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Mass multiplication and Shelf life of *Trichoderma hamatum* in different solid based carriers

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Abstract
In the present study five different formulations with *Trichoderma hamatum* i.e., Castor cake, Gypsum, Vermicompost, Talc powder & FYM were prepared and the colony forming units (cfu) was highest initially, but gradual decline was recorded with the increase in storage time. The result revealed that all the formulations contained initial cfu count in range of 5.3 to 11.62 X 10^7 cfu / g. However, maximum cfu was observed in vermicompost (5.31 X 10^9 cfu / g) followed by FYM (3.59 X 10^9 cfu / g). The maximum population of *Trichoderma hamatum* (cfu) of 5.31 x 10^9 cfu/g was observed in Vermicompost which was followed by Farm yard manure with population of 5.39 x 10^7cfu/gram formulation after 30 days of storage. After 180 days (6 months) population of *Trichoderma hamatum* cfu of 1.28 x 10^9 was found in Vermicompost followed by Farm yard manure with population (cfu) of 0.84 x 10^9 cfu/g of formulation.

Keywords: *Trichoderma hamatum*, shelf life, different carriers, mass multiplication

Introduction
Modern agriculture depends largely on the use of chemical inputs, such as pesticides and fertilizers, to control plant pathogens and to enhance crop yield. No doubt these chemicals enhances agriculture productivity but health concerns and environmental hazards associated with the use of chemical pesticides have resulted in an increasing interest in biological control as a promising alternative or a supplemental way of reducing the use of agro-chemicals. Some naturally occurring soil bacteria and fungi have demonstrated great potential to antagonize crop pathogens, hence, biological control involving the use of such beneficial microorganisms for plant protection is being considered as a viable substitute to reduce the use of chemical pesticides. These beneficial microorganisms need some suitable carrier for their delivery, which can support their life during storage and transportation. The biocontrol activity of Trichoderma is important not only to agriculture and its crops but also the environment as it does not accumulate in food chain and thus don't harm to the plants, animals and humans (Monte and Llobell, 2003; Perveen and Bokhari, 2012; Reena *et al*., 2013) [3, 4, 5]. Trichoderma as a potent fungal biocontrol agent against a range of plant pathogens has attracted considerable scientific attention (Choudhary *et al*., 2013; Santosh Reddy *et al*. 2014; Rini and Sulochana, 2007; Tewari and Mukhopadhyay, 2001) [2, 8, 6, 10]. Different organic media like neem cake, coir pith, farmyard manure, and decomposed coffee pulp also have been suggested for its multiplication (Saju *et al*., 2002) [7]. Combinations of different carriers with different proportion were found effective in maintaining the population (cfu) of *Trichoderma spp* for the period of 120 days.

Material and method
Castor oil cake, Gypsum, Talc powder, Vermicompost and FYM used during present investigation, were collected from local agricultural product-processing units and university vermicompost units. These materials were cleaned to remove any unwanted debris such as stones and foreign plant matter. Cakes were crushed in a heavy pestle & mortar to prepare a coarse powder (particles of approximately 1 mm diameter). Castor oil cake was mixed with sterilized distilled water (SDW) (10: 2.5, w/v); to maintain 25% moisture (w/v) and placed in conical flasks of 250 ml capacity @100.00 gram/flask and other carriers were autoclaved at 121.6°C (1.1 kg/cm2) for 20 minutes.
Trichoderma hamatum fermented biomass was formulated in castor oil cake, gypsum, Talc power, vermicompost and farmyard manure. Upon harvest at 7th day, the biomass was mixed with fine powder of these five carriers in 1:10 proportion and kept for three days under shade for drying.

The final formulated products contain 10% moisture were packed in polythene bags @ 500 g / pack and then stored at 30ºC for 6 months. Viability of these formulated products was tested for 6 months. Samples from these packets were drawn at 15 days intervals and tested for viability of the formulated product. Populations of these biocontrol agents were tested monthly taking 100mg of the product and diluted to 10^7 times. One gram biomass was taken in 1ml sterile water and mixed thoroughly by vortex mixture and allowed to stand. Then from the supernatant, serial dilution was prepared up to 10^7 times. From the 10^7 dilution, 100 µl of suspension was taken and spread uniformly on Petri dishes containing potato dextrose agar medium using a spreader without disturbing surface of the medium. Five replications were maintained for each formulation. The petriplates were incubated at 28ºC for two days and numbers of colonies were counted.

Later, the populations of the bacterial and fungal biocontrol agents were evaluated by serial dilution technique using formula given by Aneja (2003) [1].

$$\text{Number of cfu/g} = \frac{\text{Number of colonies}}{\text{Amount plated x dilution}}$$

Result and discussion

Screening of different carriers for mass multiplication of *Trichoderma hamatum*

The data pertaining to the effect of various substrates on the population dynamics of *Trichoderma hamatum* on different carriers have been presented in Table 1. It is evident from the table, that among the substrates tested after 30 days of incubation (DAI), the highest mean population of T. *hamatum* in the form of CFUs (5.3×10^9) was supported by Vermicompost, which was significantly higher than the mean population (3.59×10^9) supported by Farm yard manure followed by the mean population (2.51×10^9) supported by Talc powder, while the least mean population cfu count (1.16×10^9) supported by Gypsum which was at par with the mean population count (1.19×10^9) supported by castor oil cake.

In the present study five different formulations with *Trichoderma hamatum* i.e., Castor cake, Gypsum, Vermicompost, Talc powder & FYM were prepared and the colony forming units (cfu) was highest initially, but gradual decline was recorded with the increase in storage time. Longevity of *Trichoderma hamatum* was studied up to 180 days and the mean population of *Trichoderma hamatum* was higher enough in vermicompost and FYM which were found easily by farmers, ecofrindly as well as cheap.

Based on these findings it can be concluded that any of these carriers i.e., Vermicompost and FYM can be readily used for mass multiplication of Trichoderma hamatum with a very high level of population dynamics of fungal antagonist. Utilization of Vermicompost and FYM as substrate for mass multiplication will help in enhancing fertility status of field soil in addition to minimizing the risks of disease occurrence.

Ramji singh *et al.*, (2015) has reported that De-oiled cakes of four trees born oilseeds (TBOs) viz., Neem, Jatropha, Mahua and Karanja were tested for their suitability for mass multiplication of *T. Harzianum.* In addition to these four de-oiled cakes, two composts i.e., FYM and Vermicompost were also tested for their suitability and to have a comparison with these de-oiled cakes in supporting population dynamics and longevity of *T. harzianum*.

Table 1: Shelf life of *Trichoderma hamatum* in different formulations

***Trichoderma hamatum* Formulations in different carriers (Base)**	**CFU of *Trichoderma hamatum* (x 10^7)**					
	Days after Incubation (DAI)					
	30 DAI	**60 DAI**	**90 DAI**	**120 DAI**	**150 DAI**	**180 DAI**
T_1-Castor cake	119.75 (10.97)	97.75 (9.67)	87.75 (9.41)	82.25 (9.17)	55.00 (7.47)	46.00 (6.85)
T_2 -Gypsum	116.25 (10.82)	100.0 (10.63)	93.00 (9.65)	78.75 (8.90)	56.75 (7.59)	45.75 (6.82)
T_3-Talc powder	251.50 (15.88)	194.25 (13.89)	180.00 (13.38)	156.25 (12.44)	67.75 (8.26)	57.00 (7.58)
T_4-Vermicompost	530.50 (22.99)	429.75 (20.73)	399.75 (20.00)	320.50 (17.92)	220.25 (14.85)	128.00 (11.34)
T_5-FYM	359.50 (18.98)	288.75 (17.08)	252.75 (15.92)	201.00 (14.20)	120.00 (10.98)	84.50 (9.23)
SE(m) ±	21.29	14.40	13.66	11.86	7.50	5.38
CD (0.05)	64.77	43.80	41.55	36.08	22.83	16.38

(Figures in parentheses indicate corresponding square root transformation values)

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