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Survival ability of *Trichoderma* spp and *Pseudomonas* in Different carrier Materials

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A B S T R A C T

The investigation is carried about talc based biocontrol agents for formulation and delivery system for microorganism that enable them for efficient disease control in Soilborne disease. In the present investigation, the carrier materials viz., talc, gypsum, vermiculate, flyash, talc + gypsum, talc + vermiculate, talc + flyash, Talc + Neem cake, Talc+ Decomposed coir pith and Talc + FYM were tested to assess the survival of *T. viride* (TVB1), *T. harzianum* (THB1) *Pseudomonas fluorescens* and *Bacillus subtilis* The results revealed that *T. viride* and *T. harzianum* showed the maximum level of population in talc at the end of 120th Days after storage followed by neem cake, Bacterial antagonist for *Pseudomonas fluorescens* and *Bacillus subtilis* showed the maximum level of population in talc at the end of 120th Days after storage followed by Talc + Farmyard Manure

Introduction

The talc based powder formulations are popular in India and elsewhere, it is used for larger area application using different substrates for multiplication biocontrol agents and increasing shelf life microorganism due integrated disease management with different substrates, longer shelf life, greater protection against environmental stresses and increased field efficacy. The mass production systems should be compatible with industrial and commercial development methods and field application. So formulations of *Trichoderma viride* 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. Production of adequate quantities of good quality inoculums is an essential component of the biocontrol programme. Therefore the present study was aimed to find out the storability of *Trichoderma viride* and Pseudomonas in different formulations with the various substrates. wheat straw, wheat straw dust and sorghum grain-peat-bran (Roiger and Jeffers, 1991). Lewis *et al.,* (1991) developed a bulk type formulation In this system vermiculite containing small amounts of bran was amended with biomass of *Gliocladium* and *Trichoderma* and stored dry for as long as 24 weeks. Before use, the dry vermiculite-bran-biomass formulation was moistened with dilute acid and incubated for 2 days to allow the fungi to germinate and colonize the substrate *Trichoderma viride* against Root rot pathogens (Paramasivan *et al.,* 2007), Sangeetha Panickar and Jeyarajan (1993) used different substances like rice bran, wheat bran, peat soil, farm yard manure (FYM) and rice straw and they reported that the FYM and wheat bran were the best substrates for mass multiplication of *Trichoderma* spp.. Prasad and Rangeshwaran (1999) studied the modified granular formulation containing powdered wheat bran, kaolin, acacia powder and biomass of isolates of *T. hamatum, T. virens* and G. *aeliguescens.* Granules with all isolates of bioagents significantly reduced the damping-off in chickpea. The storage of bioagent *T. harzianum* promoted initial growth ($10^{7.6}$ to $10^{8.5}$ cfu g^{-1}) of the fungus upto 30 days and the population declined thereafter but retained substantial number of viable propagules even at 90 days (Prasad and Rangeshwaran, 2000).

Selvakumar *et al.,* (2001) studied the various substances *viz.,* coirpith, maize cob medulla, peat, saw dust, talc powder and wheat bran for the mass multiplication of C. *globosum* and they reported that peat soil was found to be the best in supporting the growth of C. *globosum* (7.2 x 10^8 cfu g^{-1}). Ali *et al.,* (2001) tested the survival period of *Pseudomonas aeruginosa* on mungbean seed coated with different carriers / substrates and was found best on the talc amended with CMC. On all substrates the antagonists population were declined dramatically at 120 days after coating.

Sivakumar *et al.,* (2000) tested different carrier substrates *viz.,* talc powder, blackgram shell, coir pith, peat, shelled maize cob. Among these carriers, peat and talc were found to maintain the population at 19.5 x 10^7 and 18.3 x 10^7 cfu g^{-1} of the product respectively after 40 days of storage. Manjula and Podile (2001) developed a formulation supplemented with 6.5 per cent chitin of *Bacillus subtilis* (AF 1) effectively reduced *Aspergillus niger* and *Fusarium udum* caused crown rot of groundnut and wilt of Pigeon pea, respectively. *Bacillus subtilis* AF 1 talc based formulation promoted the seed germination and biomass yield of both crops.

Shelf life of bio control agents

The efficient bio control agents should possess longer shelf life without losing its efficacy. Shelf life of an antagonist in the substrate is important in commercial production. Talc based formulation of *Trichoderma viride* retained 50 and 25% of its initial population after two and four months of storage respectively. Loganathan (2002) reported that talc was found to be best for the survival of *Trichoderma* spp. and *Pseudomonas* spp. The *Trichoderma* spp. survived with required colony forming units (10^8 cfu /ml) in talc formulation upto 120 days of storage. Both Pf1 and CHAO strains survived with 10^8 cfu per ml up to 90 days in talc formulation.

Manav and Singh (2003) tested the shelf life of *Trichoderma harzianum* for a period of 120 days under wet and dry condition. The wet formulation recorded maximum number of colony forming units than the dry formulation. The low temperature of 8 ± 2°C favoured

maximum number of propagules (18) than the high temperature of 25 ± 2°C (14).

Materials and Methods

Isolation and identification of *Trichoderma* spp and *Pseudomonas*: The rhizospheric samples were collected randomly from different locations of Tropical sugarbeet ecosystem. Effective *Trichoderma* spp and *Pseudomonas*, and *Bacillus subtilis* against *Sclerotium rolfsii* under *In vitro* condtion, these bioagents application with different carrier material tested for field application.

Preparation of talc based formulation bio control agent

A loop full of *P. fluorescens* and *B. subtilis* were inoculated into the King's B broth and Nutrient broth and incubated in a rotary shaker at 150 rpm for 72 h at room temperature (28 ± 2 °C). After 72 h of incubation the broth containing 9 x 10 ^{8}cfu/ml was used for the preparation of talc based formulation. To 400 ml of bacterial suspension, 1 kg of the talc powder, calcium carbonate 15 g (to adjust the pH to neutral) and carboxy-methyl cellulose (CMC) 10 g (as adhesive) were mixed under sterile condition following the method described by Vidhyasekaran and Muthamilan (1999). The products were shade dried to reduce the moisture content to 20 percent and then packed in polypropylene bags and sealed.

Trichoderma sp.

A mycelial disc 9mm of *T. viride* (TVB1) and *T. harzianum* (THB 1) was inoculated into 100 ml of molasses yeast medium in 250 ml conical flasks and incubated at room temperature for 14 days. The mycelial mat was homogenized and blended with talc powder at 1:2 ratio. Five gram of CMC was added to one kg of talc and mixed well. The materials were shade dried and packed in polypropylene bags, heat sealed and kept at room temperature (Ramakrishnan *et al.*, 1994).

Survival of antagonists' organism in different carrier materials

Survival of *P. fluorescens* and *B. subtilis* in different formulations

Bacterial biocontrol agents were grown in King's B and Nutrient broth for 48 h at room temperature (28 ± 2º C). Survival of the antagonist's bacterium was tested in different formulations. Talc, gypsum, flyash, vermiculate, talc + gypsum (1:1), talc + flyash (1:1), talc+ vermiculate (1:1).Ten gram of carboxy methyl cellulose was added to one kg of the carrier and mixed well. The pH of the all materials was adjusted to 7.00 by adding calcium carbonate. The carrier was autoclaved for 30 min for two consecutive days. Four hundred ml of the bacterial suspension containing 9 x 10 ^{8}cfu/ml was added to one kg of the carrier materials and mixed well under aseptic conditions. The materials were packed in polythene bags. Sealed and incubated at room temperature (28 ± 2 º C). After 20 days storage, the samples were drawn at 10 days intervals and the bacterial population was assessed using King's B and Nutrient Agar medium by dilution plate methods.

Trichoderma sp

T. viride and *T. harzianum* were grown in yeast molasses medium and it was incubated for 15 days and then the content was drained. The fungal biomass was removed and this was mixed with 1 kg of carrier materials at 1:2 ratio. The mixture was shade dried. Carboxy methyl cellulose was added to the carrier material @ 5 g/kg of carrier material. The materials were packed in polythene bags. Sealed and incubated at room temperature (28 ± 2ºC). Storage after 20 days storage the

samples were drawn at 10 intervals and the *Trichoderma* spp population was assessed using *Trichoderma* special medium by dilution plate methods.

Mass multiplication of antagonistic bacteria and *Trichoderma* in different substrates

The antagonistic bacteria *P. fluorescens* isolate SBHRPF2 and *B. subtilis* isolate SBHRBS1and *T. viride* (TVB1) and *T. harzianum* (THB1) were mixed with at the rate of 10g/kg of sterilized Neemcake, decomposed coir pith and farmyard manure. Moisture content of the farmyard manure was adjusted to 50 per cent. The population of both bacteria and *Trichoderma* were recorded at an interval of 10 day to assess its shelf life.

Results and Discussion

Fungal antagonists

In the present investigation, the carrier materials viz., talc, gypsum, vermiculate, flyash, talc + gypsum, talc + vermiculate, talc + flyash, Talc + Neem cake, Talc+ Decomposed coir pith and Talc + FYM were tested to assess the survival of *T. viride* (TVB1) and *T. harzianum* (THB1) The results revealed that *T. viride* and *T. harzianum* showed the maximum level of population in talc at the end of 120th Days after storage followed by neem cake (Table 1 and Fig. 1). The population of *T. viride* showed decreasing trend from the initial level population level in the carrier materials viz., Talc + Neem, gypsum, Talc + gypsum (1:1) whereas the population increased up to 20 days after storage from the initial population in case of talc. The population of *T.harzianum* decreased from the initial population over time in all the carrier material except talc. Where the population increased up to 20 DAS and later it showed a decreasing trend. Paramasivan *et al.,* (2013) stated that the Rhizosphere *Trichoderma* sp against *Sclerotium rolfssi* in tropical sugarbeet and was recorded in *Trichoderma* talc formulation 3 months of rhizosphere and declined in nature. The earlier work by Jeyarajan *et al.,* (1994) explained that the talc based formulation and vermiculite wheat bran based formulation of *T. viride* could be safely stored from 75 to 120 days at room temperature. Loganathan (2002) reported that talc based formulation was best over other carriers tested and retained required cfu until120 and 90 days in *Trichoderma* and *Pseudomonas* sp. respectively.

Bacterial antagonists

The results revealed that the population level of *P. fluorescens* (SBHRPF2) and *B. subtilis* (SBHRBS1) was the maximum in talc followed by Talc+FYM, vermiculate at the end of 120 days after storage. The population level showed decreasing trend in the carrier materials viz., talc + gypsum, talc + vermiculate, talc + flyash, and Decomposed coir pith. Whereas the population increased upto 20 days from the initial population, later it showed decreasing trend (Table 2 and Fig. 2).

Vidhyasekaran and Muthamilan (1995) developed a talc-based formulation of *P. fluorescens* for controlling chickpea wilt. The carrier materials viz., peat and talc maintained the population level of the antagonists up to 40 days of storage (Siva Kumar *et al.,* 2000). Sangita Bapat and Shah (2000) reported that a formulation of *B. brevis* with vermiculite as a carrier had a shelf life of at least six months.

The development of the powder formulation of the bacteria, with a shelf life of more than eight months may be highly useful for large scale field application of the product and effective control of the diseases (Vidhyasekaran and Muthamilan, 1999).

Table.1 Survivals of *T. harzianum* in different carrier materials

S. No	Carrier materials	*Survival of *T. harzianum* in carrier materials 10^3CFU/g								
		Number of Days (21 Days after)								
		0	20	30	40	50	60	70	80	90
1	Talc	50.00	48.20	42.50	42.50	40.50	38.75	36.76	34.00	**30.33**
2	Gypsum	46.50	42.50	40.00	37.50	34.00	30.50	26.50	23.00	**14.33**
3	Flyash	38.50	35.50	34.00	27.00	23.50	19.50	16.50	14.30	**10.00**
4	Vermiculate	38.00	35.26	33.50	30.75	27.50	23.50	19.50	16.50	**9.00**
5	Talc + Gypsum	49.50	50.00	43.50	40.50	38.50	36.75	31.50	28.50	**20.50**
6	Talc +Flyash	45.50	41.26	39.00	37.50	33.50	30.33	24.50	20.50	**13.00**
7	Talc +Vermiculate	39.50	38.50	37.56	35.50	30.50	27.50	20.75	17.50	**11.50**
8	Talc + Neem cake	48.00	49.50	43.50	40.50	37.00	35.50	30.75	26.50	**18.50**
9	Talc + Decomposed coir pith	40.50	34.50	31.50	29.50	24.00	20.00	17.50	15.40	**7.50**
10	**Talc + FYM**	**44.00**	**41.50**	**40.50**	**38.50**	**35.50**	**30.25**	**27.50**	**24.50**	**15.00**

*Mean of three replications

CD (P=0.5)

Carrier materials	0.878
Days	0.878
Carrier materials x Days	2.779

Fig.1 Survival of biocontrol agents in different carrier materials

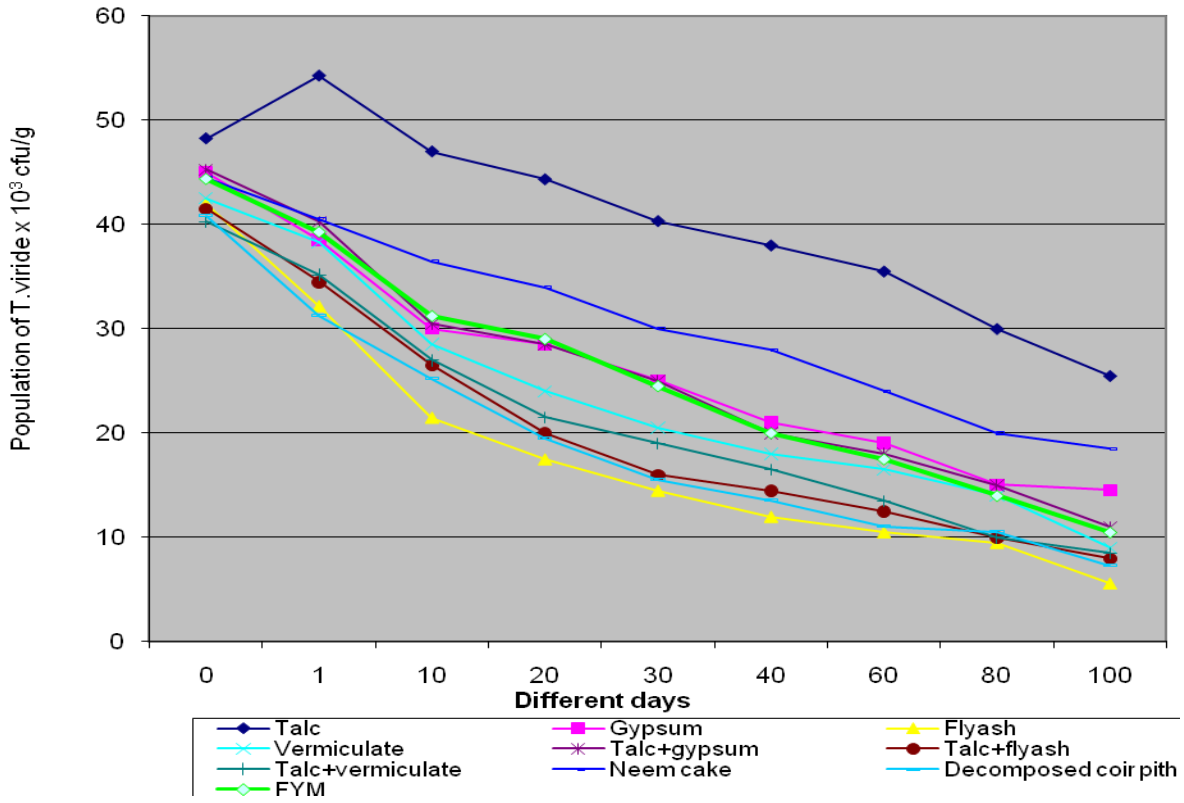


Table.2 Survivals of *Bacillus subtilis* in different carrier materials

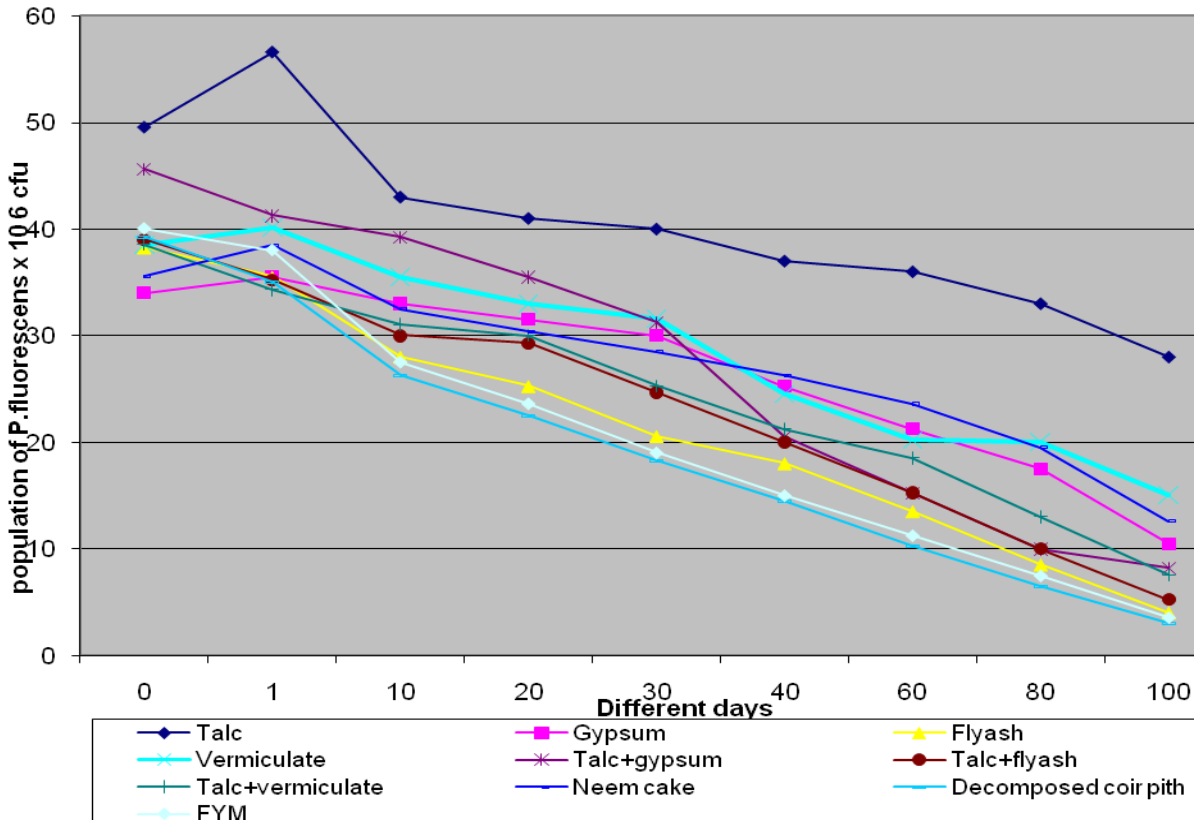
S. No	Carrier materials	*Survival of *Bacillus subtilis* in carrier materials 10^7CFU/g								
		Number of Days (21 Days after)								
		0	20	30	40	50	60	70	80	90
1	Talc	46.50	47.50	44.50	40.50	38.50	35.50	30.50	28.50	**24.00**
2	Gypsum	42.33	43.00	38.00	34.50	30.00	24.50	21.00	18.75	**12.33**
3	Flyash	41.5	40.50	37.50	34.50	27.50	23.00	20.00	15.50	**9.50**
4	Vermiculate	42.50	43.60	40.50	37.50	35.00	31.50	26.50	24.00	**18.50**
5	Talc + Gypsum	44.25	43.00	41.50	38.50	35.50	30.00	27.00	21.50	**16.60**
6	Talc +Flyash	40.25	39.00	36.50	33.00	29.25	25.00	21.00	15.00	**10.00**
7	Talc +Vermiculate	39.25	36.00	34.50	30.00	26.50	21.50	17.00	13.00	**8.50**
8	Talc + Neem cake	41.50	40.00	38.00	35.50	31.50	27.50	24.50	20.50	**16.50**
9	Talc + Decomposed coir pith	40.50	38.50	33.20	30.00	25.40	21.50	18.00	14.5	**7.50**
10	**Talc + FYM**	**42.00**	**41.50**	**40.00**	**35.00**	**31.50**	**27.50**	**23.00**	**16.50**	**10.50**

*Mean of three replications

CD (P=0.5)

Carrier materials	0.780
DAYS	0.780
Carrier materials x Days	2.469

Fig.2 Survival of Pseudomonas fluorescens in different carrier materials



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