUs) per gram dry cake (Table 1). Viability decreased with the period of storage. *T. harzianum* population in dry cake after 10 months of storage was found to be 5.0 x10^6 CFU g^{-1} and thereafter it was less than 2x10^6 CFU g^{-1}, the recommended standard. Thus, the viability could be maintained for 10 months in the dry TCPC formulation. Various types of agricultural wastes and crop residues have been reported as effective carrier media for mass multiplication and field application of antagonists (Kousalya and Jeyarajan, 1990; Saju *et al*., 2002). Rini and Sulochana (2007) reported that when coir pith was used to grow *T. harzianum* there was only scanty growth of the fungus. But, when coir pith was mixed with neem cake at 1:1 ratio, superior growth and sporulation of the fungus were observed, which completely covered the substrate in 8 days. They further noticed a population of 112.3x10^8 CFU g^{-1} in the coir pith + neem cake formulation after 10 days of inoculation. Though there are several reports on the growth and sporulation of *Trichoderma* spp. in various organic substrates, most of these were prepared for immediate field application. Talc formulation of *Trichoderma* spp. is mainly used as a commercial product. Talc formulation prepared from *T. harzianum* grown in coconut water medium had an initial population of 5.5x10^6 CFU g^{-1} immediately after preparation and it was only 3x10^6 CFU g^{-1} after a storage period of 6 months (Ambilikuttyamma, 2011). But, the *T. harzianum* population in dry TCPC formulation was 5x10^6 CFU g^{-1} even after 10 months of storage.

Table 1. Shelf life of coir pith cake formulation of *T. harzianum* stored at room temperature after oven drying continuously for 4 days at 38-40 °C

Period of storage (months)	*T. harzianum* population (x 10^6 CFU g^{-1})*
0 **	132.33[a]
1	30.00[b]
2	29.33[b]
3	24.67[b]
4	14.00[c]
5	12.00[c,d]
6	7.67[e,d]
7	6.67[e,d]
8	5.67[e]
9	5.33[e]
10	5.00[e]
11	1.67[e]
12	1.33[e]

CD (P≤0.05): 5.78; CV (%) 16.23

Means followed by the same letter are not significantly different at (P=0.05) by DMRT; *mean of 3 replications; **Immediately after drying for 4 days at 38-40 °C (before storing)

Dry TCPC was activated by moistening with water and incubating for 2 days at room temperature. After 2-day-incubation period, the TCPC was covered by thick growth of *T. harzianum* which appeared as white to green colour fluffy growth (Fig. 1). The studies on population of *T. harzianum* in TCPC dried in oven and sun dried under a glass

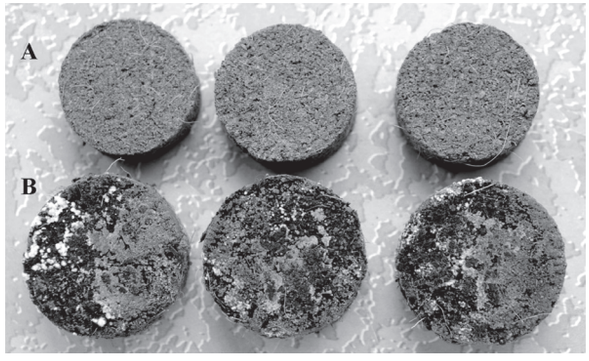

Fig. 1. Trichoderma coir pith cake (TCPC)
A: Before activation; B: After activation

roof during summer months revealed significant variation in CFUs before and after activation. The CFUs in oven dried and sun dried TCPC after 12 months of storage were 1.6 and 6.8 CFU g^{-1} before activation whereas it was 5.2 and 17.4 CFU g^{-1} after activation (Table 2). Since the mean population of *T. harzianum* in oven dried TCPC stored for 12 months was less than 2 x 10^6 CFUs g^{-1} it is better to recommend shelf life of 10 months as ideal for the product developed. It has been found that it is better to use TCPC after activating it by moistening with water and incubating at room temperature for 2 days. This is very simple because activation process can be accomplished in the laboratory/ordinary room without any special precaution to assure sterility. Sterilization process is only required for producing fungal biomass and processing coir pith. The TCPC product is easy to handle, store and transport due to light weight and it is an ecofriendly formulation with nutrients for multiplication of bio-control fungus on activation. The application of activated TCPC is of special interest since it contains actively growing and sporulating bio-control fungus. Coir pith cake formulations of bio-control agents are very easy to handle and apply in the crowns of palms and in soil at the base of plants in the field, nurseries and potted plants for management of pests and diseases. Thus, TCPC which can be easily activated for high level of population of *T. harzianum* is significantly advantageous as a commercial product. Coir pith accounts for about 50-60 per cent of the total weight of coconut husk (Bhowmic and Debnath, 1985). It has been estimated that in India alone 7.5 million tones of coir pith is produced annually (Kamaraj, 1994) but a major portion of this waste material remains unutilized in the coconut growing regions leading to problems associated with its disposal and associated environmental pollution problems. The popularization of the technology developed for production of coir pith cake containing suitable microbial bio-agents in the coconut growing regions and establishment of small scale industries for commercial production will help to a great extent not only in plant and soil health management but also in getting 'wealth from waste' and solving the problem of unemployment to some extent.

Table 2. Population of *T. harzianum* in coir pith cake (TCPC) formulation activated by moistening with water and incubated at room temperature for 2 days (48 h)

Period of storage (months)	*T. harzianum* population (x10^6 CFU g^{-1})*			
	Continuous oven drying at 38-40 °C for 4 days		Sun drying under glass roof	
	Before activation	After activation**	Before activation	After activation**
0	135.2	-	215.8	-
6	10.8	24.2	44.2	209.4
10	5.0	14.4	8.8	44.8
12	1.6	5.2	6.8	17.4

*Mean of 5 replications; **Dried TCPC activated by moistening with water and incubated for 2 days at room temperature

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