

Original Research Article

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## Evaluation of Substrates and Carrier Material for Mass Multiplication and Shelf Life of *Trichoderma viride*

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### ABSTRACT

In eco-friendly management of soil borne plant diseases the *T. viride* plays an important role. Traditionally *T. viride* is multiplied on solid and liquid fermentation. Solid fermented biomass of *Trichoderma* consisted mainly of chlamydospores and conidia with some amount of mycelia fragments. High costs of substrate and storage methods are major problems in accelerate the production. Therefore, experiments were carried out to screen out locally available agricultural wastes and grains for solid fermentation process. Colony forming units (CFU) were maximum on sorghum and least on wheat 67.18 per g and  $31.38 \times 10^{-6}$  per g. in soybean bran showed wishes CFU and least on amongst the carriers tested talcum and gypsum supported the survival of *T. viride* which showed high CFU even after 180 days  $2.0 \times 10^7$  per g respectively. Charcoal and lignite supported the *T. viride* up to 150 days after 150 days the CFU started to decline at room temperature.

#### Keywords

*T. viride*, Colony forming unit (CFU), Mass multiplication

#### Article Info

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### Introduction

Extensive uses of agrochemicals have created certain environmental health problems and resistance in plant pathogens. Recently government has banned some chemicals. Therefore, the bio-control agents has gained importance and *Trichoderma* spp. are one of the most common fungal bio-control agent which is widely used to manage various soil borne diseases (4,5). In country like India solid substrate fermentation is preferred as it has some added benefits like use of large

number of grain and agro wastes can be used as a substrate. It requires the low capital investment, low energy expenditure, less water usage and lower waste output. Also it has high potential of productivity and reproducibility with less fermentation space. The biomass obtained from solid fermentation mainly consist chlamydospores, conidia and some amount of mycelia fragments which can be ground and mixed with carrier (Singh, 2006). Development of safe, easy cost based formulation which keeps the microorganisms live is the most important steps for production

of bio-pesticides. A final formulation must have longer shelf life at room temperature, best to handle, must be stable to temperature at the range 5-30°C.

Therefore, looking towards the need for cost effective production of eco-friendly bio-pesticide having longer shelf life present investigations were conducted to evaluate locally available substrates and carries for the bio-control agents.

### Materials and Methods

Rice grain, wheat grain, sorghum grains and maize grains, soybean bran, saw dust and groundnut shell were collected from department of Agronomy of COA, Parbhani. The 100 g grains were presoaked in distilled water for 15-20 minutes. In 250 ml capacity conical flasks the selected food grains and substrates (100 g) were taken. The mouth of the flasks was closed using cotton plugs. All conical flasks were autoclaved 121 °C (1.04 kg/cm<sup>2</sup>) for 20 minutes. The flasks were inoculated with 100 ml 7 days old broth of *T.viride* was incubated for 15 days at room temperature. Three replication of each treatment were kept. The biomass produced was evaluated with the help haemocytometer (Kumhar *et al.*, 2014).

### Shelf life of *Trichoderma viride* in different carrier material

The present study was carried out in Department of Plant Pathology, College of Agriculture, Parbhani. *Trichoderma viride* obtained from culture collection of the Department of Plant Pathology, College of Agriculture, Parbhani were used in the present studies.

In order to identify less cost base material, different carrier materials were tested for the mass multiplication of *Trichoderma. T. viride*

was multiplied in Potato Dextrose Broth medium for 12 days and entire biomass along with medium was incorporated into the sterile carriers *viz.*, talc, lignite, gypsum, fly ash, FYM, vermicompost, charcoal at 50 ml suspension per 100 g. The contents were thoroughly mixed; shade dried for two days and stored in polythene bags at room temperature (30 ± 2 °C). Viability of these formulated products was tested 30, 60, 90, 120, 150 and 180 days of storage. One gram sample was drawn from each formulation and transferred in 10 ml sterilized water in test tube and shaken thoroughly with vortex mixture for 3 minutes to make 10<sup>-1</sup> dilution. One ml suspension of stock solution was transferred in next test tube containing 9 ml distilled water by using sterilized pipette and shaken to make 10<sup>-2</sup> dilution and seven test tube to make up 10<sup>-7</sup> dilution. One mL of suspension was taken from the dilution of 10<sup>-7</sup> and transferred in petri plates containing 20 ml sterilized PDA and gently shaken to spread evenly. The experiment was conducted in Completely Randomised Design with three replications were maintained for each formulation. These petri plates were incubated at 25 ± 2 °C for two days and periodic observation were taken for the development of colonies of *T. viride*. Observations for colony forming units (CFU) were taken by using formula (Schmidt and Caldwell, 1967).

CFU per gram =

$$\frac{\text{CFU per plate} \times \text{dilution factor}}{\text{Weight of substrate (g)} \times \text{amount plated (ml)}}$$

### The experimental details

Design: Completely Randomized Design (C. R.D.)

Replication: Three

Treatment: Eight

T <sub>1</sub>	: Talc
T <sub>2</sub>	: Vermicompost
T <sub>3</sub>	: Fly ash
T <sub>4</sub>	: FYM
T <sub>5</sub>	: Charcoal
T <sub>6</sub>	: Lignite
T <sub>7</sub>	: Gypsum
T <sub>8</sub>	: Control

## Results and Discussion

The results (Table 1, Fig. 1) showed that, the seven different substrates *viz.*, rice grain, wheat grain, sorghum grain, soybean bran, saw dust, maize grain and groundnut shell were used for their mass production studies of *T. viride*. Among the different substrates evaluated significantly highest population was recorded in sorghum grain ( $67.15 \times 10^6 \text{g}^{-1}$  cfu) followed by maize grain ( $58.15 \times 10^6 \text{g}^{-1}$  cfu) and rice grain ( $41.67 \times 10^6 \text{g}^{-1}$  cfu) after 15 days of storage period on the *Trichoderma viride*.

The mean population significantly recorded was ranged from sorghum grain ( $67.18 \times 10^6 \text{g}^{-1}$  cfu) to saw dust ( $5.70 \times 10^6 \text{g}^{-1}$  cfu). Significantly highest population of *T. viride* was recorded in sorghum grain ( $67.15 \times 10^6 \text{g}^{-1}$  cfu) which was on par with maize grain ( $58.15 \times 10^6 \text{g}^{-1}$  cfu) for 15 days. Rice grain at 15 days ( $41.67 \times 10^6 \text{g}^{-1}$  cfu) was found next option. Significantly lowest and on par population of was recorded in case of saw dust ( $5.70 \times 10^6 \text{g}^{-1}$  cfu), groundnut shell ( $24.78 \times 10^6 \text{g}^{-1}$  cfu) in (Table 1, Fig. 1).

Rini and Sulochana (2007) evaluated eight different substrates for multiplication of *T. viride* and *T. harzianum* and reported that highest population of both the species of *Trichoderma* was observed in sorghum grain and lowest was on saw dust. Rini and Sulochana (2007) also concluded that sorghum grain with jaggery served as nutritional supplements and enhanced the conidial yield of *Trichoderma viride*. Sorghum

grain was found as superior grain substrate as it gave maximum population and proved very useful and effective for mass multiplication. Similar type of observation also observed at present investigation.

Results (Table 2, Fig. 2) showed that among the different carriers tested, gypsum and talc were significantly superior in supporting the survival of *T. viride* and showed that mean populations of  $12.3 \times 10^7$  and  $11.6 \times 10^7$  cfu  $\text{g}^{-1}$  product respectively.

All the carriers tested were showed decreasing trend in retaining the viability of *T. viride* with increasing period of storage. The mean population of *T. viride* gradually decreased from  $23.5 \times 10^7$  cfu  $\text{g}^{-1}$  product on the day of preparation to  $0.6 \times 10^7$  cfu  $\text{g}^{-1}$  product 180 days after storage.

Among the carriers tested, the reduction in survival of *T. viride* was minimum in talc and gypsum based product and these were on par with each other by recording significantly higher populations of respectively  $2.0 \times 10^7$  and  $1.5 \times 10^7$  cfu  $\text{g}^{-1}$  product even after 180 days of storage at room temperature. Charcoal and lignite carriers were observed viability till 150 days after storage but decline and was observed absence of *T. viride* after 150 days after storage ( Fig. 2).

Papavizas *et al.* (1984) recorded 90 per cent viable propogules of *Trichoderma* spp. in powder formulation 180 days after storage when stored at 5 °C. The alginate-pyrose pellets of *Trichoderma viride* retained 93 per cent of the original population 90 days after storage at 5 °C (Fravel *et al.*, 1985).

The minimum population of *Trichoderma* spp. required for an effective powder formulation was  $20 \times 10^6$  cfu  $\text{g}^{-1}$  as standardised by Jeyarajan *et al.* (1994).

**Table.1** Evaluation of substrates for mass multiplication of *Trichoderma viride* ( $\times 10^6 \text{g}^{-1}$ ) cfu

Sr. No	Substrates	Population ( $\times 10^6 \text{g}^{-1}$ ) cfu Days after incubation (15 Days)
1.	Rice grain	41.67
2.	Wheat grain	31.38
3.	Sorghum grain	67.18
4.	Soyabean bran	34.85
5.	Saw dust	5.70
6.	Maize grain	58.15
7.	Groundnut shell	24.78
	Mean	<b>37.67</b>
	SE $\pm$	<b>1.45</b>
	CD @ 1%	<b>4.32</b>

**Table.2** Effect of different carriers on the population of *Trichoderma viride* ( $\times 10^7 \text{cfu g}^{-1}$ )\*

Carriers	Days of storage ( $\times 10^7 \text{cfu g}^{-1}$ )*							Mean
	0	30	60	90	120	150	180	
Talc	23.8	19.2	16.2	12.3	8.3	4.4	2.0	12.3
Vermi-compost	23.4	18.4	13.2	8.7	6.6	2.9	0.4	10.5
Fly ash	23.3	18.3	12.3	8.1	4.6	1.9	0.2	9.8
FYM	23.7	18.4	12.6	8.0	6.2	2.6	0.1	10.2
Charcoal	23.5	18.2	12.1	7.5	4.7	2.0	0.0	9.7
Lignite	23.2	17.4	12.0	7.4	4.2	1.8	0.0	9.4
Gypsum	23.3	18.9	15.2	11.2	7.7	3.9	1.5	11.6
Mean	23.5	18.4	13.4	9.0	6.0	2.8	0.6	
SE $\pm$	<b>0.819</b>	<b>0.782</b>	<b>1.080</b>	<b>0.835</b>	<b>0.810</b>	<b>0.523</b>	<b>0.125</b>	
CD @ 1%	<b>2.438</b>	<b>2.329</b>	<b>3.215</b>	<b>2.487</b>	<b>2.413</b>	<b>1.559</b>	<b>0.375</b>	

\*Mean of three replications

Fig.1 Evaluation of substrates for mass multiplication of *Trichoderma viride* ( $\times 10^6 \text{g}^{-1}$ ) cfu

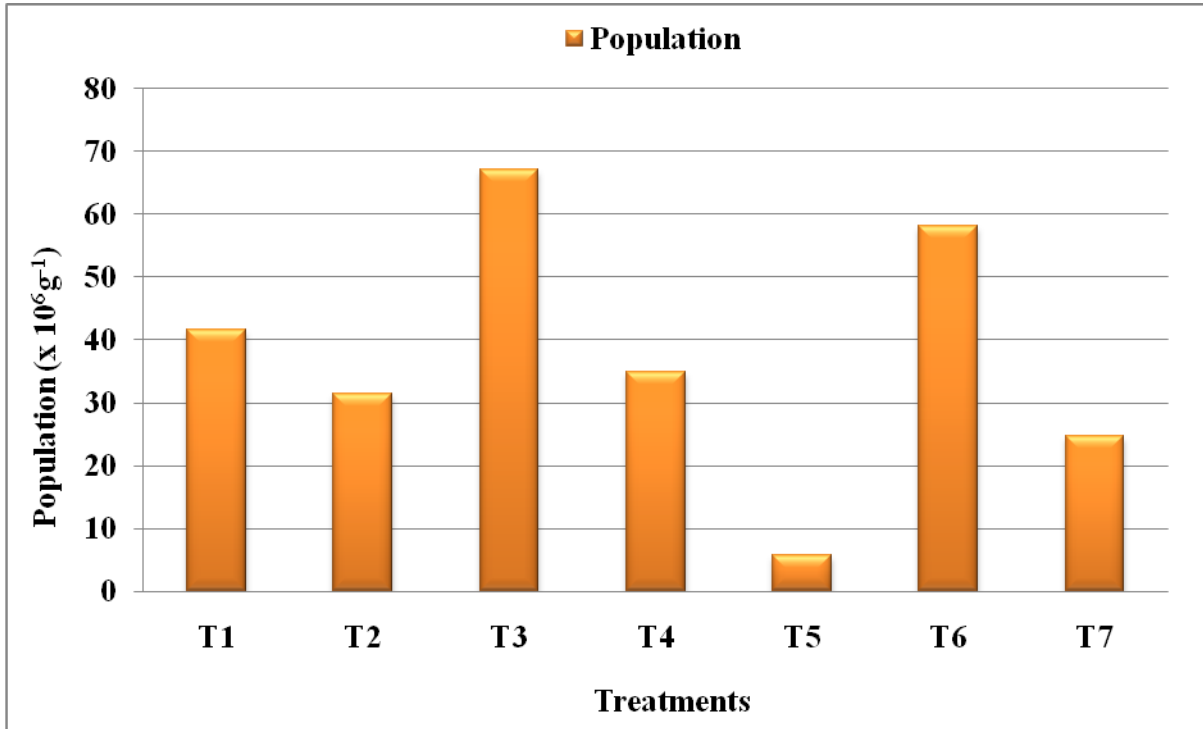
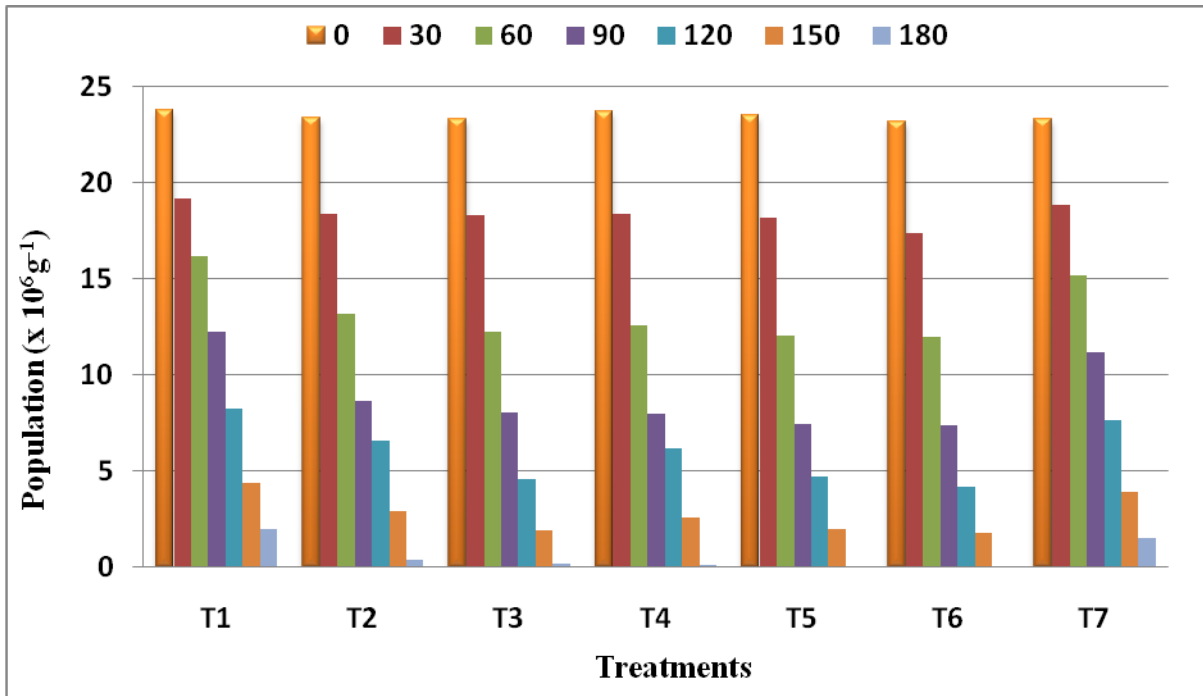


Fig.2 Effect of different carriers on the population of *Trichoderma viride* ( $\times 10^7 \text{cfu g}^{-1}$ )\*



The result of the present study indicated the suitability of gypsum and talc as carrier material for the commercial preparation of *Trichoderma*. An ideal carrier material for the mass multiplication of biocontrol agents should be inexpensive and easily available (Gaind and Gaur 1990).

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