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Short communication

Substrate evaluation for multiplication of *Trichoderma* spp.

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Abstract

Cow dung, neem cake, coir pith, sorghum grains, saw dust, and rice bran, either alone or in certain combinations, with or without additives such as jaggery and wheat flour, and having differential moisture levels were evaluated as substrates for mass production of *Trichoderma harzianum* and *T. viride*. Pre-boiled sorghum grains, coir pith + neem cake (1:1), cow dung + neem cake (1:1) + wheat flour (10%) maintained high populations of *T. harzianum* and *T. viride* within 10 days of inoculation. Jaggery and wheat flour served as nutritional supplements and enhanced the conidial yield from 23.66 x 10^8 to 34 x 10^8 and 45.6 x 10^8 colony forming units g^{-1} respectively. An increase in the number of viable propagules up to 30 days was noted regardless of the substrates and its moisture levels. Although highest initial population of *Trichoderma* spp. was observed in sorghum grains, propagule viability was low in that compared to other substrates. Coir pith + neem cake (1:1) at 35% and 45% moisture gave longer shelf life for *Trichoderma* propagules.

Keywords: Biocontrol, Organic substrates, Shelf life, Substrate moisture levels.

Trichoderma as a potent fungal biocontrol agent against a range of plant pathogens has attracted considerable scientific attention (e.g., Tewari and Mukhopadhyay, 2001; Rini and Sulochana, 2007). Different organic media like neem cake, coir pith, farmyard manure, and decomposed coffee pulp also have been suggested for its multiplication (Saju et al., 2002). Yet reports on the optimum moisture levels of these substrates for high inoculum production of *Trichoderma* spp are inadequate. Therefore, a study was conducted to evaluate some locally available organic substrates and to standardize the optimum moisture levels for mass multiplication and long-term survival of *Trichoderma* spp. Attempts were also made to enhance the conidial yield of these substrates using nutritional supplements.

The substrates evaluated include pre-boiled sorghum, rice bran, cow dung, neem cake, coir pith, and saw dust either alone or in certain combinations (Table 1). Jaggery (3%) and wheat flour (10%) were used as nutritional supplements for enhancing conidial yield (Prasad et al., 2002). Triplicate samples of moistened substrates were transferred to 250 ml conical flasks, sterilized at 1.02 kg cm^{-2} for 1 h, and inoculated with 1 cm^2 of actively growing culture discs of *Trichoderma* [*T. harzianum* (TR20) and *T. viride* (TR22)]. The contents were incubated at room temperature and the treatments were arranged in completely randomized design with three replications. Visual observations on fungal growth were made daily and the propagule density estimated on potato dextrose agar (PDA) supplemented with Rosebengal @ 25 mg L^{-1} on the 10^{th} day of inoculation by dilution plate technique. Colony forming units (cfu) were counted after 2 days of incubation. The study was conducted during the period from June to August 2004.

Pre-boiled sorghum grains, coir pith + neem cake (1:1), cow dung + neem cake (1:1) + wheat flour (10%) which maintained maximum inoculum density on the 10th day

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Table 1. Effect of different substrates on the population of *Trichoderma harzianum* (TR20) and *T. viride* (TR22).

Treatments	Mean population ($\times 10^8$ cfu g^{-1}) at 10^{th} day of incubation (n=3)	
	T. harzianum	*T. viride*
Cowdung + neem cake (1 : 1)	23.7 (4.86)	15.6 (3.95)
Coir pith	13.0 (3.61)	15.3 (3.91)
Coir pith + Neem cake (1 : 1)	112.3 (10.59)[b]	69.0 (8.31)[b]
Sorghum grains	156.3 (12.50)[a]	138.6 (11.77)[a]
Saw dust	10.3 (3.21)	12.0 (3.46)
Rice bran	31.0 (5.57)	20.6 (4.54)
Cow dung + neem cake (1: 1) + jaggery (3%)	34.0 (5.83)	49.3 (7.02)
Cow dung + neem cake (1: 1) + wheat flour (10%)	45.6 (6.75)[c]	53.6 (7.32)[c]
CD (0.05)	1.04	1.15

Figure in parenthesis indicates $\sqrt{x}$ transformed values; values indicated by superscripts are significantly superior.

were selected and further evaluated for the effect of different moisture levels on spore viability. Initial moisture content of materials were estimated by oven dry method and known quantities of water considering the initial moisture content were added to obtain the desired substrate moisture levels. For example, the coir pith – neem cake mixture was moistened to obtain 35, 45, and 55% moisture by weight, 35% being the initial moisture content. For cow dung + neem cake (1:1) + wheat flour (10%) combination 20, 40, and 60% moisture levels were chosen. The substrates were taken in conical flasks and sterilized at 1.02 kg cm^{-2} for 1 h and inoculated with 1 cm^2 discs of actively growing culture of *Trichoderma*, followed by incubation at room temperature (28±2ºC). Population estimation of *Trichoderma* was done fortnightly using PDA – Rosebengal medium following the dilution plate technique. Three replications were maintained and the data analyzed using the analysis of variance technique.

Among the different substrates tested, sorghum grains showed the highest growth of *T. harzianum.* White mycelial growth was observed on sorghum grains on the third day of incubation and it covered the entire surface of the substrate with profuse green sporulation in 6 days. Maximum biomass production of *T. harzianum* (156.3 x 10^8 cfu g^{-1}) at 10 days of incubation also was noticed on this substrate, which was significantly superior to others (Table 1). In case of cow dung + neem cake (1:1) mixture, mycelial growth was visible over the surface on the fourth day and it took 8 days to cover the whole substrate. A population of 23.66 x 10^8 cfu g^{-1} was recorded on this substrate 10 days after incubation. However, an increase in the growth rate and population were observed when wheat flour 10% (45.6 x 10^8 cfu g^{-1}) and jaggery 3% (34 x 10^8 cfu g^{-1}) were used as nutrient supplements. On coir pith and saw dust, when used alone, scanty growth of the fungus was noted. Nevertheless, coir pith when used in combination with neem cake (ratio 1:1), superior growth and sporulation of the fungus were observed, which completely covered the substrate in 8 days. A spore load of 112.3 x 10^8 cfu g^{-1} was noticed in the coir pith – neem cake combination as against 13 x 10^8 cfu g^{-1} in coir pith alone. Rice bran recorded a population of 31 x 10^8 cfu g^{-1}, which was statistically on par with cow dung - neem cake mixture.

Growth rate of *T. viride* on different organic substrates was similar to that of *T. harzianum*. Sorghum grains maintained the maximum growth and spore count of 138.6 $\times$ 10^8 cfu g^{-1} followed by coir pith + neem cake (1:1) mixture (69 $\times$ 10^8 cfu g^{-1}) and cow dung + neem cake + 10 % wheat flour (53.6 $\times$ 10^8 cfu g^{-1}). Coir pith and saw dust recorded lower spore counts (15.3 $\times$ 10^8 and 12 $\times$ 10^8 cfu g^{-1} respectively) compared to other substrates (Table 1).

Spore viability of both *T. harzianum* and *T. viride* were highest at 30 days and it declined thereafter (Table 2). Furthermore, as the moisture content of the substrate

Table 2. Spore viability of *Trichoderma harzianum* (TR20) and *T. viride* (TR22) at different moisture levels.

Treatments	Mean population ($\times 10^8$ cfu g^{-1}) at 30, 60, and 90 days after inoculation (n=3).					
	T. harzianum			*T. viride*		
	30	60	90	30	60	90
Sorghum grains	951 (30.81)	1.82 (1.4)	0.023 (0.15)	847 (29)	1.67 (1.27)	0.033 (0.18)
Coir pith + neem cake (1:1) - 35% moisture (original)	667 (25.8)	0.87 (0.9)	3.67 (1.9)	1487 (39)	0.97 (0.99)	2.43 (1.56)
Coir pith + neem cake (1:1) 45% moisture	1473 (38.4)	1.47 (1.2)	9.5 (3.11)	1537 (39)	0.64 (0.8)	2.45 (1.56)
Coir pith + neem cake (1:1) 55% moisture	1753 (41.9)	1.72 (1.3)	1.51 (1.23)	1530 (39)	0.87 (0.93)	1.17 (1.08)
Cow dung + neem cake (1:1) + wheat flour (10%) 20% moisture	850 (29.3)	0.43 (0.67)	0.53 (0.73)	847 (29)	1.01 (1.0)	0.70 (0.84)
Cow dung + neem cake (1:1) + wheat flour (10%) 40% moisture	1193 (34.5)	0.5 (0.7)	0.36 (0.60)	1630 (40)	0.93 (0.96)	0.54 (0.73)
Cow dung + neem cake (1:1) + wheat flour (10%) 60% moisture	1743 (41.0)	0.24 (0.5)	0.08 (0.28)	1680 (41)	0.64 (0.8)	0.52 (0.72)
CD (0.05)	0.93	0.085	0.229	1.16	0.077	0.09

Figures in parenthesis indicates $\sqrt{x}$ transformed value.

increased, the spore load also increased steadily. Maximum population at 90 days was noted in the coir pith + neem cake at 45% moisture (9.5×10^8 cfu g^{-1}), followed by 35% (3.67×10^8 cfu g^{-1}). Overall, the results indicate that locally available organic media viz., coir pith, cow dung, and neem cake are excellent sources of nutrition for antagonistic fungi like *T. harzianum* and *T. viride*, which is consistent with the findings of Saju et al. (2002). Of the media tested, cow dung – neem cake mixture is already a recommended practice for field multiplication of *Trichoderma* (KAU, 2002). Our study, however, confirms that coir pith – neem cake mixture at both its original (35%) and enhanced (45%) moisture levels forms an ideal and cheap substrate for mass multiplication and long-term survival of *T. viride* and *T. harzianum*.

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