

Mass multiplication of *Trichoderma* sp. on various agricultural and forest byproducts

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Abstract

Nineteen agricultural and forest byproducts were tested as substrates (single substrates) for mass production of *Trichoderma virens*, *T.harzianum*, *T. viride*, and *T. hamatum*, the promising antagonists of the pathogen of coconut stem bleeding disease viz., *Thielaviopsis paradoxa*. Among these, rice bran supported maximum growth of all antagonists followed by wheat bran and neem cake (703.33, 843.33, 753.67 and 660.0 x 10⁶ cfu/g substrate respectively). Among the ten combination substrates (containing two promising substrates) tested, rice bran + neem cake (1:1 w/w) showed maximum growths of all antagonists after 15 days of incubation (806.67, 926.67, 873.33 and 786.67 x 10⁶ cfu/g substrate respectively). Among the six composts tested, coffee husk vermicompost showed maximum growths of all antagonists after 15 days of incubation (563.33, 716.67, 616.67 and 663.33 x 10⁶ cfu/g substrate respectively). Based on these studies rice bran + neem cake (1:1 w/w) was selected as the best substrate for mass production of the four antagonists for soil application in the field trials for the management of stem bleeding disease of coconut.

Key words : *Trichoderma virens*, *T.harzianum*, *T. viride*, and *T. hamatum*, Antagonists, Mass production, Agricultural and forest by-products

Introduction

Stem bleeding disease of coconut caused by the soil borne fungus *Thielaviopsis paradoxa* (de Seynes) von Hohnel is a debilitating disease prevalent in almost all coconut growing areas of the country causing considerable economic loss. Since the pathogen is soil borne, it is essential to adopt an integrated approach involving antagonistic organisms. *Trichoderma virens*, *T.harzianum* (Kallangai isolate), *T.viride* (Calicut isolate) and *T.hamatum* (IARI, New Delhi isolate) were identified as potential antagonists of *Thielaviopsis paradoxa* based on *in vitro* screening of several fungi (Ramanujam *et al.*, 2000). Application of antagonistic fungi to the rhizosphere of crop plants for the control of soil borne diseases requires mass production of the antagonistic fungi within a short time using commonly available cheap substrates. Several attempts in this direction have been reported (Backman and Rodriguez Kabana 1975; Kelly, 1976). In the present investigation, eighteen commonly available agricultural/ plantation /forest byproducts and

composts were evaluated as substrates for mass multiplication of the four antagonistic fungi for soil application in the IDM for stem bleeding disease of coconut.

Materials and Methods

Nine oil cakes viz., neem, pongamia, castor, mahua, marotti, coconut, groundnut, gingelly and sunflower, two brans viz., rice and wheat and seven other substrates such as farm yard manure (FYM), saw dust, coconut leaf fronds (dried), coconut coir pith, arecanut leaf (dried), arecanut husk and cocoa pod husk (dried) were evaluated as single substrates for mass production studies. Oil cakes were ground to a fine powder, while coconut leaf fronds, arecanut leaf, arecanut husk and cocoa pod husk were chopped into small bits of 1-2 cm length before using them as substrates. 25 g of each substrate in three replications were taken in conical flasks (250 ml capacity) and the moisture was adjusted to 50-100% (w/v) depending on the substrate and sterilized at 121°C for two hr on two successive days. Each flask

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was inoculated with six mm discs of two-day old antagonistic fungus and incubated at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 45 days. One gram substrate was drawn aseptically after thorough mixing after 15 days of incubation and the number of colony forming units (cfu/g) produced in each substrate was estimated by dilution plate method on PDA.

The following combination substrates (mixed substrates) were also evaluated for mass production studies:

1. Rice bran + Neem cake (1:1 w/w)
2. Rice bran + Wheat bran (1:1 w/w)
3. Rice bran + Farm yard manure (1:1 w/w)
4. Rice bran + Saw dust (1:1 w/w)
5. Wheat bran + Neem cake (1:1 w/w)
6. Wheat bran + Saw dust (1:1 w/w)
7. Wheat bran + Saw dust (1:1 w/w)
8. Neem cake + Farm yard manure (1:1 w/w)
9. Neem cake + Saw dust (1:1 w/w)
10. Farm yard manure + Saw dust (1:1 w/w)

Six composts viz., coconut coir pith, arecanut leaf compost, arecanut leaf vermicompost, cocoa pod husk compost, coffee husk compost and coffee husk vermicompost were also evaluated for mass production of these antagonists. The composts were prepared using the methods described by Biddappa *et al.*, 1998.

Results and Discussion

Growth on single substrate

The growth measurements of *T. virens*, *T. harzianum*, *T. viride* and *T. hamatum* were estimated in terms of colony forming units produced per gram substrate (cfu/g) on eighteen substrates are presented in Table-1. Maximum growth of *T. virens* was noticed on rice bran (703.33×10^6 cfu/g), followed by that on neem cake, wheat bran sunflower cake and coffee husk (313.33 , 300.00 296.67 and 281.33×10^6 cfu/g respectively). Maximum growth of *T. harzianum* was noticed on rice bran and wheat bran (843.33 and 833.33×10^6 cfu/g respectively), followed by that on neem cake, coconut cake and pongamia cake (760.00 , 750.00 and 736.67×10^6 cfu/g respectively). Maximum growth of *T. viride* was noticed on rice bran and wheat bran (753.67 and 736.67×10^6 cfu/g respectively), followed by that on neem cake, cake, coffee husk and saw dust (266.67 , 257.67 and 243.33×10^6 cfu/g respectively). Maximum growth of *T. hamatum* was noticed on rice bran (660.00×10^6 cfu/g), followed by that on neem cake, and wheat bran (580.00 and 563.33×10^6 cfu/g respectively).

Minimum growth of all four antagonists was observed on cocoa pod husk (0.024 , 0.027 , 0.023 and 0.033×10^6 cfu/g).

Table 1. Growth of four antagonistic fungi of *T. paradoxa* on various agricultural and forest byproducts (Single substrates) after 15 days.

Sl. No.	Substrate	Growth of antagonistic fungus ($\times 10^6$ cfu/g substrate)			
		<i>T. virens</i>	<i>T. harzianum</i>	<i>T. viride</i>	<i>T. hamatum</i>
1	Neem cake	313.33	760.00	266.67	580.00
2	Pongamia cake	19.33	736.67	0.67	50.67
3	Castor cake	0.04	0.73	0.067	0.047
4	Mahua cake	0.94	170.00	176.67	210.00
5	Marotti cake	0.33	106.67	0.83	0.53
6	Coconut cake	0.34	750.00	0.77	0.85
7	Groundnut cake	2.00	296.67	146.67	26.00
8	Gingelly cake	0.54	123.33	8.33	0.78
9	Sunflower cake	296.67	146.67	1.40	47.68
10	Rice bran	703.33	843.33	753.67	660.00
11	Wheat bran	300.00	833.33	736.67	563.33
12	Farm yard manure	213.33	330.00	200.00	246.67
13	Saw dust	180.00	406.67	243.33	160.00
14	Coconut leaf fronds	41.33	50.33	74.67	69.00
15	Coconut coir pith	8.67	10.67	17.33	2.33
16	Arecanut leaf	38.67	44.00	70.00	63.67
17	Arecanut husk	34.00	30.67	53.67	62.00
18	Cocoa pod husk	0.024	0.027	0.023	0.033
19	Coffee husk	281.33	626.67	257.67	342.67
C.D (P = 0.05)		44.18	56.83	29.78	40.61

Among the 18 substrates (single) tested, maximum growth of all four antagonistic fungi were observed on rice bran, followed by the growth on wheat bran/neem cake. These materials seemingly provided the required essential nutrients like carbohydrates, amino acids vitamins etc. for their growth and sporulation of the antagonistic fungi. Kousalya and Jeyarajan (1988) screened twenty-one substrates for mass multiplication of *T. harzianum* and *T. viride* and observed that tapioca (cassava) rind was superior to other substrates. Jacob Kuruvilla and Sivaprakasam (1993) evaluated several organic wastes and found dried effluent from gobar gas plant and farm yard manure as promising substrates for mass production of *T. harzianum* and *T. viride*. Sawant *et al.*, (1995) reported coffee waste and poultry manure as excellent substrates for mass production of *T. harzianum*. Sangeetha (1988) used rice bran for the mass multiplication of *T. harzianum* and *T. viride* for the biological control of *R. solani*. Wheat bran has been used as substrate for multiplication of *Trichoderma* spp. by Sivan *et al.*, (1984).

In addition to rice bran, wheat bran and neem cake, organics like farm yard manure and saw dust showed

good growth of all four antagonistic fungi, compared to the growths observed on other oil cakes and plantation wastes. Among the different plantation wastes tested, coconut leaf, arecanut leaf and arecanut husk supported good growth, compared to coir pith and cocoa pod husk. Although composted coir pith was reported to be a good substrate for mass production of *T. viride* (Kumar and Marimuthu, 1994), fresh coir pith did not support good growth of all four antagonistic fungi in the present study. This might be due to partial degradation of lignin in the fresh coir pith and as a result lesser amounts of nutrients were available for the growth of antagonistic fungi in the fresh coir pith, compared to the well-degraded or composted coir pith.

Growth on Composts

Among the six composts/ vermicomposts tested, coffee husk vermicompost supported maximum cfu counts of all the four antagonistic fungi followed by coffee husk compost (Table 2). Cocoa pod husk compost supported least growth of the antagonistic fungi.

Table 2. Growth of four antagonistic fungi on six composts

Sl. No.	Substrate	Growth of antagonistic fungus (x 10 ⁶ cfu/g substrate)			
		<i>T.virens</i>	<i>T.harzianum</i>	<i>T.viride</i>	<i>T.hamatum</i>
1	Coconut coir pith	134.67	308.33	268.67	203.33
2	Arecanut leaf compost	286.67	410.00	323.33	373.33
3	Arecanut leaf vermicompost	306.67	443.33	360.00	403.33
4	Cocoa pod husk compost	0.397	0.570	0.447	0.430
5	Coffee husk compost	510.00	690.00	583.33	613.33
6	Coffee husk vermicompost	563.33	716.67	616.67	663.33
	C.D.	42.76	49.32	39.96	41.27

In general, the composts provided better growth of all four antagonists compared to the fresh material.

Growth on mixed substrates

The growths of four antagonistic fungi on ten mixed substrates containing rice bran, neem cake, FYM and sawdust are presented in Table 3. Among the ten mixed substrates tested, rice bran + neem cake (1:1 W/W) showed maximum growth of all four antagonistic fungi like *T. virens*, *T. harzianum*, *T.viride*, and *T. hamatum* (806.67, 926.67, 873.33 and 786.67 X 10⁶ cfu/g respectively;). Rice bran + wheat bran supported the second highest growth of three antagonists like *T. virens*, *T. harzianum* and *T.viride*. The growth rates on these combination substrates were found high compared to that observed in each of the respective substrate. This may be due to the additive effect of varied nutrients available

in the combination substrates. Several combination substrates were used for mass production of *Trichoderma* spp by different workers. Wheat bran along with saw dust/peat was used for mass production of *T. harzianum* by Mukhopadhyay *et al.*, 1986 and Sivan *et al.*, 1984. In the present study, rice bran + neem cake (1:1 w/w) and rice bran + wheat bran (1:1 w/w) were found to be the best among the ten combination substrates tested for mass production of the four antagonistic fungi. These combination substrates could be used for large-scale production of the antagonistic fungi for field applications.

Table 3. Growth of four antagonistic fungi on mixed substrates

Sl. No.	Substrate	Growth of antagonistic fungus (x 10 ⁶ cfu/g substrate)			
		<i>T.virens</i>	<i>T.harzianum</i>	<i>T.viride</i>	<i>T.hamatum</i>
1	Rice bran + Neem cake (1: 1 W/W)	806.67	926.67	873.33	786.67
2	Rice bran + Wheat bran (1: 1 W/W)	576.67	916.67	856.67	756.67
3	Rice bran + FYM (1: 1 W/W)	546.67	723.33	516.67	776.67
4	Rice bran + Saw dust (1: 1 W/W)	576.67	793.33	536.67	756.67
5	Wheat bran + Neem cake (1: 1 W/W)	473.33	880.00	816.67	670.00
6	Wheat bran + FYM (1: 1 W/W)	340.00	656.67	490.00	423.33
7	Wheat bran + Saw dust (1: 1 W/W)	316.67	690.00	510.00	296.67
8	Neem cake + FYM (1: 1 W/W)	353.33	596.67	336.67	476.67
9	Neemcake + Saw dust (1: 1 W/W)	320.00	556.67	346.67	293.33
10	FYM + Saw dust (1: 1 W/W)	253.33	493.33	280.00	250.00
	C.D. (P=0.05)	59.41	74.76	57.84	48.67

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