Int.J.Curr.Microbiol.App.Sci (2018) *7*(11): 225-230

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 11 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.711.028

Shelf Life Study and Antagonistic Activity of *Trichoderma viride* in Different Oil Formulations

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Keywords

Trichoderma viride, Paraffin oil, Soybean oil, Liquid formulations

Article Info

Accepted:
04 October 2018
Available Online:
10 November 2018

A B S T R A C T

An investigation entitled "Evaluation of different oils on shelf life study of *Trichoderma viride*" was carried out during 2017-2018 in the department of Plant Pathology, College of Agriculture, Nagpur. Different oils viz., Paraffin oil, soybean oil, mineral oil, Potato Dextrose broth and talc was extensively used as carrier for *Trichoderma viride*. As compared to solid based carrier material the survival of *Trichoderma viride* in liquid based formulations is quite high and has the ability to limit the heat transfer high water holding capacity and maintain water around the cells for their metabolism. Shelf life studies clearly indicated significant differences at all the intervals. Population density of *Trichoderma viride* in the paraffin oil was 28.06 x 10^8 CFU/ml in the 1st month whereas the population density of *Trichoderma viride* in the 6th month was 18.33 x 10^8 CFU/ml. It was found significantly superior over all other treatment. The effect on percent growth inhibition on *Fusarium oxysporum* f. sp. *ciceri* is 79.30 per cent on *Rhizoctonia bataticola* is 84.85 per cent and *Sclerotium rolfsii is* 79.14 per cent. It was recorded maximum in formulation containing paraffin oil it was found significantly superior over all the treatments. The effect of liquid formulations on spore germination of *Trichoderma viride* was also recorded the maximum spore germination are found in formulation containing paraffin oil after six months of storage.

Introduction

Trichoderma is a genus of asexually reproducing fungi that are often the most frequently isolated soil fungi; nearly all temperate and tropical soils contain 101-103 culturable propagules per gram. These fungi also colonize woody and herbaceous plant materials, in which the sexual Teleomorph (genus *Hypocrea*) has most often been found. They show a high level of genetic diversity, and can be used to produce a wide range of products of commercial and ecological interest. They are prolific producers of extracellular proteins, and are best known for their ability to produce enzymes that degrade cellulose and chitin—although they also produce other useful enzymes (Harman and Kubicek., 1998). *Trichoderma* is considered as most efficient biocontrol agents and have attracted considerable scientific attention as they are considered as promising alternative to

chemical fungicides against many plant pathogens. Major mechanisms involved in the biocontrol activity of *Trichoderma spp*. are competition for space and nutrients, production of diffusible and/ or volatile antibiotics and hydrolytic enzymes like chitinase and β-1, 3- glucanase. These hydrolytic enzymes partially degrade the pathogen cell wall and leads to its parasitization (Navaneetha *et al.,* 2015). Oil is used as carrier for liquid formulation. They are prepared by mixing the conidia harvested from the solid/ liquid state fermentation with a combination of vegetable/ mineral oils in stable emulsion formulation. In such formulations, microbial agents are suspended in a water immiscible solvent such as petroleum fraction (diesel, mineral oil), and vegetable oils (groundnut, etc) with the surfactive agent. This can be dispersed in water to form a stable emulsion. The oil used should not have toxicity to the fungal spores, plants, humans and animals. Such formulation of *Trichoderma, Pseudomonas, Beauveria* are now being used as foliar sprays. Oil-based formulations are supposed to be suitable foliar sprays under dry weather condition and to have prolonged shelf life (Ramanujam *et al.,* 2010)

Materials and Methods

The present study was conducted in Plant Pathology Laboratory, College of Agriculture, Nagpur during the year 2017-2018.Pure culture of *Trichoderma viride* was collected from Plant Pathology Section, College of Agriculture Nagpur. The pure culture was mass multiplied for further studies.

Oil base liquid formulation

Mass multiplied *Trichoderma viride* was transferred in to mixing tank to harvest the spore and mycelium. Mixed *Trichoderma viride* formulation was poured into presterilized plastic bottles. Each treatment contained Glycerol (10ml), Dispersant (1ml), Surfactant (3ml), Suspender (3ml). Three oils are used viz., paraffin oil, soybean oil and mineral oil were incorporated into the *Trichoderma viride* formulation in each plastic bottles as per the given in treatments from T_1 to T_8. Whereas T_9 was talc base departmental culture, T_{10} was liquid formulation market product. The bottles were packed with the help of caps and kept for a storage for six month at 27 ± 1^0 C. CFU count was under taken at monthly interval by serial dilution followed by pour plate method.

Treatment details

Each treatment contained with Glycerol (10ml) + Dispersant (1ml) + Suspender (3ml) + Surfactant (3ml)

Results and Discussion

Effect of different liquid formulations on the shelf life of *Trichoderma viride* (CFU/ml) at various interval

It was revealed from the data that there were significant differences in *Trichoderma viride* at all the interval. The initial population of *T. viride* (Table 1) in first month was found maximum i.e. 28.06 x 10^8CFU/ml in T_1 (*Trichoderma* filtrate (30ml) + Paraffin oil (53ml) which was significantly superior over all treatments followed by T_2 (*Trichoderma* filtrate (20ml) + Paraffin oil (63ml) where 26.40 x 10^8CFU/ml was noticed in 1st month. The observations recorded in the treatments T_8 (24.63 x 10^8CFU/ml), T_3 (24.56 x 10^8CFU/ml) and T_9 (20.16 x 10^8CFU/ml) was also significantly superior. At the end of six months the maximum population density of *T. viride* was observed in T_1 (18.33 x 10^8CFU/ml) which was followed by T_2 (10.40 x 10^8CFU/ml), T_3 (5.46 x 10^8CFU/ml) and T_4 (3.06 x 10^8CFU/ml).

Treatment Details

Treatment No.	Treatment details
T_1	*Trichoderma* filtrate (30 ml) + Paraffin oil (53ml)
T_2	*Trichoderma* filtrate (20 ml)+ Paraffin oil (63ml)
T_3	*Trichoderma* filtrate (30 ml)+ Soybean oil (53ml)
T_4	*Trichoderma* filtrate (20 ml)+ Soybean oil (63ml)
T_5	*Trichoderma* filtrate (30 ml)+ Mineral oil (53ml)
T_6	*Trichoderma* filtrate (20 ml)+ Mineral oil (63ml)
T_7	*Trichoderma* filtrate (30 ml)+ PD broth (53ml)
T_8	*Trichoderma* filtrate (30 ml) + PD broth (63ml)
T_9	Departmental culture (Talc based)
T_{10}	Market product (liquid formulation)

Table.1 Effect of different liquid formulation on the shelf life of *Trichoderma viride* ($\times 10^8$CFU/ml) at various interval

Tr. No.	Treatment	Month					
		I	II	III	IV	V	VI
T_1	*Trichoderma* filtrate (30 ml) + Paraffin oil (53ml)	28.06	24.36	22.50	21.76	20.63	18.33
T_2	*Trichoderma* filtrate (20 ml) + Paraffin oil (63ml)	26.40	22.80	19.76	21.20	12.40	10.40
T_3	*Trichoderma* filtrate (30 ml) + Soybean oil (53ml)	24.56	21.20	11.56	10.70	7.70	5.46
T_4	*Trichoderma* filtrate (20 ml) + Soybean oil (63ml)	13.13	12.23	11.36	10.10	6.43	3.06
T_5	*Trichoderma* filtrate (30 ml) + Mineral oil (53ml)	8.83	8.13	7.43	5.56	2.86	1.06
T_6	*Trichoderma* filtrate (20 ml) + Mineral oil (63ml)	10.70	9.26	8.30	7.30	1.26	0.76
T_7	*Trichoderma* filtrate (30 ml) + PD broth (53ml)	18.63	17.50	13.36	11.00	9.46	1.66
T_8	*Trichoderma* filtrate (20 ml) + PD broth (63ml)	24.63	21.30	18.00	10.70	9.93	2.40
T_9	Departmental culture (Talc)	20.16	17.43	16.43	13.23	12.83	2.23
T_{10}	Market Product (liquid)	10.6	7.96	6.96	4.46	3.60	0.96
	F test	Sig	Sig	Sig	Sig	Sig	Sig
	SE ± m	2.9	3.6	3.8	4.5	3.8	3.4
	CD (P=0.01%)	11.6	14.2	14.8	17.9	14.8	13.6

Table.2 Effect of *Trichoderma viride* liquid formulations on per cent growth inhibition on 8th DAI

Tr. No.	Treatment	Mycelial growth (mm)			% Growth inhibition		
		Fusarium oxysporum* f. sp. *ciceri	***Rhizoctonia bataticola***	***Sclerotiumrolfsii***	***Fusarium oxysporum* f. sp. *ciceri***	***Rhizoctonia bataticola***	***Sclerotiumrolfsii***
T_1	*Trichoderma* filtrate (30 ml) + Paraffin oil 53ml)	18.63	13.63	18.77	79.30	84.85	79.14
T_2	*Trichoderma* filtrate (20 ml) + Paraffin oil (63ml)	21.23	16.43	21.67	76.41	81.74	75.92
T_3	*Trichoderma* filtrate (30 ml) + Soybean oil(53ml)	22.30	25.30	28.67	75.22	71.88	68.14
T_4	*Trichoderma* filtrate (20 ml) + soybean oil (63ml)	24.13	30.87	31.20	73.18	65.70	65.33
T_5	*Trichoderma* filtrate (30 ml) + Mineral oil (53ml)	33.38	43.64	43.60	62.91	51.51	51.56
T_6	*Trichoderma* filtrate (20 ml) + Mineral oil (63ml)	34.67	42.67	43.36	61.47	52.58	51.82
T_7	*Trichoderma* filtrate (30 ml) + PD Broth (53ml)	35.27	37.57	41.57	60.81	58.25	53.81
T_8	*Trichoderma* filtrate (20 ml) + PD Broth (63ml)	33.00	41.77	43.00	63.33	53.58	52.22
T_9	Departmental culture (Talc)	25.16	32.43	40.30	72.03	63.97	55.22
T_{10}	Market Product(liquid)	42.93	38.97	43.67	52.30	56.70	51.48
	Control	90.00	90.00	90.00	100	100	100
	F test	Sig	Sig	Sig			
	SE ± m	0.65	0.59	0.83			
	CD (P=0.01%)	2.55	2.31	3.24			

During the shelf life study Reddy *et al.,* (2017) calculated that *T. harzianum* in the form of CFU on 56thday of observation paraffin oil (20 x 10^7) and in soybean oil (2.1 x 10^7) gave the best result of spore viability. Nadare *et al.,* (2018) revealed that the colony forming unit of *Trichoderma viride* were maximum in paraffin oil which was followed by soybean oil. Sathiyaseelan *et al.,* (2009) showed that application of paraffin oil increases the shelf life of *Trichoderma* which was used as a bio fungicide comparing to liquid formulation of *Trichoderma* was more effective to control phytopathogen.

Effect of *Trichoderma viride* liquid formulation on per cent growth inhibition on 8th DAI

All the treatments significantly inhibited the radial mycelial growth of *Fusarium oxysporum* f. sp. *ciceri, Rhizoctonia bataticola* and *Sclerotium rolfsii* over control. Observations recorded in Table 2 showed that treatment T_1 (*Trichoderma* filtrate (30ml) + Paraffin oil (53ml)) was found significantly superior to the rest of treatments in checking the growth of *Fusarium oxysporum* f. sp. *ciceri.* It showed 18.63 mm mean colony diameter against the control (90 mm) with per cent inhibition of 79.30 at 8th DAI, respectively it was followed by the treatment T_2 (*Trichoderma* filtrate (20ml) + Paraffin oil (63ml), T_3 (*Trichoderma* filtrate (30ml) + Soybean oil (53ml)) andT_4 (*Trichoderma* filtrate (20ml) + Soybean oil (63ml)) with mean mycelial growth of the organism 21.23 mm in T_2, 22.30 mm in T_3, 24.13mm in T_4, with per cent inhibition of 76.41 in T_2, 75.22 in T_3, 73.18, 65.70 in T_4 respectively in *Fusarium oxysporum* f. sp. *ciceri.*

In *Rhizoctonia bataticola* T_1 (*Trichoderma* filtrate (30ml) + Paraffin oil (53ml) is significantly superior to rest of the treatments. The mycelial growth of T_1 over the control (90 mm) was 13.63 with per cent inhibition 84.85 at 8th DAI. T_1 was followed by T_2, T_3 and T_4 showed 16.43, 25.30 and 30.87 with per cent inhibition 81.74, 71.88 and 65.70 showed in Table 2.

In case of *Sclerotium rolfsii* T_1 (*Trichoderma* filtrate (30ml) + Paraffin oil (53ml)) treatment is significantly superior over control (90 mm) mean colony growth 18.77 with per cent inhibition 79.14 followed by T_2, T_3 and T_4 with mean colony mycelial growth were 21.67, 28.67 and 31.20 per cent inhibition 75.92, 68.14 and 65.33 showed in Table 2). The present investigation are in accordance with the results of earlier workers like Rajput *et al.,*(2010), Siameto *et al.,* (2010), Perveen *et al.,* (2012), Srivastava *et al.,* (2012), Tapwal *et al.,* (2015), Dixit *et al.,* (2015) showed that the bioagent like *Trichoderma viride* inhibit the mycelial growth of the soil born pathogen. They revealed that the maximum growth reduction in the *Fusarium oxysporum* f. sp. *ciceri, Rhizoctonia bataticola.* Perveen *et al.,* (2012) showed that the antagonistic activity of *Trichoderma viride* against *Fusarium oxysporum* was excellent. Similar results were also obtained by Siameto *et al.,* (2010) and Srivastava *et al.,* (2012), Seema and Devaki (2012).

Acknowledgement

Authors are thankful to Professor and Head, Department of Plant Pathology, College of agriculture Nagpur for providing facilities for conducting the present research work.

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How to cite this article:

Taral Akshata, L., D.D. Guldekar, S.R. Potdukhe, S.S. Kale and Ashwini Kumar. 2018. Shelf Life Study and Antagonistic Activity of *Trichoderma viride* in Different Oil Formulations. *Int.J.Curr.Microbiol.App.Sci.* 7(11): 225-230. doi: https://doi.org/10.20546/ijcmas.2018.711.028