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Comparison of different oil formulations on shelf life of *Trichoderma asperellum*

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Abstract

An investigation entitled "Comparison of different oil formulations on shelf life of *Trichoderma asperellum*" was carried out during 2018-2019. The laboratory experiment was carried out in Completely Randomized Design with nine treatment in three replication. Paraffin oil, soybean oil, groundnut oil, potato dextrose broth and talc powder were extensively used as carrier for *Trichoderma asperellum* at an intervals of 30, 60, 90, 120, 150 and 180 DAI. It was revealed from shelf life studies that there were significant differences at all the intervals over uninoculated control. Maximum CFU count was recorded with the treatment Paraffin oil 28×10^8 CFU at 30 DAS and it was significantly superior over all other treatment except Talc based culture 27.33×10^8 CFU and was gradually increased upto 90 DAS and thereafter found to be declined from 120 to 180 DAI in all the treatments. It was further noticed that maximum spore germination per cent was noticed in Paraffin oil 59.18 percent at 30 DAT followed by Talc based culture 54.06 percent. There were significant reduction in spore germination from 60 DAI till 180 DAI in all the treatment. As regards of percent growth inhibition at 8TH DAI, among three plant pathogenic fungi *F. oxysporum* inhibited maximum growth 74.44 per cent followed by *R. bataticola* 81.85 per cent and *S. rolfsii* in paraffin oil treatment.

Keywords: *Trichoderma asperellum*, Paraffin oil, Soybean oil, Liquid formulations

Introduction

Trichoderma spp., a genus of filamentous fungi, are among the microorganisms most commonly used as biological control agents are marked as bio-pesticides, biofertilizers, growth-enhancers and stimulants of natural resistance, owing to their ability to protect plants, enhance vegetative growth and check pathogen populations under varied agricultural conditions, as well as to act as soil amendments/inoculants for improvement of nutrient uptake, decomposition and biodegradation. The live microorganisms can be impregnated into various formulations as pure spores or conidial suspensions, in liquid culture filtrates, and can be integrated with various inert components and stored for months without losing its efficacy. The *Trichoderma* formulations are applied as a pre-planting application to seed or propagating material, foliar spray, post pruning, treatment, incorporation in the soil during seeding or transplanting irrigated or applied as root dip or drench. One of the advantages of water or oil emulsions over other methods of formulation is that the oil traps water around the organism and slows water evaporation once applied. This is particularly beneficial for organisms that are sensitive to desiccation (Jean *et al.*, 2006) [7]. Chandra (2011) [4] studied oil based formulations in the field as well as in laboratory and found the highest shelf life of *Trichoderma* for more than four years with average of 10^9 to 10^{10} cell ml⁻¹. These products are able to survive 6 to 2 years depending upon the formulation. *Trichoderma harzianum* and *Trichoderma viride* are the widely used species and have been exploited on about 87 different crops and about 70 soil borne and 18 foliar pathogens, respectively (Sharma *et al.*, 2014) [13]. Keeping in view of the growing market for long shelf life products with very high CFU counts, oil based formulations is developed for biocontrol fungus like *Trichoderma*.

Materials and Methods

Studies on liquid formulation entitled "Comparison of different oil formulations on shelf LIFE OF *Trichoderma asperellum*" was conducted in Plant Pathology Laboratory, College of Agriculture, Nagpur during the year 2018-2019.

Mass multiplied *Trichoderma asperellum* was transferred in to mixing tank to harvest the spore and mycelium. *Trichoderma asperellum* formulation was poured into pre sterilized plastic bottles.

Each treatment contained glycerol (10ml), dispersant (1ml), surfactant (3ml), suspender (3ml). Three oils were used viz., paraffin oil, soybean oil and groundnut oil incorporated into the *Trichoderma asperellum* formulation in each plastic bottles as per the given in treatments from T₁ to T₆. Whereas T₇ was talc base departmental culture, T₈ was liquid formulation market product. The Treatment details were T₁- Paraffin oil (60 ml), T₂- Soybean oil (60 ml), T₃- Groundnut oil (60 ml), T₄- Paraffin oil (30 ml)+ Soybean oil (30 ml) T₅ - Paraffin oil (30 ml)+ Groundnut oil (30 ml), T₆ -Soybean oil (30 ml)+ Groundnut oil (30 ml), T₇. Talc based culture (Departmental culture) T₈ Li Liquid culture (Market product) and T₉. The observations were recorded on CFU count spore germination at 30, 60, 90, 150, and 180 DAI and percent growth inhibition. The bottles were packed with the help of caps and kept for a storage for 180 days at 27±1⁰ C. CFU count was under taken at various interval by serial dilution followed by pour plate method.

Results and Discussion

Effect of different liquid formulations on the shelf life of *Trichoderma asperellum* (×10⁸CFU/ml) at various interval

It was revealed from the data (Table 1) that there were significant differences in *Trichoderma asperellum* at all the interval over uninoculated control. The initial population of *T. Asperellum* at 30 DAI was maximum i.e. 28 x 10⁸CFU/ml in T₁ Paraffin oil (60 ml) which was significantly superior over all treatment except T₇ Talc based culture (27.33 x 10⁸ CFU/ml). At the 180 DAI maximum population density of *T. asperellum* was observed in T₁ (18.33 x 10⁸CFU/ml) followed by T₇ treatment. These result correlates with the findings of

Reddy *et al.* (2017) who calculated *T. harzianum* in the form of CFU on 56th day of observation in treatment of paraffin oil (20 x 10⁷) and in soybean oil (2.1 x 10⁷) imilarly Rai and Tewari (2016), Bhai and Anandraj (2014), Mbarga *et al.* (2014), Chandra (2011)^[4], Khan *et al.* (2011), Taweil *et al.* (2010), Nadare *et al.* (2018) and Mujtaba and Kulkarni (2017)^[10] reported maximum CFU of *Trichoderma viride* in paraffin oil which was followed by soybean oil. Batta (2004)^[2], Kolombet *et al.* (2008)^[8], Bhatet *et al.* (2009)^[3] revealed that the formulation retained good numbers of viable propagules (above 10⁶cfu/g) for more than 150 days of storage.

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

All the treatments significantly inhibiting the radial mycelial growth of *Fusarium oxysporum* f.sp. *cicerei*, *Rhizoctonia bataticola* and *Sclerotium rolfsii* over uninoculated control (Table 2) control. The results showed that treatment T₁ was found significantly superior to all the other treatments the growth of *Fusarium oxysporum* f. sp. *cicerei*. It showed 23 mm mean colony diameter against the control (90 mm) with per cent inhibition of 74.44 percent at 8th DAI, In *Rhizoctonia bataticola* T₁ Paraffin oil (60ml) is significantly superior to all other treatments. The mycelial growth of T₁ over the control (90 mm) were 24.66 with per cent inhibition 81.85 at 8th DAI. Maximum percent growth inhibition was noticed with T₁ treatment 74.44 per cent by *Fusarium oxysporum* f.sp. *cicerei* followed *Rhizoctonia bataticola* 81.85 per cent. However, minimum per cent growth. Similar findings have been reported by cherkupally *et al* (2017)^[5], Patole *et al* (2017)^[11] and Reddy *et al.* (2017).

Table 1: Effect of different liquid formulation on the shelf life of *Trichoderma asperellum* (×10⁸ CFUml⁻¹) and spore germination at various interval

T Treatment	<i>Trichoderma asperellum</i> (×10 ⁸ CFUml ⁻¹)						S Spore germination (per cent)					
	3 30	6 60	9 90	120	1 150	1 180	3 30	6 60	9 90	1 120	1 150	1 180
Paraffin oil	28.00	23.66	22.33	21.33	20.00	18.33	5 59.18	5 55.10	4 45.28	3 38.98	3 35.84	3 30.18
S Soybean oil	12.00	11.33	10.00	8.66	6.66	5.33	46.15	3 37.28	2 28.49	2 25.67	2 22.60	2 21.32
Groundnut oil	10.33	9.33	8.66	7.00	5.66	2.00	2 26.43	2 25.58	1 17.43	1 16.66	9. 9.64	8. 8.56
Paraffin oil + Soybean oil	9.00	8.00	7.66	6.66	5.00	1.66	3 32.70	2 23.18	2 21.15	1 16.98	1 16.01	1 10.48
Paraffin oil + Groundnut oil	8.00	7.33	7.00	6.33	4.66	1.33	3 32.05	2 23.07	1 16.96	1 16.02	1 14.96	8. 8.29
Soybean oil + Groundnut oil	7.6	6.66	6.33	5.33	3.66	1.00	2 29.09	2 25.45	1 16.66	1 15.64	1 12.5	7. 7.43
T Talc based culture	27.33	25.33	20.32	19.36	18.66	16.00	5 54.06	5 51.20	4 42.79	3 23.95	2 22.00	2 20.20
L Liquid culture	9.33	7.00	6.33	4.33	2.33	1.66	4 43.57	3 31.40	2 25.77	2 20.75	10.84	^{20.20}
Control	24.66	21.00	18.00	10.33	9.33	2.33	3 38.31	3 30.94	2 26.54	2 23.80	1 18.64	8 8.33
SE ± (m)	0.86	0.98	0.76	0.44	1.05	0.57	2 2.2	2. 2.2	1. 1.4	1. 1. 9	1. 1.2	0. 0.66
CD (P=0.01)	3.41	3.86	2.99	1.73	4.13	2.26	8 8.7	8. 8. 6	5 5.5	7. 7.6	4. 4. 7	2. 2. 6

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

Tr. No.	Treatment	Mycelial growth (mm)			% Growth inhibition		
		<i>F. oxysporum</i> f. sp. <i>cicerei</i>	<i>R. bataticola</i>	<i>S. rolfsii</i>	<i>F. oxysporum</i> f. sp. <i>cicerei</i>	<i>R. bataticola</i>	<i>S. rolfsii</i>
T ₁	P Paraffin oil	23.00	16.33	24.66	74.44	81.85	72.6
T ₂	S Soybean oil	28.66	19.00	30.3	68.15	70.37	66.33
T ₃	G Groundnut oil	26.66	25.66	27.3	70.37	71.48	69.66
T ₄	P Paraffin oil + Soybean oil	30.33	27.33	30.00	66.3	69.63	66.66
T ₅	P Paraffin oil + Groundnut oil	29.33	29.66	31.00	61.3	67.04	65.55
T ₆	S Soybean oil + Groundnut oil	34.00	35.00	34.3	62.22	61.11	61.88
T ₇	T Talc based culture	21.33	33.66	43.33	76.3	62.6	51.85
T ₈	Li Liquid culture	40.00	37.33	40.66	55.55	58.52	54.82
T ₉	C Control	90.00	90.00	89.00	-	-	-
	SE ± (m)	0.13	0.13	0.09			
	C CD (P= 0.01)	0.54	0.53	0.39			

Table 3: Effect of different liquid formulation on the shelf life of *Trichoderma asperellum* ($\times 10^8$ CFUml⁻¹) at various interval.

Tr. No.	Treatment	DAI					
		30	60	90	120	150	180
T1	Paraffin oil	28.00	23.66	22.33	21.33	20.00	18.33
T2	Soybean oil	12.00	11.33	10.00	8.66	6.66	5.33
T3	Groundnut oil	10.33	9.33	8.66	7.00	5.66	2.00
T4	Paraffin oil + Soybean oil	9.00	8.00	7.66	6.66	5.00	1.66
T5	Paraffin oil + Groundnut oil	8.00	7.33	7.00	6.33	4.66	1.33
T6	Soybean oil + Groundnut oil	7.6	6.66	6.33	5.33	3.66	1.00
T7	Talc based culture	27.33	25.33	20.32	19.36	18.66	16.00
T8	Liquid culture	9.33	7.00	6.33	4.33	2.33	1.66
T9	Control	24.66	21.00	18.00	10.33	9.33	2.33
	SE \pm (m)	0.86	0.98	0.76	0.44	1.05	0.57
	CD (1%)	3.41	3.86	2.99	1.73	4.13	2.26

Table 4: Effect of *Trichoderma asperellum* liquid formulations on per cent growth inhibition on 8th DAI.

Tr. No.	Treatment	Mycelial growth (mm)			% Growth inhibition		
		F. oxysporum f. sp. ciceri	R. bataticola	S. rolfsii	F F. oxysporum f. sp. ciceri	R. bataticola	S. rolfsii
T ₁	P Paraffin oil	23.00	16.33	24.66	74.44	81.85	72.6
T ₂	S Soybean oil	28.66	19.00	30.3	68.15	70.37	66.33
T ₃	G Groundnut oil	26.66	25.66	27.3	70.37	71.48	69.66
T ₄	P Paraffin oil + Soybean oil	30.33	27.33	30.00	66.3	69.63	66.66
T ₅	P Paraffin oil + Groundnut oil	29.33	29.66	31.00	61.3	67.04	65.55
T ₆	S Soybean oil + Groundnut oil	34.00	35.00	34.3	62.22	61.11	61.88
T ₇	T Talc based culture	21.33	33.66	43.33	76.3	62.6	51.85
T ₈	Li Liquid culture	40.00	37.33	40.66	55.55	58.52	54.82
T ₉	C Control	30.66	42.66	42.66	65.93	52.6	52.6
	Control	90.00	90.00	90.00	100	100	100
	SE \pm (m)	0.13	0.13	0.09			
	C CD (P= 0.01)	0.54	0.53	0.39			

Table 5: Effect of *Trichoderma asperellum* formulation on seed germination, root length, shoot length and SVI of Bengal gram, seedinoculated with *Fusarium oxysporum*, *Rhizoctonia bataticola*, *Sclerotium rolfsii*.

Tr. No.	Treatment	<i>Fusarium oxysporum</i>				<i>Rhizoctonia bataticola</i>				<i>Sclerotium rolfsii</i>			
		Germination (%)	Root length (cm)	Shoot length (cm)	SVI	Germination (%)	Root length (cm)	Shoot length (cm)	SVI	Germination (%)	Root length (cm)	Shoot length (cm)	SVI
T1	Paraffin oil	87.33	21.66	31.33	4611.02	74.33	19.66	30.66	3740.28	73.00	18.66	30.00	3552.18
T2	Soybean oil	83.66	19.00	30.00	3990.58	67.66	17.66	28.33	3111.68	64.33	16.66	27.66	2851.10
T3	Groundnut oil	81.33	16.33	28.33	3594.78	63.33	15.33	27.00	2680.75	60.66	14.66	26.33	2486.45
T4	Paraffin oil + Soybean oil	74.66	17.33	28.00	3329.8	57.66	15.66	26.66	2440.17	52.33	15.00	26.00	2145.53
T5	Paraffin oil + Groundnut oil	71.66	15.00	24.33	2823.4	51.33	13.66	24.33	1950.02	49.33	13.00	23.66	1808.43
T6	Soybean oil + Groundnut oil	68.00	12.00	21.00	2162.4	50.33	11.33	19.66	1559.72	48.00	10.66	19.00	1423.68
T7	Talc based culture	74.33	20.00	27.00	3344.85	66.00	19.33	26.33	3013.56	64.00	18.66	25.66	2836.48
T8	Liquid culture	67.33	9.00	16.00	1669.78	63.00	8.33	1.30	606.69	59.33	7.33	8.00	909.52
T9	Control	64.66	9.60	16.66	1697.97	61.30	9.33	14.66	1470.58	60.00	8.66	14.00	1359.6
	SE \pm (m)	-	0.61	1.04			0.76	0.97			0.70	0.96	
	CD (1%)	-	2.51	4.24			3.13	3.81			2.86	3.94	

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