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Low cost bioformulation of *Trichoderma harzianum* for biological control of plant disease

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Thirteen organic by product and waste materials were screened during 2007 - 2008 as substrate cum carrier for mass multiplication of *T. harzianum* of which only wheat bran, rice bran, pulse bran and orange peel supported good growth of the antagonist. Besides these four oil cakes viz., neem cake, groundnut cake, mustard cake and linseed cake were also tested as an additive or enricher with the bran. Wheat bran alone produced significantly higher population of the antagonist at all the age of incubation except 14 days where it was statistically insignificant with rice bran. Initially at 7 days of incubation wheat bran showed highest population (21.6 x 10^6 c.f.u./g of substrate) followed by rice bran (17.6 x 10^6 c.f.u./g of substrate) and pulse bran (15.0 x 10^6 c.f.u./g of substrate) respectively. When the four organic by products were assayed in combination with oil cakes at a concentration of 5, 10 and 20% (w/w) it was found that wheat bran + mustard cake (20% w/w) gave highest population (272.6 x 10^6 c.f.u /g) followed by rice bran + mustard cake (20%). It was also noticed from the result that the growth of the antagonist increased in term of c.f..u./g with increase in concentration of the oil cakes

Key words: Mass multiplication, *Trichoderma harzianum*, agricultural by product

INTRODUCTION

Successful biological control depends on the use of appropriate formulations and delivery system of the bioagent. Formulation is necessary to retain the viability during preparation, storage and ease of application and it favours survival of bioagent in the environment. If wide spread biological control of soil borne plant pathogens is to be achieved by augmentation or seed treatment, it is necessary to mass production of promising antagonist rapidly in the form of spores, mycelia or mixture (Pan and Jash, 2004). Several isolates of *Trichoderma* can develop large amount of biomass containing conidia and chlamydospore in both liquid and solid media containing expensive ingredients (Lewis and Papavizas, 1983). Solid media for the experimental production of *Trichoderma* have frequently been used in laboratory, green house and as well as in the field (Davet *et al*, 1981). Various types of crop residues, agricultural wastes, by product and number of inert materials have been reported to act as carrier media for *Trichoderma* (Kousalya and Jeyarajan, 1990; Whips, 1992). Bhai *et al*, (1994) have evaluated a number of agricultural wastes, which could be used as carrier and multiplication media, simultaneously. The conidia and chlamydospores of *T. harzianum* produced by sold state and liquid fermentation, respectively, do not prove significant in reducing root rot incidence in chick pea caused by *R. solani* (Prasad *et al.*, 2002). However, earlier studies (Papavizas and Lewis, 1989; Lewis *et al.*, 1990) have revealed that chlamydospore preparation of *Trichoderma* are more effective than conidial preparations in controlling disease caused by *S. rolfsii* and *R. solani*. The conidia produced on solid state fermentation are known to have thicker cell walls compared to those produced in liquid medium (Munoz *et al.*, 1995) and survive longer than chlamydospores and exhibit almost equal bioefficacy as compared to chlamydospores produced under submerged fermentation (Prasad *et al*, 2002).

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Many scientists have comprehensively worked on various agricultural wastes and byproducts for multiplication of Trichoderma (Anandaraj and Sarma, 1997; Prakash *et al*, 1999; Godwn-Egein and Arinzae, 1999). Godwn-Egein and Arinzae (2001) used different food wastes viz. ripe plantain, unripe plantain, cassava, yam, sweet potato, orange and yam peels in powder form for media preparation and as well as soil amendment in pot. Besides, these agricultural byproducts and food waste materials increased the organic matter content in soil (Saju *et al.*, 2002; Dubey and Patel, 2002). It has been shown by Saju *et al.* (2002) that addition of *T. harzianum* into organic media like neem cake, coir pith, farm yard manure and decomposed coffee pulp increase the population of antagonist in 3 days. So, the present investigations have been made to use low cost agricultural by-product for mass multiplication of *T. harzianum* by adopting farmer friendly technique.

MATERIALS AND METHODS

Biocontrol fungus *T. harzianum* was isolated from rhizosphere soil on TSM medium (Elad and Chet, 1983; modified by Saha and Pan, 1997) using dilution plate techniques (Harris and Sommers, 1968). Antagonistic potentiality of the biocontrol agent was assessed against many soil borne plant pathogens (Bose *et al*, 2005).The isolate was maintained on PDA slant at 4°C for further use.

Rapid screening of various locally available low cost agricultural waste materials was done for selection of substrate. Fifty gm of each material was moistened with distilled water and autoclaved at 121°C. for 30 min for consecutive two days in conical flask. After inoculation with *T. harzianum* the growth and sporulation were noticed and based on the performance only four organic waste materials were selected.

Four agricultural waste materials ,viz. rice bran (RB), wheat bran (WB), pulse bran (PB), orange peel (OP)j four oil cakes ,viz; neem cake (NC), mustard cake (MC), groundnut cake (GC) and linseed cake (LC) and various combinations of NC, MC, GC and LC (at 5,10 and 20% w/w ratio) with RB, WB, PB, and OP were used as substrate for mass multiplication of the *T. harzianum.* The moisture content of these substrates was maintained at 60% mhc. Substrates were inoculated with 3 mycelial discs of 6 mm diameter of *T. harzianum* and incubated in B.O.D. incubator at 28 ± 1°C. Observations were made in terms of colony forming unit (c.f.u)/g of substrate at 7 days interval after inoculation. On the basis of theses observations, suitable substrate was selected for large scale mass multiplication. The data recorded in the experiments were analyased according to Duncan's Multiple Range Test by transforming into their corresponding log values.

RESULT AND DISCUSSION

Among the thirteen substrates screened for mass multiplication of *Trichoderma harzianum* only wheat bran, pulse bran, rice bran, orange peel and sugarcane bagasse supported rapid growth and sporulation (Table 1). Though initially sugarcane bagasse supported good growth but still it was not considered in the experiment for the cause as high residual sugar content of sugarcane, it invited early

Table 1: Rapid screening of various low cost agricultural waste materials for growth of *T. harzianum*

Substrate	Growth*
Sugarcane bagasse	++
Paddy straw	+
Wheat straw	+
Banana peel	+
Orange peel	++
Wheat bran	+++
Pulse bran	+++
Shelled maize cob	+
Rice bran	+++
Tea waste	+
Coirpith	+
Potato peel	+
Saw dust	++

* The intensity of population. +=Medium; ++ = Good; + + + = very good

Table 2 : Effect of different agricultural by product on growth of *T. harzianum*

Substrate	Population of *T. harzianum* (c.f.ux10/g of substrate)		
	7 days	14 days	21 days
Wheat bran	21.6 (1.332)^{g}	103.8 (2.015)^{d}	201.4 (2.503)^{a}
Orange peel	12.4 (1.089^	61.8 (1.790)^{f}	140.0 (2.145)^{c}
Rice bran	17.2 (1.234)^{h}	91.4 (1.960)^{d}	174.8 (2.242)^{b}
Pulse bran	15.0 (1.173)'	71.4 (1.852)^{c}	153.8 (2.186)^{bc}

Figures in parenthesis are log transformed values
Means with same letter are not significantly different according to Duncan's multiple range test (P=0.05)

growth of many saprophytic organisms and thereby preventing further growth of *T. harzianum*. Four organic by-products i.e., wheat bran, rice bran, pulse bran and orange peel were assayed as low cost substrate for mass multiplication of *T. harzianum* as a means of evaluating a suitable delivery system of the antagonist under field scale (Table 2). It could be revealed from the result that wheat bran alone produced significantly higher population of the antagonist at all the age of incubation except 14 days where it was statistically insignificant with rice bran. Initially at 7 days of incubation wheat bran showed highest population (21.6 x 10^6 c.f.u./g of substrate) followed by rice bran (17.6 xlO^6 c.f.u./g of substrate) and pulse bran (15.0 x 10^6 c.f.u./g of substrate) respectively. At 21 days of incubation growth in terms of c.f.u. followed almost similar trend in all the substrates where the population of wheat bran (201.4 x 10^6 c.f.u/g of substrate) significantly differed from other three substrates, among which orange peel appeared to be least suitable for the growth of the organism producing only 140.0 x 10^6 c.f.u/g of substrate.

Table 3 : Effect of different oil cakes on growth of *T. harzianum*

Substrate	Population (c.f.u x 10^6/g of substrate)		
	7 days	14 days	21 days
Groundnut cake	40.6 (1.606)^{f}	77.8 (1.889)^{bc}	87.8 (1.943)^{b}
Neem cake	38.6 (1.585)^{f}	65.2 (1.813)^{d}	77.0 (1.886)^{bc}
Mustard cake	51.2 (1.709)^{c}	86.4 (1.936)^{b}	102.6 (2.010)^{a}
Linseed cake	26.4 (1.415)^{g}	48.8 (1.686)^{c}	68.0 (1.831)^{cd}

Figures in parenthesis are log transformed values
Means with same letter are not significantly different according to Duncan's multiple range test (P=0.05)

A comparison of four oil cakes, viz. ground nut cake, neem cake, mustard cake and linseed cake individually were also tested as substrates for supporting the growth of the antagonist (Table 3). Mustard cake appeared to be the best suitable substrate followed by groundnut cake throughout the experiment. Population of antagonist in ground nut cake (40.6 x 10^6 c.f.u./g of substrate) and neem cake (38.6 x 10^6 c.f.u. /g of substrate) at 7 days of incubation were statistically insignificant whereas the population of other two cakes, i.e. mustard cake (51.2 x 10^6 c.f.u./g of substrate) differed significantly. At 14 days of incubation the growth of the antagonist in terms of c.f.u. was accelerated and appeared to be the best one in this respect in mustard cake (86.4x 10^6 c.f.u./g) and this was sequentially followed in descending order by ground nut cake (77.8 x 10^6 c.f.u./g), neem cake (65.2 x 10^6 c.f.u./g) and linseed cake (48.8 x 10^6 c.f.u./g) and at this stage the population dynamics varied significantly among themselves. While it could also be observed from the same table that similar trend of growth was also maintained at 21 days where mustard cake (102.6 x 10^6 c.f.u./g) proved significantly superior followed by groundnut cake (87.8 x 10^6 c.f.u./g). Although population of the antagonist in neem cake was numerically higher than linseed cake but had no significant difference among them at 0.05% level.

Table 4 : Effect of wheat bran-oil cakes amended media on growth of *T. harzianum*

Substrate	Concentration of Cakes (w/w)	Population (c.f.u x 10^6/g)		
		7 days	14 days	21days
Wheat bran + Neem cake	5	63.4 (18.01)^{u}	119.6 (2.077)^{TM1}	214.2 (2.331)^{dcf}
	10	72.6 (1.859)st	132.6 (2.122)^{kt}	222.8 (2.348)^{de}
	20	66.6 (1.822)^{tu}	118.8 (2.074)^{mn}	203.2 (23.08)^{efe}
Wheat + Groundnut cake	5	76.8 (1.884)^{rs}	128.2 (2.107)^{lm}	221.4 (2.345)^{de}
	10	91.8 (1.962)^{q}	145.2 (2.162)*	230.8 (2.363)^{bod}
	20	102.8 (2.01 l)^{p}	159.6 (2.203)^{l}	252.6 (2.402)^{ab}
Wheat bran + Mustard cake	5	82.2 (1.914)^{r}	135.2 (2.131)^{kl}	228.8 (2.359)^{cd}
	10	96.4 (1.983)^{pq}	152.8 (2.184)^{l}	246.6 (2.392)^{bc}
	20	106.4 (2.025)^{op}	176.2 (2.246)^{l}	272.6 (2.435)^{a}
Whet bran + Linseed cake	5	48.6 (1.683)^{v}	I0b.6 (2.023)^{op}	183.8 (2.264)^{hi}
	10	61.2 (1.785)^{u}	115.2 (20.60)^{no}	190.4 (2.279)^{ghi}
	20	66.8 (1.823)^{tu}	135.4 (2.131)^{kl}	197.4 (2.295)^{fgh}

Figures in parenthesis are log transformed values
Means with same letter are not significantly different according to Duncan's multiple range test (P=0.05)

In a next set of experiment, wheat bran along with different combinations of oil cakes (Table 4) was evaluated for the growth of the antagonist. If we rapidly scan over the result, it could be stated that wheat bran amended with 20% (w/w) mustard cake (106.4 x 10^6, 176.2 x 10^6 and 272.6 x 10^6 c.f.u./g at

Table 5 : Effect of rice bran-oil cakes amended media on growth of *T.harzianum*

Substrate	Concentration of Cakes (w/w)	Population (c.f.u x 10 /g of substrate) 7 days	14 days	21days
Rice bran + Neem cake	5	55.6 (1.743)st	*108.4* (2.034)^{kl}	*192.6* (2.284)^{efg}
	10	59.20 (1.770)^{rs}	117.6 (2.070)^{jk}	203.6 (2.309)^{def}
	20	57.80 (1.760)^{s}	118.8 (2.074)^{jk}	201.4 (2.304)^{def}
Rice bran + Groundnut cake	5	63.80 (1.804)^{qr}	117.8 (2.071)^{k}	207.4 (2.317)^{de}
	10	65.80 (1.817)^{pq}	135.4 (2.131)'	221.6 (2.346)^{bcd}
	20	74.80 (1.873)°	142.2 (2.153)*	242.8 (2.385)^{ab}
Rice bran + Mustard cake	5	71.40 (1.852)^{op}	123.2 (2.090)'	*217.8* (2.338)^{cd}
	10	77.40 (1.888)"°	141.8 (2.151)*	235.6 (2.372)^{abc}
	20	83.20 (1.920)"	166.6 (2.211)"	253.6 (2.404)^{a}
Rice bran + Linseed cake	5	4O60 (1.606)"	*95.60* (1.980)^{m}	178.6 (2.252)^{gh}
	10	52.20 (1.716)'	101.2 (2.005)$^{1"1}$	184.8 (2.267)*
	20	61.20 (1.786)^{qrs}	112.2 (2.049/*	190.8 (2.280)^{efg}

Figures in parenthesis are log transformed valuesMeans with same letter are not significantly different according to Duncan's multiple range test (P=0.05)

7, 14 and 21 days of incubation) supported highest growth of the antagonist at all three days of assessment and this was sequentially followed by ground-nut cake, neem cake and linseed cake wheat bran combination. While considering the concentration of oil cakes, a 20% (w/w) combination always supported better growth than two other concentrations irrespective of the nature of the cakes and the population gradually increased with the incrément of the period of incubation at least up to 21 days, the highest period considered in this experiment.

Similarly the result of other three substrates viz. rice bran (Table 5), pulse bran (Table 6) and orange peel (Table 7) was also evaluated with three different concentrations (5, 10 and 20% w/w) of same oil cakes as before. The growth pattern with respect to bran-oil cake combinations at different concentrations and period of incubation followed the same pattern like that of wheat bran-oil cake combination . Twenty per cent mustard cake, irrespective of substrate provided maximum growth of the antagonist followed by ground nut cake, neem cake and linseed cake. A similar trend was also noted with respect to concentrations of oil cakes and days of incubation.

Commercial success of a biocontrol agent not only depends on its bio-efficacy or shelf life but also ease with which it can be mass multiplied on a suitable substrate that is easily available and relatively inexpensive. Godwin-Egein and Arinze (2001) used different food wastes,viz. ripe plantain, unripe plantain, cassava, yam, sweet potato, orange and yam peel in powder form for media preparations and as well as soil amended in pots. A wheat bran preparation was used as a food base for the growth and application of *T. harzianum* to soil as biocontrol agent by Chet *et al.* (1979).In present study growth and sporulation of *T. harzianum* in rice bran and wheat bran were very high as compared to pulse bran and orange peel. This may be due to presence of some growth promotion substances in rice bran

Table 6 : Growth of *T. harzianum* in pulse bran-oil cake amended media.

Substrate	Concentration of Cakes (w/w)	Population (c.f.u x 10^6/g of substrate) 7 days	14 days	21days
Pulse bran + Neem cake	5	41.8 (1.619)^{tu}	80.4 (1.905)^{mn}	155.8 (2.192)^{fg}
	10	47.2 (1.669)st	89.4 (1.950)^{lm}	189.4 (2.277)^{cd}
	20	50.6 (1.701)^{rs}	92.8 (1.966)^{lm}	185.6 (2.268)^{cd}
Pulse bran + Groundnut cake	5	50.6 (1.701)^{rs}	102.2 (2.009)^{kl}	181.6 (2.259)^{cde}
	10	61.8 (1.789)^{pq}	120.4 (2.080)^{ij}	201.2 (2.303)^{abc}
	20	68.4 (1.833)^{op}	136.6 (2.135)^{ghi}	220.2 (2.343)^{ab}
Pulse bran + Mustard cake	5	57.6 (1.757)^{qr}	112.6 (2.05iy^{k}	181.6 (2.259)^{cde}
	10	69.4 (1.839)^{op}	135.6 (2.132)^{hi}	196.8 (2.294)^{bc}
	20	75.2 (1.875)"°	150.0 (2.175)^{fgh}	229.6 (2.360)^{a}
Pulse bran + Linseed cake	5	31.6 (1.494)^{v}	76.6 (1.88)"°	159.0 (2.200)^{ef}
	10	38.6 (1.584)^{u}	84.2 (1.922)^{mn}	168.6 (2.226)^{def}
	20	42.2 (1.621)^{tu}	91.8 (1.962)^{lm}	182.2 (2.260)^{cde}

Figures in parenthesis are log transformed values
Means with same letter are not significantly different according to Duncan's multiple range test (P=0.05)

Table 7: Growth of *T. harzianum* on orange peel media amended with different concentrations of oil cakes

Substrate	Concentration of Cakes (w/w)	Population (c.f.u x 10^6/g of substrate) 7 days	14 days	21days
Orange peel + Neem cake	5	34.40 (1.533)^{r}	71.80 (1.855)^{k}	129.2 (2.110)^{ef}
	10	39.40 (1.594)^{pq}	83.40 (1.921)^{i}	149.4 (2.174)^{c}
	20	42.20 (1.624)^{p}	88.40 (1.946)^{i}	142.8 (2.154)^{cd}
Orange peel + Groundnut cake	5	37.60 (1.573)^{q}	68.40 (1.835)^{kl}	139.6 (2.144)^{cde}
	10	43.40 (1.637)^{p}	80.40 (1.905)^{ij}	171.8 (2.235)"
	20	48.60 (1.686)^{o}	112.6 (2.05 l)^{gh}	193.8 (2.287)^{a}
Orange peel + Mustard cake	5	41.60 (1.618)^{p}	88.60 (1.947)^{1}	144.0 (2.158)^{d}
	10	58.80 (1.769)^{m}	109.2 (2.038)^{h}	184.2 (2.265)^{ab}
	20	63.60 (1.803)'^{m}	121.6 (2.084)^{fg}	201.2 (2.303)^{a}
Orange peel + Linseed cake	5	28.00 (1.446)	53.60 (1.728)^{n}	115.8 (2.062)^{8*1}
	10	31.80 (1.500)	74.80 (1.873)^{ik}	131.8 (2.119)^{def}
	20	41.20 (1.615)^{pq}	84.60 (1.927/	144.8 (2.160)^{cd}

Figures in parenthesis are log transformed values
Means with same letter are not significantly different according to Duncan's multiple range test (P=0.05)

and wheat bran that lacks in pulse bran and orange peel.

This result supports the finding of Kousalya and Jeyarajan (1990) who observed that growth and sporulation of *T. harzianum* in chickpea husk medium was, very low due to poor nutritive quality. When wheat bran mixed with sawdust and tap water (3: 1:4 v/v) was head, it was found to be effective against *S. rolfsii* and *R. solani* under field condition (Kaur and Mukhopadhyay,1992). In same mixture, *T. viride* might have established and grown faster by secreting some cellulolytic enzymes which degraded the cellulose present in the saw dust (Mathivanan *et al.*, 1998). Gopalakrishnan *et al.* (2003) reported that brewery waste amended spent malt was found to be optimum for the growth of *T. harzianum* in solid state fermentation. Coconut water amended coir pith, shelled maze cob powder and black gram shell powder (Gandhi Kumar *et al.*, 2001), tea waste (Prakash *et al.* 1999) and oven dried orange peel (Godwin-Egein and Arizae, 2000) showed very good result in solid state technology.

Though nutritional quality in oil cake is very high in compared to different brans, the growth and sporulation of *T. harzianum* in oil cake was not as high as in brans. This is due to poor aeration and clump formation in oil cakes. Again the growth of the antagonist was very low in neem cake, pongmia cake and groundnut cake as compared to wheat bran, FYM etc. (Jahagirdar *et al*, 1998). Bandyopadhyay *et.al.* (2001) obtained good growth and sporultion in sesame cake followed by linseed and niger cake. It could be conclusively stated that aeration in the substrate appeared to be the important factor for optimum growth and sporulation of this antagonist when organic substrates are being used as growth media.

When different brans were mixed with oil cakes as conditioner, the growth of the antagonist showed the synergistic effect. Even growth and population of *T. harzianum* increased with increasing concentration of oil cakes. Oil cake being rich in nitrogen enhanced the initial establishment and subsequent spread of the biocontrol fungi through out the medium surface. Besides nitrogen, oil cake supplied many other nutrient i.e. phosphorus and sulphur that stimulated many enzymatic activity in fungus. However, addition of oil cakes in suitable concentrations with bran did not reduce the aeration of substrate and made it granular form. This result is in accordance with Jahagirdar *et al.* (1998) who reported combination of neem cake and organic waste material produced highest growth of antagonist. Addition of neem cake at 20% (w/w) concentration did not increase the population significantly from 10% concentration. This may be due to some inhibitory substance present in neem cake (Nimbidin which has been conclusively proved to be an antifungal substance) that suppress or stabilize the growth and sporulation of the strain used in the total experiment.

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