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STUDIES ON *TRICHODERMA VIRIDE* FORMULATIONS FOR ENHANCING ITS STORABILITY

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ABSTRACT
The present investigation was carried out to evaluate the viability in different formulations of *T. viride* and also assessment of moisture content present in using formulations *of T. viride*.Seven different carrier base formulations such as chalk powder, talc powder, charcoal powder, chalk tablet, talc tablet and charcoal tablet with three concentrations 5%, 10%, 15%, one capsule formulation*T.viride* and culture of *T. viride* (ISO-1) were evaluated on different period to observe their viability. Different formulations of *T. viride* maximum (80×10^7 cfu/g) was recorded in 15% charcoal powder followed by capsules (78.67×10^7cfu/g),upto 260 days storage. However, least recorded in 5% *T. viride*(1×10^7cfu/g) in talc powder. Viability were absent in chalk powder and tablets at different concentration observed till 100 DAI but decline to zero viability. In moisture content, Maximum (34.7%) moisture content was retained up to 260 days of storage in 15% charcoal powder followed by 10% *T. viride* charcoal powder (30.28%) while it was least recorded in 5% talc powder(2.2%).It was found that the storability maximum in *T. viride* 15% in charcoal powder with high moisture.

INTRODUCTION

The use of biological control agents as an alternative to fungicides is increasing rapidly in the present day agriculture due to the deleterious effects of chemical pesticides. *Trichoderma* spp. and *Pseudomonas* spp. have been reported to secrete an array of chemically diverse anti-microbial secondary metabolites and hydrolytic enzymes such as proteases, cellulases, chitinases, lipasees etc., which help it in host recognition and pathogen control pesticides (Singh *et al.*, 2013). *Trichoderma* species have been investigated as biological control agents (BCAs) for over 70 years but it is only recently that strains have become commercially available. Knowledge concerning the behavior of these fungi as antagonists is essential for their effective use since they can act against target organisms in several ways by producing extracellular enzymes antifungal antibiotics and also be competitors to fungal pathogens (Mathews *et al.,* 2010). *Trichoderma viride*, a fungal biocontrol agent is widely used for the management of soil-borne diseases (Chung and Choi 1990; Ramakrishnan *et al.,* 1994; Sankar and Jeyarajan, 1996). The major aspects of successful biological control technologies include the establishment of product, formulation and delivery system for microorganism that enable them for efficient disease control. The mass production systems should be compatible with industrial and commercial development methods and field application. So formulations of *Trichodermaviride* required to find out suitable media therefore, the first step for the formulations of any bio-control agent is to identify the suitable substrates, The type and form of substrate i.e. broth and solid may also vary according to the specific purpose for which bio-control agent biomass is required. The quality of a microbial bio-protectant is dependent on the propagate density in the biomass and its ability to survive processing (Harman *et al.*, 1991). Production of adequate quantities of good quality inoculum is an essential component of the biocontrol programme. Therefore the present study was aimed to find out the storability of *Trichoderma viride* in different formulations with the various concentrations of *Trichoderma viride* and assessment of moisture content in charcoal and talc powder in formulation of *T. viride*.

MATERIALS AND METHODS

Viability of *T. viride* different formulations under *in vitro* condition

Seven carrier base formulations of *T. viride* i.e. chalk, talc and charcoal powder and their tablet form with the three concentrations and one capsule form were evaluated for viability form 20 days to 260 days and observed at 20 days interval by adopting the following methods (Elad and Chet 1983).Different formulation of *T. viride* were examine *T. viride* 5% in chalk powder, *T. viride* 10% in chalk powder, *T. viride* 15% in chalk powder, *T. viride* 5% in talc powder, *T. viride* 10% in talc powder, *T. viride* 15% in talc powder, *T. viride* 5% in charcoal powder, *T. viride* 10% in chalk powder, *T. viride* 15% in charcoal powder, *T. viride* 5% in chalk tablet, *T. viride* 10% in chalk tablet , *T. viride* 15% in chalk tablet, *T. viride* 5% in talc tablet, *T. viride* 10% in talc tablet , *T. viride* 15% in talc tablet , *T. viride* 5% in charcoal tablet , *T. viride* 10% in charcoal tablet, *T. viride* 15% charcoal tablet, *T. viride*

capsule and fresh culture as control treatment for compare.

One gram sample was drawn from each formulation and transferred in 10mL sterilized water in test tube and shaked thoroughly with vortex mixture for 3 minutes to make 10^{-1} 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^{-2} dilution and seven test tube to make up 10^{-7} dilution. One mL of suspension was taken from the dilution of $10^{-7}$and transferred in petri plates containing 20mL sterilized PDA and gently shaken to spread evenly. These petri plates were incubated at 25 ± 2ºC 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 and Aneja, 2003)

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

Assessment of moisture content in charcoal and talc powder in formulation of *T. viride* after 260 days

For estimation of moisture content some formulations selected were*T. viride* 5% in charcoal powder, *T. viride* 10% in charcoal powder, *T. viride* 15% in charcoal powder *T. viride* 5% in talc powder, *T. viride* 10% in talc powder and *T. viride* 15% in talc powder base formulation of *T. viride* at 260 days of storage was studied. For this purpose, 10g of each formulation was taken in glass petridishes. These formulation were dried in oven at 40ºC till constant weight. Thereafter moisture content was determined by dry weight of sample for each formulation.

RESULTS AND DISCUSSION

The present investigation was carried out to evaluate the viability in different formulations of *T. viride*.

Seven carrier base formulations with three concentrations and one culture of *T. viride* (ISO-1) were evaluated on different period to observed their viability and data are presented in the table-1.It is evident from the data that among the different formulations of *T. viride* maximum (80 × 10^7 cfu/g) was recorded in 15% charcoal powder followed by capsules (78.67 × 10^7cfu/g), *T. viride* 15% charcoal tablets (77 × 10^7cfu/g), *T. viride*10 % charcoal tablets (67.67 ×10^7cfu/g) and *T. viride* 10% charcoal powder (67 × 10^7cfu/g)upto 260 days storage, (1 × 10^7cfu/g). However, least recorded in 5% *T. viride* (1 × 10^7cfu/g) in talc powder and next were found in *T. viride* 5% and 10% in talc powder tablets. In other formulation the population of *T. viride* was varied from 2- 5 X 10^7cfu/g.at 260 days.

Viability were absent in chalk powder and tablets at different concentration observed till 100 DAI but decline to zero viability, showed absence of *T. viride* after 100 DAI. Figure 1 present upto 260 days storage highest to lowest rate of cfu.

A biocontrol formulation should posses several desirable characteristics such as ease of preparation and application, stability, adequate shelf life, abundant viable propagules and low cost of production. Inthe present findings are matched

Table 1: Evaluation of viability of *T. viride* in different formulations

S. No.	Formulation	x 10^7CFU per gram 20 DAI	40 DAI	60 DAI	80 DAI	100 DAI	120 DAI	140 DAI	160 DAI	180 DAI	200 DAI	220 DAI	240 DAI	x 10^7CFU per gram 260 DAI
1	*T.viride* 5% chalk powder	83.00	+	+	+	+	-	-	-	-	-	-	-	0.00
2	*T.viride* 10% chalk powder	83.67	+	+	+	+	-	-	-	-	-	-	-	0.00
3	*T. viride* 15% chalk powder	85.67	+	+	+	+	-	-	-	-	-	-	-	0.00
4	*T.viride* 5% Talc powder	92.00	+	+	+	+	+	+	+	+	+	+	+	1.00
5	*T.viride* 10% Talc powder	93.00	+	+	+	+	+	+	+	+	+	+	+	2.00
6	*T. viride* 15% Talc powder	94.00	+	+	+	+	+	+	+	+	+	+	+	2.00
7	*T. viride* 5% charcoal powder	93.00	+	+	+	+	+	+	+	+	+	+	+	47.67
8	*T. viride* 10% charcoal powder	94.00	+	+	+	+	+	+	+	+	+	+	+	67.00
9	*T.viride* 15% charcoal powder	95.00	+	+	+	+	+	+	+	+	+	+	+	80.00
10	*T.viride* 5% chalk powder in tablets	83.67	+	+	+	+	-	-	-	-	-	-	-	0.00
11	*T.viride* 10% chalk powder in tablets	86.00	+	+	+	+	-	-	-	-	-	-	-	0.00
12	*T. viride* 15% chalk powder in tablets	88.00	+	+	+	+	-	-	-	-	-	-	-	0.00
13	*T. viride* 5% Talc powder in tablets	93.00	+	+	+	+	+	+	+	+	+	+	+	1.67
14	*T.viride* 10% Talc powder in tablets	94.00	+	+	+	+	+	+	+	+	+	+	+	1.67
15	*T.viride* 15% Talc powder in tablets	96.00	+	+	+	+	+	+	+	+	+	+	+	5.00
16	*T. viride* 5% charcoal powder in tablets	94.00	+	+	+	+	+	+	+	+	+	+	+	59.00
17	*T.viride* 10% charcoal powder in tablets	95.00	+	+	+	+	+	+	+	+	+	+	+	67.67
18	*T. viride* 15% charcoal powder in tablets	96.67	+	+	+	+	+	+	+	+	+	+	+	77.00
19	*T.viride* Capsule	98.00	+	+	+	+	+	+	+	+	+	+	+	78.67
20	Fresh culture	99.00	+	+	+	+	+	+	+	+	+	+	+	95.00

+ = Present of *T.viride*,- = Absent of *T. viride*

Table 2: Determination of moisture content in charchoal and talc powder formulations

S.NO.	Treatment	Moisture Percent			
		24 hr	48hr	72hr	96 hr After
1	Charchoal powder 5%	13.82	19.35	22.76	22.77
2	Charchoal powder 10%	18.67	27.53	30.24	30.28
3	Charchoal powder 15%	26.21	32.75	34.66	34.70
4	Talc powder 5%	0.55	1.36	2.19	2.20
5	Talc powder 10%	2.60	3.20	3.85	3.87
6	Talc powder 15%	0.84	1.61	2.40	2.40

Figur 1: formulations of *T. viride*

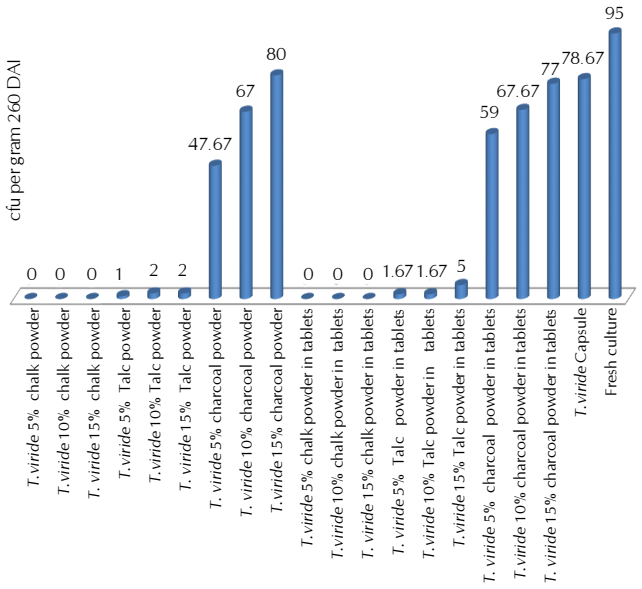

Figure 2: Evaluation of viability of *T. viride* in different formulations at 260 days

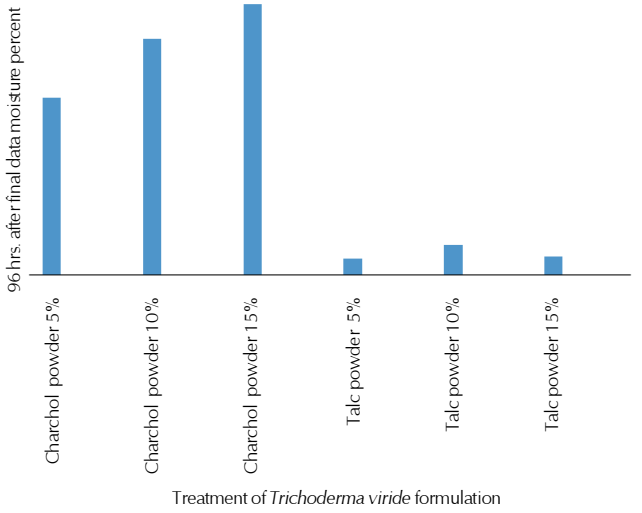

Figure 2: Effect of Percentage moisture in charchol powder and talc powder formulations after 96 hr

with the results of Sanjiv *et al.* (2013) who reported sorghum grains charchoal based formulations of *T. viride* survive upto 120 days. Similar types of results were also worked out by Shafa *et al.* (2007) on laic and gypsum based formulation of *T. viride* with excellent viability (195-255 day).

Assessment of moisture percent in charcoal and talc powder

This study was conducted to determine the moisture content in charcoal and talc powder formulation. The data are presented in Table 2 clearly that maximum (34.7%) moisture content was retained up to 260 days of storage in 15% charcoal powder followed by 10% *T. viride* charcoal powder (30.28%) and 5% *T .viride*in charcoal powder (22.77%) while it was least recorded in 5% *T. viride* (2.2%) and 2.4 and 3.8 % was noticed in 15 and 10% *T. viride* in talc powder respectively. Fig. 2 present moisture percent after 96 hr .

These findings are in agreement with results of Rini and

Sulochana (2010) who suggested at 35% and 45% moisture level longer shelf life of *Trichoderma* propagules on sorghum grain base formulation.

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