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Original Research Article

Assessment of Shelf Life of *Trichoderma viride* on Different Liquid Formulations

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A B S T R A C T

An experiment was conducted during 2016-17 in the department of Plant Pathology, College of Agriculture, Nagpur. Shelf life studies of *Trichoderma viride* was executed by using different carriers viz. paraffin oil, mustard oil, diesel, soybean oil, sunflower oil, talc powder mixed with broth 40ml + 5ml dispersant + 3ml suspender + 8ml surfactant in each oil. Shelf life of formulations was tested up to 6 months at room temperature (15- 350). Among the different liquid formulations in treatment T2 i.e. paraffin oil retained maximum viability (31.2 × 105 CFU/ml) followed by soybean oil (21.5×105 CFU/ ml) and groundnut oil (19×105 CFU/ml.). Based on present investigation it can be inferred that application of paraffin oil increase the shelf life of *Trichoderma* which was used as a biofungicide comparing to the solid formulation of biofungicide used, liquid formulation of *Trichoderma* was more effective to control phytopathogen.

Keywords

T. viride, shelf life, and liquid formulations

Introduction

Trichoderma is a genus of asexually reproducing fungi that is present in all type of soils. It belong to the family of Hypocreaceae. Recent discoveries show that they are opportunistic, avirulent plant symbionts, as well as being parasites of other fungi. At least some strains establish robust and long-lasting colonizations of root surfaces and penetrate into the epidermis and a few cells below this level. They produce or release a variety of compounds that induce localized or systemic resistance responses. These root microorganism associations cause substantial changes to the plant proteome and metabolism. Plants are protected from numerous classes of plant pathogen by responses that are similar to systemic acquired resistance and rhizobacteria-induced systemic resistance. Root colonization by *Trichoderma* spp. also frequently enhances root growth and development, crop productivity, résistance to abiotic stresses and the uptake and use of nutrients. As most of the crops are infected by the soil borne plant pathogens that primarily attack the vulnerable seeds or seedlings, *Trichoderma* can be applied directly to target area, i.e., to seeds or seedlings and a single application using an existing delivery system (seed treatment, biopriming, furrow treatment) can significantly reduce crop losses. A number of successful products based on different species of *Trichoderma* have been commercialized in India and elsewhere (Kumar *et al.* 2013).

The carrier based (Solid) biofertilizers are used since a long time. The traditional nitrogen fixing biofertilizers have suffered from the problems of short shelf life, instability to ambient temperature and laborious large scale application. Whereas liquid inoculants could be produced with minimum labour, space and energy. Also the quantity of inoculum required for application is less as compared to carrier based formulations. It is easier for farmers to handle and apply liquid biofertilizer's formulation which could be considered as one potential strategy for improving the shelf life of biofertilizer. Unlike solid carrier based biofertilizers, liquid formulations allows the manufacturer to include sufficient amount of nutrients, cell protectant and inducers responsible for cell/spore/cyst formation to ensure prolonged shelf life. Very high enzymatic activity since contamination is nil.

Materials and Methods

The present study was conducted in Plant Pathology Laboratory, College of Agriculture, Nagpur during the year 2016-2017.Pure culture of *Trichoderma viride* was collected from Plant Pathology Section, College of Agriculture Nagpur. The pure culture was mass multiplied for further studies.

Shelf life study in Oil base liquid formulation

Mass multiplied *Trichodrma viride* was transferred in to mixing tank to harvest the spore and mycelium. Mixed *Trichoderma viride* formulation was poured into pre streilized 100ml of conical flask by broth (40ml), with Dispersant (5ml), Surfactant (8ml), Suspender (3ml). Different oil (44ml) were incorporated into the *Trichoderma viride* formulation in each conical flask as per given in treatment details from T_2 to T_7. Whereas T_8 was talc base departmental culture, T_9 was liquid formulation market product and T_{10} was utilized as pure broth. Flask was plugged by non absorbing cotton and these were kept for a storage for six month. CFU count was under taken at monthly interval by serial dilution followed by pour plate method. (Chandra 2011)

Results and Discussion

Oil Based Formulation of *Trichoderma viride*

The shelf life study revealed that there were significant differences in *Trichoderma viride* at all the interval. The initial population of *T. viride* in first month was found maximum (31.2×10^5 CFU/ml) in T_2 (T_1 + Paraffin oil) which was significantly superior over all other treatments. Sathiyaseelan *et al*. (2009) showed that application of paraffin oil increase the shelf life of *Trichoderma* which was used as a biofungicide comparing to the solid formulation of biofungicide used, liquid formulation of *Trichoderma* was more effective to control phytopathogen. They also showed thatthe second best treatment in terms of colony forming units of *T. viride* was T_6 (T_1 + Soybean oil) where 21.5×10^5 CFU/ml population was noticed in first month.This result also correlated with the Rai and Tewari (2016). These treatments were followed by treatment T_8 (Departmental culture, 19.6 CFU/ml) and T_3 (T_1 + Mustard oil, 18.9 CFU/ml). The population of *T. viride* was found to be gradually decreased over a period of time. At the end of six month maximum population of *T. viride* (24.5 CFU/ml) was observed in the treatment T_2 (T_2 + Paraffin oil) which was significantly superior over all other treatments, it was followed by T_4 (T_1 + Groundnut oil, 14.6 CFU/ml) and T_8 (Departmental culture (Talc) 13.6 CFU/ml). The result of present investigation are in full

agreement with reports of earlier workers. Kumar *et al.* (2013) observed similar result about talc base *Trichoderma viride* formulation. Decrease in *Trichoderma viride* colony was observed after one month. But it was observed that CFU count in talc is comparatively high. These result also correlated with, Chandra (2011), Khan *et al.* (2011), Bhai and Anandraj (2014). The similar results are also found to other scientists viz., Hartsell (1947), Mugnier and jung (1985), Sanker and Jeyrajan (1996), Inamdar *et al*. (2000), Singleton *et al*. (2002), Mahaveer *et al.* (2011), Yadav and Chandra (2014), Batta *et al*. (2004), Sridhar et al. (2004), Jeyrajan (2006), Kolombet *et al.* (2008). These results are in accordance with earlier studies of scientist (Ramkrishnan and Raghuchandar *et al.*1994). They showed that in talc based formulation the colony of *Trichoderma viride* were maintained during the first 15 day of storage after which there was gradual reduction in number of CFU. Talc based formulations yielded the maximum CFU. Prasad *et al.* (2002) had reported conidial formulation which retained a viable count of above 10^6 CFU/gm even after 180 day of storage.

Table.1 Effect of different liquid formulation on the shelf life of *Trichoderma viride* ($\times 10^5$CFU/ml) at various interval

Tr. No.	Treatment	Month					
		I	II	III	IV	V	VI
T_1	Broth + Dispersant + Suspender + Surfactant	15.3	12.5	11.00	10.5	10	5
T_2	T_1 + Paraffin oil	31.2	30.4	29.5	27.7	25.0	24.5
T_3	T_1 + Mustard oil	18.9	17	15	14.5	13.5	11.5
T_4	T_1 + Groundnut oil	20	17.3	17.7	16.2	15.8	14.6
T_5	T_1 + Diesel	14.4.	13.0	13.4	13.00	12.9	11
T_6	T_1 + Soybean oil	21.5	20.9	20.0	19.5	18.4	17
T_7	T_1 + Sunflower oil	10.2	8.3	7.8	3.4	3.0	2.1
T_8	Departmental culture (Talc)	19.6	18.1	17.3	16.4	13.8	13.6
T_9	Market Product (liquid)	14.7	14.6	14.5	14	13	12.0
T_{10}	Broth	16.9	14.9	14.5	13	10	9
	F test	Sig	Sig	Sig	Sig	Sig	Sig
	SE ± m	0.19	0.27	0.32	0.33	0.42	0.39
	CD (P=0.01%)	0.76	1.05	1.27	1.28	1.64	1.52

In conclusion, all the liquid formulations retained optimum viability. Paraffin oil liquid formulations gave higher shelf life of *Trichoderma viride* compared to other formulations. Application of liquid formulation of bio-control agent in orchards and in field would help the farmer in promising better yield.

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