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Evaluation of shelf life of some value added organic formulations of *Trichoderma harzianum*

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A B S T R A C T

Shelf life of *Trichoderma harzianum* (Th_1AN) was studied during 2007-08 in some organic formulations like vermicompost, leaf manure, rice bran and FYM and also their combinations with two oil cakes, neem and mustard cake. The growth was estimated in terms of colony forming unit (c.f.u.)/g of substrate up to 120 days at 30 days interval. In all organic products or their amendment with oil cakes c.f.u. increased up to 60 days and then gradually declined. Among the four organic media, vermicompost retained 21.3 x 10^7 c.f.u./g at 120 days where as leaf mold, FYM and rice bran retained 20.1 x 10^7 c.f.u./g, 17.3 x 10^7 c.f.u./g and 15.1 x 10^7c.f.u. /g of substrate respectively at 120 days of incubation. When oilcake was amended with organic material, vermicompost + mustard cake produced high population of the antagonist upto 60 days.

Introduction

Shelf life of a biocontrol agent plays a significant role in storing a formulated product and generally varies depending upon the nature of food base (Papavizas, 1985). A sharp decline in population of *Trichoderma* was noticed by Prasad & Rageshwaran (2000b) in talc, kaoline based formulation that retained more than 10^6 viable propagules upto 90 days and the population decline below the optimum level by 120 days while bentonite appeared to be least suitable as a carrier showing a drastic reduction in population of *Trichoderma*. However significant difference in number of viable propagule between kaoline based conidial and chlamydospore formulation were observed at 60-180 days (Prasad *et al*. 2002). Antagonist multiplied in an organic food base has greater shelf life than that on inert inorganic carrier materials (Jahagirdar *et al*. 1998). There was an increase in bioagent propagule in wheat bran-kaoline granule upto 30 days followed by slow decline in number of viable propagule thereafter and at 90 days the granule retained substantial number of viable propagules.

In all formulations the organism survived more when stored at low temperature than at high and room temperature (Manav & Singh, 2003; Prasad & Rageshwaran, 2000a). Variable shelf of *T. virens* in carboxy methyl cellulose powder formulation was recorded when stored at room temperature (20-30ºC) and refrigerated temperature (5ºC) (Tewari & Mukhopadhyay 2001). Very scanty information was available to evaluate the shelf life of low cost media for long term storage and better disease management.

Materials and Methods

Biocontrol fungus *Trichoderma harzianum* was isolated from the rhizosphere soil of black pepper collected from Andaman and Nicobar Islands on *Trichoderma* specific medium (TSM) (Elad & Chet, 1983) modified by Saha and Pan (1997) using dilution plate technique (Harris & Sommers 1968). Antagonistic potentiality of the biocontrol agent was assessed against many soil borne plant pathogens (Bose *et al*. 2005; Pan, 2009; Pan & Jash, 2009). The isolate of *Trichoderma* was maintained on potato dextrose agar (PDA) at 4°C for subsequent use.

Four agricultural waste materials viz. Rice bran (RB), Vermicompost (VC), Leaf manure (LM), Farm yard manure (FYM) and two oil cakes Neem cake (NC), Mustard cake (MC) and their combinations at 20% and 10% w/w with RB, VC, LM and FYM were used as substrate for multiplication of the *T. harzianum* (Th_1 AN). The moisture content of these substrates was maintained at 60% moisture holding capacity (mhc). Substrates were mulched under white polythene cover in direct sun for 3 consecutive days, packed in polypropylene bags @ 100gm/bag and were inoculated with 5 ml of mycelia-conidial suspension using a disposable syringe, incubated in B.O.D. incubator at 28 ±1ºC with a subsequent sliring at 7 days. Observations were recorded in terms of colony forming unit (c.f.u)/gm of substrate on TSM at 30 days interval after inoculation upto 120 days for determination of shelf life.

Results and Discussion

Results (Table 1) showed that in all organic substrates or of their oil cakes amended media colony count (c.f.u./g) was increased upto 60 days and then gradually declined at 120 days. Among the four organic waste media, vermicompost retained 21.28 x 10^7 c.f.u. /g at 120 days where as rice bran, FYM and leaf mold maintained only 15.14 x 10^7 c.f.u. /g and 20.12 x 10^7 c.f.u./g and 17.34 x 10^7 c.f.u. /g respectively at 120 days. When storage time and media considered jointly it was found that vermicompost and leaf manure both were statistically insignificant.

Table 1.

Shelf life of *T. harzianum* in various organic substrates at room temperature

Substrate	Population (cfu x 10^7/g of substrate)			
	30days	60 days	90 days	120 days
Rice bran	22.74 (1.356)	26.08 (1.416)	21.16 (1.325)	15.14 (1.180)
Vermicompost	32.02 (1.505)	35.84 (1.554)	29.48 (1.469)	21.28 (1.327)
Leaf manure	30.54 (1.484)	35.12 (1.545)	28.04 (1.447)	20.12 (1.303)
FYM	24.28 (1.385)	28.74 (1.458)	22.90 (1.359)	17.34 (1.239)
SEm ± :	Treatment x days 0.007			
CD (P= 0.05):	Treatment x days 0.014			

*Figure in the parentheses indicates log transformed values

In different vermicompost amended media (Table 2) the population level of *T. harzianum* did not differed significantly upto 120 days. Vermicompost + mustard cake (20%) media produced 30.22 x 10^7 c.f.u. /g while

vermicompost+ neem cake (10%) showing significant difference (27.16 x 10^7 c.f.u. /g) at 120 days. Vermicompost + mustard cake (10%) retained low population (23.10 x 10^7 c.f.u. /g) at 120 days.

Leaf manure produced high colony count when different oil cakes were amended with it at 120 days (Table 3) though rate of declination was fast as compared to vermicompost.

In FYM amended media population of *T. harzianum* was also increased upto 60 days and then declined slowly (Table 4). Highest population of the biocontrol fungus was recorded in FYM + mustard cake (20%) media (29.12 x 10^7 c.f.u. /g) at 60 days of storage period followed by FYM + neem cake (20%) (27.84 x 10^7 c.f.u. /g). The other substrate rice bran (Table 5) follows the same pattern of result.

Table 2.

Shelf life of *T. harzianum* in vermicompost-oilcakes amended substrate at room temperature

Substrate	Population (cfu x 10^7/g of substrate)			
	30days	60 days	90 days	120 days
Vermicompost+ Neemcake 20%	33.2 (1.521)	39.04 (1.591)	32.16 (1.507)	27.16 (1.433)
Vermicompost+ Neemcake 10%	34.02 (1.531)	39.54 (1.597)	33.08 (1.519)	28.30 (1.451)
Vermicompost+ Mustardcake 20%	36.56 (1.563)	40.54 (1.607)	35.98 (1.556)	30.22 (1.480)
Vermicompost+ Mustardcake 10%	31.52 (1.498)	33.26 (1.521)	29.54 (1.470)	23.10 (1.363)
SEm ± :	Treatment x days 0.012			
CD (P= 0.05):	Treatment x days 0.020			

*Figure in the parentheses indicates log transformed values

Table 3.

Shelf life of *T. harzianum* in leaf manure-oilcakes amended substrate at room temperature

Substrate	Population (cfu x 10^7/g of substrate)			
	30 days	60 days	90 days	120 days
Leaf manure + Neemcake 20%	31.94 (1.504)	35.08 (1.545)	30.72 (1.487)	26.14 (1.417)
Leaf manure + Neemcake 10%	32.62 (1.513)	37.12 (1.569)	31.56 (1.499)	24.12 ()1.382
Leaf manure + Mustardcake 20%	34.24 (1.534)	38.68 (1.587)	29.12 (1.464)	28.10 (1.448)
Leaf manure + Mustardcake 10%	30.42 (1.483)	34.52 (1.538)	29.36 (1.467)	20.70 (1.315)
SEm ± :	Treatment x days 0.010			
CD (P= 0.05):	Treatment x days 0.034			

*Figure in the parentheses indicates log transformed values

Table 4.

Shelf life of *T. harzianum* in FYM-oilcakes amended substrate at room temperature

Substrate	Population (cfu x 10^7/g of substrate)			
	30 days	60 days	90 days	120 days
FYM+ Neemcake 20%	25.26 (1.402)	27.84 (1.444)	21.36 (1.329)	15.92 (1.201)
FYM + Neemcake 10%	23.68 (1.374)	25.74 (1.410)	21.62 (1.334)	17.12 (1.233)
FYM + Mustardcake 20%	25.46 (1.405)	29.12 (1.464)	24.38 (1.387)	19.60 (1.292)
FYM + Mustardcake 10%	22.84 (1.358)	24.64 (1.391)	20.26 (1.306)	13.88 (1.142)
SEm ± :	Treatment x days 0.011			
CD (P= 0.05):	Treatment x days 0.028			

*Figure in the parentheses indicates log transformed values

Since even after 120 days of storage the formulations recorded 13.88 – 30.22 x 10^7 c.f.u. /g, the product can be stored safely for 120 days. The high rate of survival in all the organic

substrates may be due to continuous support of nutritions from the media, high C/N ratio, absent of toxic materials and dessication tolerance of different spore forms in the media vis-a-vis production of very high rate of dormant propagules chlamydospores in compared to conidia in liquide fermentation technology. This result are in agreement with with the findings of Papavizas *et al.* (1984) where in they recorded 90% of viable propagules in the powder formulation even after 180 days. The alginate + pyrose pellets of *T. viride* retained 93% of original population after 90 days of storage (Fravel *et al*. 1985). Nakkeeran *et al*. (1997) standardized the storage condition to increase the shelf life of *Trichoderma* formulation and found that vermiculite bran acid fermented biomass of *T. viride* recorded the highest mean population in milky white bag and 20-30°C was optimum to store formulation. In the experiment, the increase in population upto 60 days indicated that using the different organic food base, *T. harzianum* continue to multiply during storage. The population of *Trichoderma* in the product is an important factor in deciding the quantity of the product necessary to apply for one ha of land and these types of product formulation could be easily prepared and stored for longer periods. These formulations could be conveniently used in nursery seed bed, horticulture, kitchen garden including fruit plantation and directly in field along with seed.

Table 5.

Shelf life of *T. harzianum* in Rice bran-oilcakes amended substrate at room temperature

Substrate	Population (cfu x 10^7/g of substrate)			
	30days	60 days	90 days	120 days
Ricebran + Neemcake 20%	23.12 (1.363)	27.78 (1.443)	21.68 (1.336)	15.88 (1.200)
Ricebran + Neemcake 10%	26.04 (1.415)	31.78 (1.502)	28.48 (1.454)	19.80 (1.296)
Ricebran + Mustardcake 20%	30.36 (1.482)	35.48 (1.549)	31.08 (1.492)	21.84 (1.339)
Ricebran + Mustardcake 10%	22.48 (1.351)	26.76 (1.427)	22.06 (1.343)	13.76 (1.138)
SEm ± :	Treatment x days 0.013			
CD (P= 0.05):	Treatment x days 0.028			

*Figure in the parentheses indicates log transformed values

Literature Cited

Adams PB. 1990 The potential of mycoparasites for biological control of plant disease. *Annual Review of Phytopathology* **28**: 59-2.

Bose S Jash S Roy M Khalko S Pan S. 2005 Evaluation of different isolates of *Trichoderma harzianum* against soil borne plant pathogens. *Journal of Interacademicia* **9**: 329-34.

Elad Y Chet I. 1983 Improved selective media for isolation of *Trichoderma* spp. or *Fusarium* spp. *Phytoparasitica* **11**: 55-8.

Fravel DR Marois JJ Lumdsen RD Connick WJ. 1985 Encapsulation of potential biocontrol agents in an alginate-clay matrix. *Phytopathology* **75**: 774-77.

Harris GE Sommers LE. 1968 Plate dilution technique for assay of microbial ecology. *Applied Microbiology* **16**: 330-34.

Jahagirdar S Siddaramaiah AL Narayanaswamy H. 1998 Screening of substrates for mass multiplication of *Trichoderma viride*. *Karnataka journal of Agricultural Science* **11**: 233-36.

Manav M Singh RS. 2003 Shelf life of different formulation of mutants and parent strain of *Trichoderma harzianum* at variable temparatures. *Plant Disease Research* **18**: 144-46.

Nakkeeran S Sankar P Jeyarajan R. 1997 Standardization of storage conditions to increase the shelf life of *Trichoderma* formulations. *Journal of Mycology and Plant Pathology* **27**: 60-63.

Pan S. 2009 Variability in induction of defense response in Bengal gram *(Cicer arietinum)* by *Trichoderma* species. *Journal of Mycology and Plant Pathology* **39**: 320-27.

Pan S Jash S. 2009. Production and regulation of cell wall degrading hydrolytic enzymes in mycoparasitic *Trichoderma* spp. *Journal of Mycology and Plant Pathology* **39**: 208-215.

Papavizas GC. 1985 *Trichoderma* and *Gliocladium*: biology, ecology and potential for biocontrol. *Annual Review of Phytopathology* **23**: 23-54.

Papavizas GC Dunn MT Lewis JA Beagle-Ristaino J. 1984 Liquide fermentation technology for experimental production of biocontrol fungi. *Phytopathology* **74**: 1171-75.

Prasad RD Rangeshwaran R. 2000a An improved medium for mass production of the biocontrol fungus *Trichoderma harzianum*. *Journal of Mycology and Plant Pathology* **30**: 233-35.

Prasad RD Rangeshwaran R. 2000b Shelf life and bioefficacy of *Trichoderma harzianum* formulated in various carrier materials. *Plant Disease Research* **15**: 38-42.

Prasad RD Rageshwaran R Anuroop CP Phani Kumar PR. 2002 Bioefficacy and shelf life of conidial and chlamydospore formulation of *Trichoderma harzianum* Rifai. *Journal of Biological Control* **16**: 145-48.

Saha DK Pan S. 1997 Qualitative evaluation of some specific media of *Trichoderma* and *Gliocladium* and their possible modifications. *Journal of Mycopathological Research* **34**: 7-13.

Tewari AK Mukhopadhyay AN. 2001 Testing of different formulations of *Gliocladium virens* against chickpea wilt complex. *Indian Phytopathology* **54**: 67-71.