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EVALUATION OF SHELF LIFE OF *TRICHODERMA HARZIANUM* AND *GLIOCLADIUM VIRENS* ON DIFFERENT ORGANIC SUBSTRATES FOR BETTER USE EFFECIENCY

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ABSTRACT
Production of *Trichoderma harzianum* and *Gliocladium virens* on different substrate is essential for mass multiplication and better management of plant pathogens but it is more important that the shelf life of the product should have the capacity to effectively manage the pathogens. In this study shelf life of the organism has been evaluated. Some locally available organic substrate *viz*., Farm Yard Manure (FYM) Mushroom Littre (ML), Wheat Bran (WB), Rice Bran (RB), Maize Meal (MM) with their various combinations were evaluated for shelf life of the two bio control agents *viz*., *T. harzianum* and *G. virens*. Some modifications in the composition of substrates were also made to increase the qualitative merit in terms of spore population and chlamydospore formation. Molasses (Mol) @ 2% (v/w), ammonium tartarate (AT) @ 0.3% (w/w), $CaSo_4$ @ 0.03% (w/w) were used as amendments of substrate. Highest sporulation occurred in RB, WB and MM substrates. In addition to this, medium containing ML+FYM+2% molasses also gave good sporulation ($X10^{11}$). Chlamydospore formation was more in those substrates that were fortified with molasses, $CaSo_4$ and AT. The antagonists had a shelf life of 150 days (+) with cfu count ($x10^7$ cfu g^{-1}) in all amended substrates. However, antagonists grown in RB appeared superior over the other substrates both in terms of quality and quantity of spore formed.

KEYWORDS: *T. harzianum*, *G. virens*, organic substrate, mass multiplication, shelf life, cfu.

INTRODUCTION

The antagonistic potentiality of bio control agents has been considered to be directly dependent upon the food base used for mass multiplication (Lewis and Papavizas, 1987; Knudsen *et al.,* 1991). The use of conidial bio mass which is not only least fragile with limited chance of survival unless precolonised in a protected energy-rich habitat (Lewis and Papavizas 1987; Papavizas, 1985). The more durable survival structure appears to be the chlamydospore which takes a long time to develop in any medium (Lewis and Papavizas 1983). An attempt therefore has been made here to develop a technique for rapid production of chlamydospores assuming that some stress may act as an inducer for their development.

Shelf life of biological control agent is necessary to increase the viable cfu *i.e*. ability of the organism to control of plant pathogens and development of suitable delivery system at field scales (Mathivnan *et al*., 1998).The viable inocula must be produced in an inexpensive and commonly available agricultural produced substrates that provide a long shelf life to the antagonists (Mathivnan *et al*., 1998). It has been claimed that the economic mass production of biocontrol agent could be achieved by utilizing readily available crude agricultural wastes (Sangeetha and Jeyarajan,1993) and long shelf life is one of the pre requisite criteria for any commercial product (Prasad and Rangeshwaran, 2000). In this study various low cost media *viz*. Well decomposed FYM, Rice Bran (RB), Wheat Bran (WB), Maize Meal (MM) *etc*. with or without supplements like calcium sulphate ($CaSo_4$), ammonium tartarate and molasses were used for mass production and to increase the sporulation and enhancement of chlamydospore formation vis- a- vis to increase the shelf life of the product. This experiment was undertaken to find out the qualitative merit of the different substrates that support the prolonged shelf life as well as a good delivery system.

MATERIALS & METHODS

T. harzianum and *G. virens* were isolated from the soil on Trichoderma specific medium (TSM) (Elad *et al*., 1981) with modification (Saha and Pan, 1996) and purified in PDA medium.

1.Preparation of substrates for mass production of antagonists

Different organic substrates *viz.* Rice Bran (RB), Farm Yard Manure (FYM),Wheat Bran (WB), Maize Meal (MM) and various amendments like molasses (Mol) Calcium sulphate ($CaSo_4$), Ammonium tartarate (AT) were used for mass multiplication of *T. harzianum* and *G. virens*. Ammonium tartarate (AT) @3%(w/w) of the substrate was added in some treatments while molasses @2% (v/w) was added to some other treatments or their combinations and incubated for 10 days to stabilise the nutritional properties of the amended substrates.

The treatments were arranged as follows, T_1 =RB, T_2=WB, T_3=MM, T_4=RB+AT, T_5=WB+AT, T_6 =MM +AT, T_7= FYM, T_8= FYM +Mol, T_9=FYM+Mol+AT, T_{10}= FYM+ Mol+ AT+Caso4, T_{11}= FYM+Mol +AT +$CaSo_4$. Some of the treatments were supplemented with $CaSo_4$ @ 0.03% (w/w) to bring down the pH of the substrate at 5.5-6.0 for better growth. The moisture

content of the substrates were adjusted nearly 60% of its WHC.

Each of the treatments was replicated thrice. The substrates were packed @ 50gm in doubled layered polypropylene bags plugged with cotton. The packets were sterilized for two consecutive days at 15 psi for 20 minutes.

2.Inoculation of Substrate in polypropylene bags

Each of the sterilized polypropylene bag containing substrate was inoculated with four 6 mm diameter mycelia plugs of the selected antagonists (*T. harzianum* and *G. virens*) taken from the periphery of the actively growing 6 days old cultures.

The bags were incubated at a fixed temperature (28 $\pm 1^0$C) for a period of 21 days under 12 hrs alternate light and dark conditions in a BOD incubator.

3. Determination of Shelf-life

The shelf life of the antagonists in different substrates was estimated by following methods.

Spoonful of substrate was scooped out at random from different locations and diluted in 10ml sterile distilled water and shake vigorously in a wrist action shaker. This gave a mother suspension. The mother suspension was serially diluted up to 10^{-5} dilutions. The dilutions at 10^{-3} and 10^{-5} were used for determination of shelf life (Viability) on TSM as found suitable for respective treatment. The developing colonies were counted under a colony counter. This gave the total number of colony forming unit (cfu) which included all the viable propagules. Observations were recorded after different periods of incubations 30, 60, 90, 120, 150 DAI (Table 1 and Table 2; Fig.1 and 2).

RESULTS & DISCUSSION

1. Enumeration of conidia: chlamydospores ratio

The ratio of conidia and chlamydospore was enumerated by direct count of the total conidia with total chlamydospores using Haemocytometer under high power objective(40X) of a compound microscope from different microscopic field and thereafter calculating the ratio of conidia and chlamydospores developed (Table 3 and Table 4).

TABLE 1: Chlamydospores: Conodia ratio of *T.harzianum* on different substrates at different time intervals[#]

Substrates	7 DAI[*]	14 DAI	21DAI	28DAI
RB	-	-	1:81.5	1:11.8
WB	-	-	-	1:11.5
MM	-	1:5.6	1:1.7	1:1.4
RB +AT	-	1:91.2	1:75.0	1:42.8
WB + AT	-	1:29.5	1:15.6	1:12.1
MM + AT	-	1:5.1	1:1.2	1:1.2
FYM	-	1:12.3	1:6.5	1:5.3
FYM + Mol 2%	1:40.0	1:15.0	1:66.0	1:37.6
FYM + Mol2% + AT	1:1.8	1:1.7	1:3.6	1:3.9
FYM + Mol2% + CaSo4	1:41.2	1:21.0	1:13.6	1:9.8
FYM + Mol2% + CaSo4+AT	1:1.1	1:1.8	1:2.5	1:1.3

* Days after Inoculation,

Each insertion is an average of 20 different observations taken from each replication.

TABLE 2: Chlamydospores: Conodia ratio of *G. virens* on different substrates at different time intervals[#]

Substrates	7 DAI[*]	14 DAI	21DAI	28DAI
RB	-	1:239.0	1:210.6	1:185.0
WB	-	-	-	1:191.3
MM	-	-	-	1:230.1
RB +AT	-	1:185.2	1:103.2	1:90.3
WB + AT	-	1:173.0	1:110.7	1:87.8
MM + AT	-	1:250.0	1:192.8	1:138.7
FYM	-	1:42.3	1:30.2	1:15.2
FYM + Mol 2%	1:30.1	1:17.0	1:62.5	1:16.1
FYM + Mol2% + AT	1:3.0	1:6.2	1:10.9	1:4.2
FYM + Mol2% + CaSo4	1:32.0	1:23.2	1:15.6	1:8.7
FYM + Mol2% + CaSo4+AT	1:2.5	1:3.7	1:4.3	1:1.5

* Days after Inoculation

Each insertion is an average of 20 different observations taken from each replication.

TABLE 3: Quantitative enumeration of cfu* of *T, harzianum* on different substrates (x 10^6 g^{-1})**

Substrates	30DAI***	60DAI	90DAI	120DAI	150DAI
RB	430.00	331.90	160.00	43.40	27.00
WB	510.00	421.00	230.00	78.20	3.10
MM	322.00	124.00	35.00	29.00	13.00
RB +AT	420.50	310.40	150.20	71.30	25.50
WB + AT	540.00	410.00	220.50	98.75	45.50
MM + AT	320.50	150.50	71.00	30.00	15.20
FYM	20.50	12.50	4.20	2.00	1.20
FYM + Mol 2%	21.00	3.40	1.90	1.30	0.47
FYM + Mol2% + AT	86.00	8.60	0.90	0.80	0.70
FYM + Mol2% + CaSo4	90.00	20.10	2.50	0.92	0.85
FYM + Mol2% + CaSo4+AT	61.00	4.20	3.20	0.70	0.30
CD(P=0.01)	75.78	58.80	23.83	20.92	5.89

*colony forming unit (spore + mycelial fragments)
** Each insertion is an average of 3 replications.
*** Days after Inoculation

TABLE 4: Quantitative enumeration of cfu* of *G.virens* on different substrates (x 10^6 g^{-1})**

Substrates	30DAI***	60DAI	90DAI	120DAI	150DAI
RB	5100.00	4735.90	4433.30	65.10	30.90
WB	5600.00	6359.00	6100.00	333.00	42.00
MM	349.10	21.90	41.00	32.80	15.00
RB +AT	5271.00	4175.20	3481.00	180.20	40.20
WB + AT	5438.00	5210.20	5020.00	482.50	67.25
MM + AT	455.20	1200.20	60.00	30.20	14.25
FYM	120.00	62.50	20.50	8.20	4.10
FYM + Mol 2%	92.00	47.50	6.80	5.60	3.40
FYM + Mol2% + AT	16.20	8.30	5.70	5.20	4.40
FYM + Mol2% + CaSo4	110.50	58.20	10.50	5.20	2.50
FYM + Mol2% + CaSo4+AT	24.00	15.33	13.33	9.10	8.90
CD(P=0.01)	137.62	1086.59	15.97.96	88.92	16.40

*colony forming unit (spore + mycelial fragments)
** Each insertion is an average of 3 replications.
*** Days after Inoculation

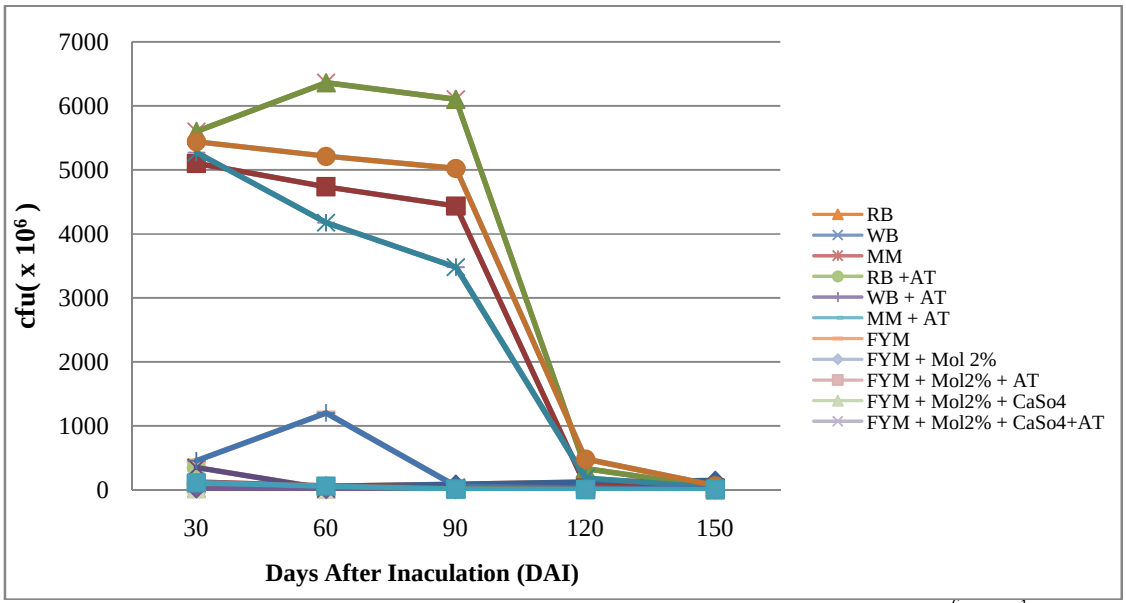

FIGURE1: Effect of different substrate on shelf life of *Trichoderma harzianum* (X10^6 cfu g^{-1})

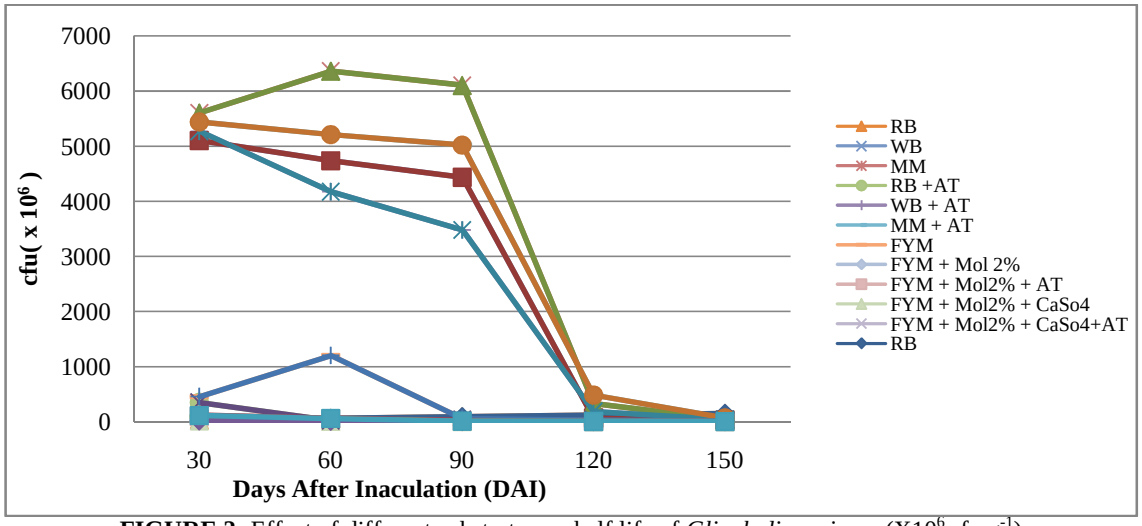

FIGURE 2: Effect of different substrate on shelf life of *Gliocladium virens* ($X10^6$ cfu g^{-1})

The ratio between chlamydospore and conidia produced by a particular antagonist on different substrates after different periods of inoculation *(viz.* 7DAI, 14DAI, 21DAI, 28DAI) have been given in the Table 1 for *Trichoderma harzianum* and Table 2 for *Gliocladium virens*.

It was clear from the table that the population of phialospores in any substrate after a given period of incubation was always higher then chlymadospore irrespective of treatments and type of antagonist. The highest population of chlamydospore of *T. harzianum* was obtain in Maize Meal + Ammonium tartarate at 28 DAI. This was subsequently followed by same organism on same substrate at 21DAI and 14DAI in a decending order. While in other substrates,other than MM alone, the population of chlamydospore was much less. Although in substrate annulled with different additives, the population of chlamydospore was higher compare to phialospore, but the total population of chlamydospore at the same time was less than they were produced either in MM or RB particularly at the later part of incubation. When molasses was used alone @ 2%(v/w) with FYM produced more phialospores over the other treatments and this was equally similar for *G.virens*. *G.virens* has been found to produce less chlamydospore in general over *T. harzianum*.

2. Determination of shelf life

This experiment was divided into two parts in the 1st part the total spore population of a particular antagonist at a specific date after incubation was determined under Haemocytometer. The spore population was expressed in terms of $x10^8$ g^{-1} of substrate. In second part spore suspension of antagonists was plated on TSM for determination of cfu (viable spore count) from the 30th day of incubation and was expressed in terms of $x10^6$ g^{-1} of substrate. The cfu included hyphal fragments, phialospore and chlamydospore all a viable state those were able to form a colony in an agar media**.** It appeared from the result (Table 1 and Table 2) that the cfu/g of substrate was always less than that of total spore population (phialospore+ chlamydospore) g^{-1} of substrate. The cfu count (Table 3 and Table 4) decreased gradually as a function of time. Nakkeran *et al.*, 1997 observed slow decline in population in formulated product when stored in room temperature after 75 days.

Fravel *et al.* (1988) working with an isolate of *T.viride* found that 50% of the total propagules remained viable for about 30 days in prills formulated synthetically with calcium chloride ($Cacl_2$) over 84 days when the prills were prepared with calcium gluconate. Gangadharan and Jeyarajan (1990) observed that various low cost and readily available media like wheat bran, rice bran, well decomposed FYM and similar some other substrate provided good sporulation of the antagonists and subsequently Sangeetha and Jeyarajan (1993) claimed rice bran, wheat bran were the good substrate of mass multiplication of these fungal antagonists.

The present investigations are in confirmety with the earlier observation. However augmentation of the substrate either with ammonium tartarate or with molasses did not improve the situation over the earlier studies in respect to chlamydospore production.

CONCLUSION

It can be concluded from the study that both the antagonists *T. harzianum* and *G. virens* though improve the phialospore chlamydospore ratio with storage duration but losses their shelf life drastically after 90days of storage.

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