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Shelf-life of *Trichoderma viride* in talc and charcoal based formulations

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ABSTRACT

The shelf-life of *Trichoderma viride* was studied in talc and charcoal based formulations. Potato dextrose broth talc based formulation was prepared by adding three different volumes of biomass of *T. viride* along with medium @ 30, 40 and 50 ml/g talc. The initial mean CFU of *T. viride* at 30, 40 and 50 ml/100 g talc on 0 day was 227.2 × 10^9, 256.00 × 10^9 and 291.03 × 10^9 CFU/g sample, respectively. It gradually declined and at 120 days of storage the population came down to 70.33 × 10^9, 80.67 × 10^9 and 96.67 × 10^9 CFU/g which in terms of reduction in viability were 69.04, 68.48 and 66.78% respectively. Similar trends of results were recorded in all the three sorghum grains charcoal based formulations almost at par with each other ranging from 71.14 to 72.44% in 120 days. Sufficient numbers of spores were viable even after 120 days of storage in the formulation indicating that the formulation can be stored further for more time.

Key words: Charcoal, Formulations, Potato dextrose broth, Shelf-life, Talc, Sorghum, *Trichoderma viride*

During the recent past genus *Trichoderma* has gained maximum popularity as an effective and widely used biological control agent for management of soil borne plant pathogens. They are reported to have antifungal, antinematode, plant growth promoting and plant defence inducing activities (Zaidi *et al*. 2004). Some important soil borne pathogens against which species of *Trichoderma* showed promise are *Pythium*, *Fusarium*, *Rhizoctonia*, *Sclerotium* and *Macrophomina*. These pathogens cause diseases like damping off, wilt, root and collar rot and stem canker. Unlike chemical pesticides, biological agents need support even after their application to get established in targeted niche. The commercial success of biological agents depends not only on its bio-efficacy or shelf-life but also on easy availability at reasonably cheaper cost for which development of an easier and economically viable technique for mass multiplication is an important issue. The production and commercialization of biological products is a dynamic process and the most critical obstacle is the paucity of simple method of mass multiplication and delivery to seed and soil. Seed treatment with different carrier based formulations of *Trichoderma* has recently become popular among the farmers. Efforts have been made by different workers to grow *Trichoderma* spp on a wide range of substrates like pigeonpea, farmyard manure, wheat bran, neem cake, mustard cake, saw dust, coffee husk, vermicompost, sorghum grains etc but their use in mass multiplication and formulation remained little explored.

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MATERIALS AND METHODS

Talk and charcoal formulations of *Trichoderma viride* were prepared by the technique described by Jeyarajan *et al.* (1994) and Sarade *et al.* (1998). Potato dextrose broth was filled in conical flask @ 150 ml medium per flask. The medium is inoculated with 2 dishes of 5 mm of 3 days old culture of *T. viride* and incubated at room temperature 26-28°C for 15 days. The biomass along with the medium was incorporated into the talc and charcoal carriers @ 30 ml, 40 ml and 50 ml/100 g of carrier. The formulation was air dried and 500 mg CNC (carboxyl methyl cellulose)/100g carrier was mixed. The products were packed in polythene bags and stored at 28±1ºC. The initial population of *T. viride* was assessed by serial dilution technique. The product was serially diluted to obtain 10^{-9} concentration and 1ml was poured in sterilized petriplates. Thereafter, selective medium was poured @ 20ml/plate. Plates were rotated horizontally for uniform distribution of inoculum and incubated at 28±1ºC for 48 hours and observation of CFU was recorded. Colony forming units (CFU) of *T. viride* was calculated by the formula derived by Schmidt and Coldwell (1967) which is given below:

$$\text{Number of CFU of } T.\ viride \text{ per gram of sample} = \frac{\text{No. of CFU} \times \text{Dilution factor}}{\text{Dry weight of sample} \times \text{Aliquot taken}}$$

Variability of spores of *T. viride* was studied at fortnightly interval by the procedures.

Sorghum grains were pre soaked for overnight in 2% dextrose solution. Water was decanted and one hundred grams of sorghum grains were taken in 250 ml Erlenmeyer flask and autoclaved at 15 lb pressure per square inch for 15 minutes. The flask were inoculated with 5 mm disc of *T. viride* and incubated at 28±1ºC for 15 days. The biomass of sorghum grains was air dried and powdered. The powdered form of sorghum grains containing *T. viride* was incorporated into talc and charcoal @10g, 15g and 20 g/100 grams carriers. The formulations were dried at room temperature and 500 mg CMC/100 mg carrier was mixed. The product was packed in polythene bags and stored at room temperature. The initial population of *T. viride* was assessed by serial dilution technique.

RESULTS AND DISCUSSION

Shelf-life of T. viride *in potato broth talc based formulation*

Trichoderma viride was grown in potato dextrose broth for 15 days. The entire biomass was added in three different amounts, i e @ 30, 40, and 50ml/100g of talc powder. Shelf-life of *T. viride* was studied at 15 days interval in these talc based formulations packed in polythene bags.

The initial mean CFU of *T. viride* at 30ml/100g talc on 0 days was 227.2 × 10^9 CFU/g and on 15th day the CFU of *T. viride* came down to 218.2 × 10^9 CFU. It gradually declined and 120 days of storage the population reached to 70.33 × 10^9 CFU/g indicating reduction of 69.04%. The CFU of *T. viride* after 15 days was statistically at par with 0 day (Table 1)

The CFU of *T. viride* at 40 ml/ 100g talc carrier on 0 day was recorded as 256 × 10^9 CFU/gram sample which was reduced to 245.67 × 10^9 CFU at 15 days. It gradually declined and 120 days of storage the CFU recorded/g sample was 80.67 × 10^9 indicating reduction in viability by 68.48%.

At 50 ml/100g carrier on 0 day the mean CFU of *T. viride* was 291.03 × 10^9 CFU/g and 15 days the CFU came to 280.67 × 10^9 CFU/g sample. It continued to decline and reached to 96.67 × 10^9 CFU/g after 120 days of storage indicating a reduction of 66.78 percent viability of spores of *Trichoderma* in carrier. Gaur *et al.* (2005) reported that *T. harzianum* in talc based powder formulation remained viable at temperature ranging between 0 to 40ºC for 180 days. Tiwari *et al.* (2004) reported that a locally available millet, was found to be the best for maximum spore concentration, spore viability and biomass production.

Shelf-life of T. viride *in patato dextrose broth charcoal based formulation*

T. viride was grown on potato dextrose broth for 15 days. The entire biomass was mixed @ 30, 40 and 50 ml/100g charcoal, air dried and CMC was added @ 500mg/100g carrier and packed in polybags.

The mean CFU of *T. viride* in charcoal based formulation on 0 day at all three volume of biomass, i e 30, 40 and 50 ml/100g carrier were 204.0 × 10^9, 249.0 × 10^9 and 282.0 × 10^9 CFU/g, respectively which gradually declined and at 120 days of storage the population reached to 61.33 × 10^9, 74.33 × 10^9 and 84.67 × 10^9 CFU/g indicating reduction of 69.98, 70.18 and 70.01%. In charcoal based formulation the population of *T. viride* was reduced to 69.98 to 70.18% after 120 days of storage in different concentrations (Table 2). It is concluded that population of *T. viride* declined gradually in charcoal based formulation also. At 15 days of storage the reduction in CFU was minimal but later it was significant.

Table 1 Shelf-life of *T. viride* in potato broth talc based formulations

Storage period (days)	CFU of *T. viride*(× 10^9)*			Population decline (%)		
	Volume of biomass of *T. viride*/100 g carrier			Volume of biomass of *T. viride*/100 g carrier		
	30 ml	40 ml	50 ml	30 ml	40 ml	50 ml
0	227.20	256.00	291.03	0.00	0.00	0.00
15	218.20	245.67	280.67	3.80	4.00	3.50
30	202.67	227.63	258.33	10.79	11.19	11.23
45	175.33	198.33	227.33	22.83	22.52	21.88
60	150.67	170.33	193.33	33.68	33.46	33.57
75	131.33	146.67	166.67	42.19	42.70	42.73
90	121.33	137.33	157.67	46.59	46.35	45.82
105	94.33	106.67	121.67	58.48	58.33	58.19
120	70.33	80.67	96.67	69.04	68.48	66.78
CD (P=0.05)	9.27	6.10	7.42			
CV	3.50	2.04	2.27			

*Average of 3 replications.

Table 2 Shelf-life of *T. viride* in potato broth charcoal based formulations

Storage period (days)	CFU of *T. viride*($\times 10^9$)*			Population decline (%)		
	Volume of biomass of *T. viride*/100 g carrier			Volume of biomass of *T. viride*/100 g carrier		
	30 ml	40 ml	50 ml	30 ml	40 ml	50 ml
0	204.00	249.00	282.00	0.00	0.00	0.00
15	199.33	238.67	271.67	2.40	4.20	3.70
30	183.67	222.33	249.67	10.11	10.82	11.56
45	156.33	190.67	215.67	23.49	23.52	23.61
60	134.33	162.67	185.33	34.25	34.75	34.35
75	115.67	140.00	161.00	43.39	43.84	42.97
90	108.33	130.33	150.00	46.98	47.72	46.87
105	81.67	97.67	112.67	60.03	60.82	60.09
120	60.33	74.33	84.67	69.98	70.18	70.01
CD (P=0.05)	7.28	5.54	7.31			
CV	3.06	1.93	2.24			

*Average of 3 replications.

But still enough viable propagules remained alive in the formulations which indicated that the formulations can be stored for more time. Sarode *et al*. (1998) utilized five different substrates, i e FYM, peat soil, charcoal powder, talc powder and neem powder as carrier. Charcoal, FYM and talc were most suitable carriers for long storage of *Trichoderma.* Prasad and Rangeshwaran (2000) also reported a significant decline in *Trichoderma* population in talc, kaolin and bentonite carrier materials at 120 days.

Shelf-life of T. viride *in sorghum grains talc based formulation*

The shelf-life of *T viride* was studied in sorghum grains talc based 3 formulations of different concentrations, i e 10, 15 and 20 g/100g talc. The mean CFU of *Trichoderma viride* in 10, 15 and 20g sorghum grains biomass/100g talc on 0 day was 165.33×10^9, 202.6×10^9 and 252.6×10^9 CFU/g respectively. It continued to decline and at 120 days CFU reached to 49.0×10^9, 61.67×10^9 and 76.33×10^9 CFU/g indicating reduction of 70.36, 69.52 and 69.79%. The viable propagules in the preparations were still in higher number which indicated that formulations can be stored further (Table 3).

In talc, lignite, peat and kaolin based formulation the populations of *T. viride* were maintained during the first 15 days of storage after which there was gradual reduction in the number of CFU. Talc based formulations yielded the maximum CFU (Ramakrishnan 1994 and Raghuchander *et al*. 1995). Sathpathi (1998) prepared a mixture of *T. viride* spores bone dust and neem kernel dust. The shelf-life and

Table 3 Shelf-life of *T. viride* in sorghum grains talc based formulations

Storage period (days)	CFU of *T. viride*($\times 10^9$)*			Population decline (%)		
	Amount of biomass of powder/100 g carrier			Amount of biomass of powder/100 g carrier		
	10 g	15 g	20 g	10 g	15 g	20 g
0	165.00	202.60	252.60	0.00	0.00	0.00
15	158.67	196.33	244.67	4.00	2.96	3.16
30	143.33	177.33	222.67	13.30	12.35	11.87
45	130.67	159.67	201.33	20.96	21.08	20.31
60	110.33	136.33	173.33	33.26	32.61	31.40
75	94.00	116.00	146.33	43.14	42.66	42.08
90	87.67	108.33	135.33	46.97	46.55	46.44
105	66.33	82.67	102.00	59.88	59.14	59.33
120	49.00	61.67	76.33	70.36	69.52	69.79
CD P=0.05	4.66	5.32	6.18			
CV	2.43	2.25	2.14			

* Average of 3 replications

Table 4 Shelf-life of *T. viride* in sorghum grains charcoal based formulations

Storage period (days)	CFU of *T. viride*($\times 10^9$)*			Population decline (%)		
	Amount of biomass of powder/100 g carrier			Amount of biomass of powder/100 g carrier		
	10 g	15 g	20 g	10 g	15 g	20 g
0	136.67	186.00	248.00	0.00	0.00	0.00
15	130.33	181.67	237.33	4.30	2.30	4.30
30	118.33	160.67	211.33	14.78	13.61	14.78
45	105.67	145.33	190.67	23.11	21.86	23.11
60	89.33	124.67	165.00	33.46	32.97	33.46
75	78.67	105.33	140.67	43.27	43.37	43.27
90	72.00	98.33	129.33	47.85	47.13	47.85
105	54.33	73.33	98.67	60.02	60.57	60.21
120	38.33	53.67	68.33	70.81	71.14	72.44
CD (P=0.05)	4.67	4.63	5.67			
CV	2.3	2.15	2.00			

* Average of 3 replications.

compatibility at this formulated dust were good but the toxicity was low compared with other inorganic fungicides.

Shelf-life of T. viride *in sorghum grains charcoal based formulation*

The shelf-life of *T. viride* in sorghum grains biomass charcoal based formulations were studied (Table 4). The initial mean CFU of *T. viride* in 10, 15 and 20g sorghum grains biomass powder/100g charcoal at 0 day was 136.67×10^9, 186.00×10^9 and 248.00×10^9 respectively. It gradually declined and at 120 days CFU recorded was 53.67×10^9 and 68.33×10^9 CFU/g indicating a reduction of 71.14 to 72.44% viability. Sufficient number of viable spores was viable even after 120 days of storage in the formulation indicating that the formulation can be stored further for more time. Sarode *et al.* (1998) utilized five different substrates and reported charcoal, FYM and talc were, most suitable carriers for long storage of *Trichoderma.* Saju *et al*. (2002) used coir pith, FYM and decomposed coffee pulp for study the shelf-life of *Trichoderma viride* FYM is the best carrier for long storage of *Trichoderma.*

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