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MASS MULTIPLICATION OF *TRICHODERMA HARZIANUM* FOR BIOCONTROL OF RHIZOME ROT OF GINGER

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Agricultural wastes and by product of sugar industry were tried for mass multiplication of *Trichoderma harzianum* which is effective against rhizome rot of ginger. Substrates were evaluated individually as well as in combination in the form of liquid extract and solid. It was observed that shelled maize cob supported highest growth individually liquid and solid form as well as with other substrates and molassess proved more effective for supporting growth of *T. harzianum*. Substrates tested for pathogen suppressiveness *in vitro*, saw dust was highly inhibitory to *P. myriotylum* (69%) but had little effect on *F. solani* (29%) while combination of shelled maize cob with saw dust was quite effective in suppressing growth of both the pathogens. This formulation with *T. harzianum* was also observed more effective when tested in sterilized soil against both the pathogens. Studies on shelf life of *T. harzianum* at room temperature revealed that population increased up to 40 days and then slightly declined up to 120 days. A combination of shelled maize cob with saw dust was found effective as compared to individuals and it's suppressiveness further increased by adding Triton-x 100 (2ml/kg).

Keywords : *Fusarium solani;* Molassess; *P. myriotylum;* Saw dust; Shelled maize cob; *Trichoderma harzianum.*

Introduction

Decades of laboratory experiments on biological weapons has given birth to a potential antagonistic fungi *Trichoderma* spp. alternative to chemical arsenals. As a result, several commercial products of *Trichoderma* have appeared in the market[1]. The mass multiplication of fungal antagonists is critical for the effective implementation of biocontrol of various crop diseases. Direct application of antagonists continues to be the principal method for introducing in to soil for biological control. Rhizome rot of ginger (*Zingiber officinale* Rose) is both soil as well as rhizome born[2,3] caused by *Pythium* or *Fusarium* sp. or both. The genetic base of resistance to rhizome rot appears to be limited and resistant varieties are not available[4]. *Trichoderma* spp., *Gliocladium virens* and *Pythium acanthophoron* were found effective in *in vitro* studies in inhibiting the growth of both the pathogens through production of volatile and / or non-volatile (diffusible) antibiotics, or through mycoparasitism[5,6]. The study was undertaken to develop a simple and effective technology for mass multiplication of potential resident biocontrol agent *T. harzianum* on some agricultural and industrial waste products and their shelf life in these formulations.

Materials and Methods

Organisms : Pure cultures of the rhizome rot pathogens *Fusarium solani* and *Pythium myriotylum* isolated previously from rotten ginger rhizomes and of biocontrol agent (*Trichoderma harzianum)* from the surface of healthy ginger rhizosphere from infested ginger fields with rot. The culture of *P. myriotylum* on corn meal agar and *F. solani* and *T. harzianum* were maintained on 0.1 per cent malt exract agar.

Evaluation of different substrates for multiplication of *T. harzianum*

For mass multiplication of *T. harzianum* three agricultural waste products viz. wheat straw, shelled maize cobs (powdered) biogas slurry and three industrial by products such as saw dust, molasses and sugarcane mud individually as well as in different combinations were tested in both liquid and solid forms.

Growth and sporulation of *T. harzianum*

(a) *In liquid extract :* For preparing extract from substrates, 100 of material was boiled in 500 ml of water for 20 minutes and filtered through double layer of muslin cloth and 24 ml of the extract dispensed in 150 ml flask for each treatment kept three flasks and autoclaved at 1.056 kg/sq cm pressure for 15 minutes. Each flask was inoculated with a 2 mm bit actively growing culture of *T. harzianum* and incubated at 28± 1°c for 7 days. The mycelial mats were filtered through Whatman filter paper no. 42, and dry weight of the mycelium was determined.

(b) *On solid substrates :* For comparing the growth and sporulation of *T. harzianum* was grown on sterilized substrates, dispensed in equal amount in conical flasks of 500 ml up to 2/3 parts alone as well in combinations, in 1:1 or 1:1:1 ratio, moistened with water

and autoclaved. Flasks were inoculated with a 10 mm bit actively growing culture of *T. harzianum*, keeping three replications and were incubated at 28±1°C for 7 days. For comparing the ability of growth and sporulation on these substrates colony forming units (CFU g^{-1}) were determined by dilution plate technique[7] on selective medium suggested by Budge and Whipps[8].

Effect of different substrates on growth of pathogens

The substrates were tested for their ability to suppress the growth of pathogens. 100 g of each substrate was boiled in 500 ml distilled water for 20 minutes and filtered through muslin cloth and the 500 ml volume of the extract was maintained by adding water. 25 ml extract was mixed in 100 ml Potato dextrose agar. For all the substrates combinations 50 ml mixture added in 100 ml PDA. 20 ml of these media were dispensed in screw cap bottles and autoclaved. Media were poured in sterilized petri plates in three replication along with plain PDA as a control. *P. myriotylum* colony diameter measured after 5 days and *F. solani* after 7 days. Growth inhibition per cent was calculated by the formula.

$$I = \frac{C-T}{C} \times 100$$

Where I = Inhibition Percentage
T = Average diameter of colony in treatment
C= Average diameter of colony in control

Testing of longivity *in vitro* of *T. harzianum*

After mass multiplication viability of *T. harzianum* was tested in the selected effective formulations after 7,40,80 and 120 days. These formulation containing biocontrol agent were stored in white propolythene bags which were sealed and kept in laboratory at room temperature.The c.f.u. of *T. harzianum* was detected by drawing random samples by dilution plate technique[7].

Results and Discussion

The data (Table 1) revealed that the maximum dry weight (565 mg) of mycelium of *T. harzianum* was in shelled maize cob with 5 per cent molasses followed by shelled maize cob (322 mg). Other substrates also supported growth of fungus but the dry weight was much inferior to above mentioned treatments. It is also observed that mycelium weight in combined substrates was more than that on individuals except shelled maize cob. On the contrary, combination of shelled maize cob with any substrate, except molasses, decreased the dry weight of the mycelium. Further, the inoculum density of *T. harzianum* in solid formulation was although good in all substrates, however, the maximum c.f.u. of *T. harzianum* was observed in the combination of shelled maize cob and biogas slurry (301 g) followed by shelled maize cob (286 g). The suitability of substrate revealed that shelled maize cob individually as well as in combination with molasses or biogas slurry supported luxuriant growth and sporulation of *T. harzianum*. Thus shelled maize cob, biogas slurry were found very useful and by-product of sugar industry molasses was very promising for supporting the growth of *T. harzianum*. Saw dust alone was not very much effective but in combination of shelled maize cob or molasses + clay become much better in supporting the growth and sporulation. Earlier saw dust with wheat bran has been found useful for multiplication of *Trichoderma* spp[9, 10].Molasses has also been found very promising in deep tank fermentations[11,12]. Shelled maize cob has been found useful in combination with czapeck's dox media for multiplying *T. harzianum*[13].

It was (Table 2) revealed that all selected treatments inhibit growth little or more of both the pathogens. However Saw dust was the most effective in inhibition of *P. myriotylum* (69.4%) and *F. solani* (29.0%) followed by combination of saw dust and Shelled Maize Cob found effective to inhibit growth of *P. myriotylum* (51.2%) and *F. solani* (28.5%)

The efficacy of selected formulations of *T. harzianum* in suppression of ginger rhizome rot pathogen in soil (Table 3) revealed that the inoculum density of *T. harzianum* was maximum in shelled maize cob (183) followed by in shelled maize cob + saw dust (151). The cfu of *T. harzianum* in shelled maize cob powder formulation amended in soil, was higher where *P. myriotylum* as compared to *F. solani* was inoculated in soil. The minimum inoculum density of *F. solani* was observed in shelled maize cob + saw dust + Triton x 100 which was 206 times less as compared to control. Population density of *P. myriotylum* was 10 times less in shelled maize cob + saw dust, 5 time less in shelled maize cob and not deducted from shelled maize cob + saw dust + Triton x 100 treatment as compared to untreated control. Shelled maize cob has been found useful in this study for growth and sporulation of *T. harzianum* and saw dust highly suppressive to the two ginger rot pathogens, saw dust alone was the most and shelled maize cob was less effective, but the

Table 1. Dry weight of mycelium and population (c.f.u.) of *Trichoderma harzianum* in solid and extracts of different substrates and their combinations.

S.N.	Substrates and their combination	Dry weight (mg) of mycelium in (24 ml) extracts	c.f.u. g^{-1} solid (1×10^5)
1.	Wheat straw	31	72
2.	Shelled maize cob	322	286
3.	Wheat straw + Shelled maize cob	163	125
4.	Saw dust	64	136
5.	Wheat straw + Saw dust	72	140
6.	Shelled maize cob + Saw dust	176	231
7.	Biogas slurry	83	157
8.	Wheat straw + Biogas slurry	56	84
9.	Shelled maize cob + Biogas slurry	190	301
10.	Saw dust + Biogas slurry	84	191
11.	Sugarcane mud	27	63
12.	Wheat straw + Sugarcane mud	34	82
13.	Shelled maize cob + Sugarcane mud	122	134
14.	Saw dust + Sugarcane mud	63	153
15.	Biogas slurry + Sugarcane mud	75	155
16.	Molasses	178	281
17.	Wheat straw + Molasses	214	136
18.	Shelled maize cob + Molasses	565	263
19.	Saw dust + Molasses	246	142
20.	Wheat straw + Saw dust + molasses	173	136
21.	Wheat straw + saw dust + sugarcane mud	81	207
22.	Wheat straw + clay + molasses	173	124
23.	Saw dust + clay + molasses	284	143
	S Em ±	1.47	2.9
	C. D. at 5%	2.97	5.9

Table 2. Effect of different substrates on growth of *P. myriotylum and F. solani.*

Substrate/combination	*P. myriotylum*		*F. solani*	
	Colony diameter (mm)	Per cent inhibition	Colony diameter (mm)	Percent inhibition
Wheat straw	61.6	14.4 (22.3)	67.0	6.7 (14.9)
Shelled Maize cob	60.3	16.2 (23.7)	67.5	6.0 (14.1)
WS + SMC	55.8	22.5 (28.2)	64.6	10.0 (18.6)
Saw dust	22.0	69.4 (56.4)	51.0	29.0 (32.5)
WS+Saw dust	36.6	49.2 (44.4)	53.1	26.0 (30.0)
SMC + SD	35.1	51.2 (45.6)	51.3	28.5 (32.3)
Biogas slurry	52.5	27.1 (31.3)	66.8	7.0 (15.3)
WS+Biogas slurry	49.1	38.8 (34.2)	67.0	6.7 (14.9)
SMC + Biogas slurry	64.0	11.1 (19.4)	66.1	8.0 (16.3)
SD + Biogas slurry	31.1	56.8 (48.8)	55.0	23.4 (28.9)
Control	70.0	-	71.8	-
SEM ±		1.3	1.0	
CD at 5%		2.7	2.1	

* Figures in the paranthesis are transformed value

Table 3. Population density (c.f.u.) of *T. harzianum* and *F. solani* and P. *myriotylum* in sterilized soil.

S.N.	Formulation	*T. harzianum* in/g soil		(x10^5)	
		F. solani	*P. myriotylum*	*F. solani* (1x10^5)	*P. myriotylum* 24 drops
1.	Shelled Maize cob	166	183	10	4.0
2.	Saw dust	101	101	21	7.0
3.	SMC + saw dust	153	151	3	2.0
4.	SMC + saw dust + Triton x - 100	123	121	2	0.0
5.	Control (without *T. harzianum*)	-	-	474	20

Table 4. Shelf life of different formuatlions of *T. harzianum* stored at room temperature.

S.N.	Formulation	c.f.u. of *T. harzianum* /g formulation (1x 10^5) Days incubation			
		7	40	80	120
1.	Shelled Maize cob	286.1	1305.3	970.0	824.0
2.	Saw dust	136.2	885.00	600.00	510.00
3.	Shelled maize cob+saw dust	231.5	1126.7	828.0	748.7

combination of both was found better effective in suppressing growth of both the pathgens. The suppressing ability of saw dust against ginger rot pathogens was earlier reported by Mathur *et al*[14]. It was also used as a carrier for the application of biocontrol agents for the management of ginger rhizome rot with good results[15, 16].

The shelf life studied at different time intervals up to 120 days on different formulations (Table 4). The cfu of *T. harzianum* greatly increased between the 7 to 40 days in all the formulations. The maximum growth and sporulation was recorded in shelled maize cob powder after 40 days followed by shelled maize cob + saw dust. The decline started after 40 days but was comparatively steady in shelled maize cob and shelled maiz cob + saw dust. The shelf life of *T. harzianum* in three common formulations was effective up to 120 days. Shelled maize cob supported maximum population followed by shelled maize cob + saw dust. Saw dust alone was not very effective in supporting growth and sustaining them for long time (up to 4 months) but itself was suppressive to the ginger rot pathogens. Therefore for large scale, mass production of *T. harzianum* by autoclaving of the shelled maize cob + saw dust substrates, has been found good not only for supporting growth but also for suppressing the ginger rot pathogens.

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