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Evaluation of locally available substrates for mass production of *Trichoderma*

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The fungus *Trichoderma* is widely used as bio-control agent against several pathogenic fungi causing plant diseases throughout the world (Chet *et al.*; 1979, Elad *et al*., 1980; Sivan *et al*., 1984). These antagonistic fungi are the most commonly used biocontrol agents because of their characteristics, such as antagonism and plant-growth stimulation (Harman *et al*., 2004). *Trichoderma* is being used worldwide for suitable management of various foliar and soil borne plant pathogens (Dominguesa, *et al*., 2000). Martinez-Medina *et al*. (2009) reported bentonite–vermiculite formulation of *T. harzianum* strain CECT 20714 which was effective in reducing the incidence of Fusarium wilt in melon plants under green house condition.

Different organic media like neem cake, coir pith and decomposed coffee pulp have been suggested for *Trichoderma* multiplication (Saju *et al*., 2002). Mustaf *et al*. (2009) reported organic manures like neem cake, vermiculite and spent mushroom compost as very good substrates for multiplication of *Trichoderma*. Application of neem cake fortified with talc formulation of selected *Trichoderma* isolate has been recommended for the management of basal stem rot disease of coconut, arecanut and also for stem bleeding disease of coconut. However, during recent years, due to non availability of good quality neem cake in the market and also commercially available formulations are very expensive, farmers are in need of potential biocontrol agents with farmer friendly formulations for the effective management of diseases. Development of acceptable, easily prepared and cost effective formulations of biocontrol agents plays a major role in the success of a biological control programme. Thus, an attempt was made to evaluate the locally available and cheap substrates for mass multiplication of *Trichoderma harzianum*.

Preparation of *Trichoderma* talc formulation

Among the *Trichoderma* spp. maintained in fungal culture collection of Crop Protection Division of ICAR-Central Plantation Crops Research Institute, Kasaragod, *T. harzianum* (TD 28) was found effective against major pathogens of coconut and arecanut such as *Ganoderma* spp. and *Thielaviopsis paradoxa,* causal agents of basal stem rot and stem bleeding diseases, respectively. Biomass of the fungus was prepared by using potato jaggery broth. This broth was prepared by following the standard method of preparation of potato dextrose broth except for the use of 20 g of jaggery instead of dextrose. Exactly 100 mL of potato jaggery broth was distributed into empty bottles and plugged tightly with cotton. These bottles were autoclaved, cooled to room temperature and inoculated with 5 mm mycelial disc of three days old culture of *T. harzianum.* The inoculated bottles were incubated for 7 days at room temperature in slanting position. Biomass was prepared by blending mycelial mat with medium in an electric mixer for 1 to 2 minutes. Four hundred mL of biomass slurry was mixed with 1 kg of autoclaved talc powder and dried at room temperature for five days with intermittent mixing once in two days.

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Mass multiplication using substrates

Locally available substrates such as cowdung, coir pith, neem cake, neem seed kernel powder, vermicompost, cocoa bean shell alone and in certain combinations such as neem seed kernel powder + coir pith, cocoa bean shell + coir pith (1:1) were used for mass multiplication of *T. harzianum*. All these substrates were autoclaved, cooled to room temperature and inoculated with *T. harzianum* enriched talc formulation at the rate of 10 g per kg of substrate in plastic trays. Inoculated substrates were incubated at room temperature for seven days with the intermittent mixing once in two days and maintained the moisture level up to 50 per cent. Visual observations on fungal growth were done daily and the colony forming units gram^{-1} of substrate (cfu g^{-1}) was estimated seven days after inoculation by dilution plate technique using *Trichoderma* selective medium (Elad and Chet, 1983).

Among the six substrates evaluated for mass multiplication of *T. harzianum* (TD 28), the combination of neem seed kernel powder + coir pith showed the highest growth of *T. harzianum.* White mycelial growth of *T. harzianum* was observed on the neem seed kernel powder + coir pith, cocoa bean shell + coir pith (1:1) and neem seed kernel powder alone on third day of incubation and it covered the entire surface of the substrate with profuse green sporulation in 6 days. A population of 320.2×10^8 cfu g^{-1} was noticed in neem seed kernel powder + coir pith combination on 7 days of incubation, which was significantly superior to others (Table 1). In cocoa bean shell + coir pith combination colonies of 130.3×10^8 cfu g^{-1} was recorded. A population of 100.8×10^8 cfu g^{-1} was recorded in neem seed kernel powder alone.

In case of neem cake and cocoa bean shell alone, mycelial growth was visible over the surface on the fourth day of inoculation and it took 7 days to cover the whole substrate. A population of 57.5×10^8 cfu g^{-1} in cocoa bean shell and 24.6×10^8 cfu g^{-1} in neem cake was observed on 7 days of incubation. In cowdung, vermicompost and coir pith very scanty mycelial growth and less cfu g^{-1} were observed as compare to other substrates. However, when coir pith was used in combination with neem seed kernel powder (1:1) and cocoa bean shell (1:1), superior growth and sporulation of *T. harzianum* was observed.

Table 1. Population of *T. harzianum* on different substrates

Substrates	Colony forming units ×10^8 (g)
Neem seed kernel powder + coir pith (1:1)	320.2^a
Cocoa bean shell + coir pith (1:1)	130.3^b
Neem seed kernel powder	100.8^c
Cocoa bean shell	57.5^d
Neem cake	24.6^e
Vermicompost	2.3^f
Coir pith	1.8^f
Cow dung	1.0^f

* Mean of three replications recorded on 7th day of inoculation; Values indicated with different alphabets are significantly different

Various types of organic substrates have been reported earlier as effective carrier media for mass multiplication of antagonists (Kousalya and Jeyarajan, 1990; Saju *et al*., 2002). Rini and Sulochana (2007) noticed a population of 112.3×10^8 cfu g^{-1} in the coir pith + neem cake formulation after 10 days of inoculation. However, the present study showed that neem seed kernel powder + coir pith maintained highest population of 320.2×10^8 cfu g^{-1} *T. harzianum* on 7 days of incubation.

The present study concluded that among the substrates evaluated for mass production of *T. harzianum* (TD 28), neem seed kernel powder + coir pith (1:1) recorded highest colony forming units on 7 days of incubation and found suitable for direct application to soil. This combination is cheaper than application of *Trichoderma* enriched neem cake available in the market.

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