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Shelf life studies of different formulations based on *Trichoderma harzianum* (Th14)

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ABSTRACT

In the present study total nine types of Trichoderma based formulations were prepared using formulating materials viz. Dextrin, talc, gypsum, paraffin oil and soybean oil. Shelf life of formulations was tested up to 6 months at room temperature (15-35^{0}C) and 11 months at refrigerator (4^{0}C). Among different formulation dextrin based formulation TF. Paste8 retained maximum viability(26.10%; 4.33x10^7 CFU/ g) followed by TF.Paste9 (23.95%; 4.00x10^7 CFU/ ml), and oil based TF.LQ6 (22.43%; 9.67x10^7 CFU/ ml) after 6 months of storage at room temperature (15-35^{0}C).The formulations stored at 4^{0}C retained viability (2.06- 16.06 %) up to 11 months during storage. Maximum viability was observed in TF.Paste8 (16.06%; 2.67x10^7 CFU/ g) followed by TF.Paste9 (11.98%; 2.00x10^7 CFU/ g) and oil based TF.LQ6 (8.89%; 3.83x10^7 CFU/ ml). This study showed that there is potential in using of Trichoderma paste and liquid formulations for improving shelf life of bioformulation as well as in biological control.

INTRODUCTION

The current plant disease management strategies carried out by national and international agricultural research agencies in India have been progressively reoriented to a reduced application of pesticide chemicals while focusing on biological control methods to manage crop diseases and traditional plant breeding programs to improve host plant resistance. Under the changing agriculture scenario, the only technology that seems promising to manage the diseases without disturbing the equilibrium of harmful and useful composition of environment and ecosystem is the use of more and more biological control agents. Biological control can be achieved by either introducing biocontrol agents directly in to natural ecosystem or by adopting practices which favour population build-up biocontrol agents under natural condition. Combination of both approaches is probably the best solution. In recent years there has been a tremendous progress in this area. Among various fungal and bacterial biocontrol agents *Trichoderma* spp. have received prominent attention due to their abilities to establish large rhizosphere population sizes on the emerging root system and suppression of diseases of treated plants.

Trichoderma spp. have been widely studied, and are presently marketed as biopesticides, biofertilizers and soil amendments, due to their ability to protect plants, enhance vegetative growth and reduce pathogen populations under numerous agricultural conditions[1]. The commercial success of products containing these fungal antagonists can be attributed to the large volume of viable propagules that can be produced rapidly and readily on numerous substrates

at a low cost in diverse fermentation systems [2] . The living microorganisms, conserved as spores, chlamydospores, fragmented mycelium can be incorporated into various formulations like liquid, granules or powder etc., and stored for months without losing their efficacy [3].

Shelf life of the formulated product of a biocontrol agent plays a significant role in successful commercialization. In general, the antagonists multiplied in an organic food base have longer shelf life than the inert or inorganic food bases. Talc, peat, lignite and kaolin based formulation of *Trichoderma*, have a shelf life. The viable propagules of *Trichoderma* in talc formulation were reduced by 50 per cent after 120 days of storage [4]**.** At PDBC, Bangalore work on increasing shelf life of talc formulations of *Trichoderma* using various ingredients (chitin and glycerol) in production medium and heat shock at the end of log phase of fermentation was carried out which extended the shelf of talc formulation of successfully up to one year[5,6]. However, the problem how to maintain the CFU (10^6) and its efficacy in formulated products in viable form for one year during storage at the time of application and or throughout cropping season after application in the field still remained unsolved.

Keeping in this view, investigation was undertaken to study the shelf life of dextrin, talc, gypsum, paraffin oil and soybean oil based formulation of *Trichoderma* up to 6 months at room temperature (15-35^{0}C) and 11 months at refrigerator (4^{0}C).

MATERIALS AND METHODS

The present investigation was conducted in the Oilseed Pathology Lab of the Department of Plant Pathology, G.B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand) India.

The Antagonist (*Trichoderma harzianu*m Th 14)

The *Trichoderma harzianum* isolate Th14 obtained from culture collections of Biocontrol laboratory, Department of Plant Pathology, GBPUA&T, Pantnagar for the present investigation. The fungal antagonist was maintained on potato dextrose agar (PDA) slant and stored in refrigerator for further studies.

Developed bioformulations and their ingredients

Nine different *Trichoderma* formulations (3 Wettable,4 liquid and 2 pastes based) were prepared.

No.	Bioformulation code	1. Bioformulation description (ingredients)
1	**TF.WP1**	2. *Trichoderma* filtrate (300ml) + Dextrin (700g)
2	**TF.WP2**	3. *Trichoderma* filtrate (500ml.) + Talc (500g)
3	**TF.WP3**	4. *Trichoderma* filtrate (500ml) + Gypsum (500ml)
4	**TF.LQ4**	5. *Trichoderma* filtrate (500ml)+ Paraffin oil (500 ml)
5	**TF.LQ5**	*6. Trichoderma* filtrate (500ml)+ Soybean oil (500ml)
6	**TF.LQ6**	7. *Trichoderma* spore suspension (washed from well colonized on sorghum grains in paraffin oil) (500ml)+ Paraffin oil (500 ml)
7	**TF.LQ7**	8. *Trichoderma* spore suspension (washed from well colonized on sorghum grains) (500ml) + Soybean oil (500ml)
8	**TF.Paste8**	9. Dextrin formulation (TF.WP1) (500g) + Paraffin oil (500ml)
9	**TF.Paste9**	10. Dextrin formulation (TF.WP1) (500g) + Soybean oil (500ml)

During the preparation of wettable powder based formulations 0.2 per cent carboxyl methyl cellulose (CMC) and 0.1per cent chitosan were mixed in each formulation. In liquid based formulations 0.2 per cent carboxyl methyl cellulose, 5.0 per cent glycerol, 0.1per cent chitosan and 1.0 per cent tween 80 were properly mixed in each formulation. WP based formulations were properly dried under shade at room temperature and made it in fine powder and the final formulations were kept in air tight polyethene bags and liquid formulations in glass vials.

RESULTS AND DISCUSSION

Shelf life of the bioformulations

Prepared formulations were kept at room temperature (15-35^{0}C) and in refrigerator (4^{0}C). Viability of the antagonist in each formulation was determined at one month interval up 12 month. At each sampling time 1g or ml formulation was suspended in 10 ml sterile distilled water and diluted up to desired dilutions (countable cfu/ g or ml in 90mm petri plate). One ml suspension was poured on to the surface of petri plates containing TSM (*Trichoderma* Selective Medium). After 48 hrs incubation at 27±1^{0}C colonies were noted.

Shelf life of *Trichoderma harzianum* (Th14) in different formulations

In the present study 9 different *Trichoderma* formulations i.e. TF.WP1, TF.WP2, TF.WP3, TF.LQ4, TF.LQ5, TF.LQ6, TF.LQ7, TF.Paste8, TF.Paste9 were prepared and initial CFU of each formulation was measured. The colony forming unit (cfu) count was highest initially but gradually decline was recorded with the increases in storage time. The results (Table1) revealed that all the formulations contained initial CFU count in the range of 16.4 to 43.1x10^7 CFU/g or ml. However, maximum CFU was observed in TF.LQ6 (43.1x10^7 CFU/ ml) followed by TF.LQ7 (42.4x10^7 CFU/ ml) and was at par with each other and minimum in TF.Paste8 (16.60x10^7 CFU/ ml) formulations.

These formulations were further tested for their shelf life up to 7 months at room temperature (15-35^{0}C) 11 months at refrigerator (4^{0}C) during storage at both the temperature. The observations on CFU were recorded at 1 month interval and up to 10 months. Result shows that at room temperature (15-35^{0}C) there was a gradual decline in the CFU of *Trichoderma* in all the formulations up to 6 months and thereafter sudden decline was observed. Among different formulation dextrin based formulation TF. Paste8 retained maximum viability (26.10%; 4.33x10^7 CFU/ g) followed by TF.Paste9 (23.95%; 4.00x10^7 CFU/ ml), and oil based TF.LQ6 (22.43%; 9.67x10^7 CFU/ ml) after 6 months of storage at room temperature (15-35^{0}C). Minimum viability was observed in TF.WP3(7.72%; 2.50x10^6 CFU/ g).

The formulations stored at 4^{0}C (Table 2a& 2b) revealed that at refrigerator (4^{0}C) there was a gradual decline in the CFU of *Trichoderma* in all the formulations up to 11 months and thereafter sudden decline was observed. All the prepared formulations retained optimum viability (2.06- 16.06 %) up to 11 months during storage. Maximum viability was observed in TF.Paste8 (16.06%; 2.67x10^7 CFU/ g) followed by TF.Paste9 (11.98%; 2.00x10^7 CFU/ g) and oil based TF.LQ6 (8.89%; 3.83x10^7 CFU/ ml). Minimum viability was observed in TF.WP3 (2.02%; 0.67x10^7 CFU/ g) based formulation.

In the present finding increased shelf life in different formulations were observed both at room temperature (15-35^{0}C) & at refrigerator (4^{0}C). However, paste and liquid based formulations retained higher viability as compare to wettable based bioformultions. Earlier workers reported shelf life of different formulation up to 9 and 6 months at refrigerator & room temperature [7] Talc and gypsum based formulation up to 150 days at room [8] liquid paste formulation up to 6 months at room temperature [9] paraffin oil based formulation up to 6 at 30^{0}C [10] talc formulations (chitin and glycerol) up to one year at refrigerator (4^{0}C) [5,6] sawdust + CMC, sawdust+chitosan, and sawdust + talc powder + chitosan up to 6 months at room temperature [11] talc based formulation up to 120 days[12,13] oil based formulation shelf life 18 weeks longer than conidia in aqueous suspension [14, 15].

Table 1: Shelf life of different formulations of *T. harzianum* at room temperature (15^0-35^{0}C)

Trichoderma formulation	CFU (x 10^7 g or ml)*								
	Months (January 2013 to June 2013)								
	0	1	2	3	Viability (%)	4	5	6	Viability (%)
1. WP based (*Trichoderma* filtrate)									
TF.WP1	24.50^b	24.33^b	23.50^b	20.80^b	84.90	15.10^c	12.30^{bc}	3.83^c	15.65
TF.WP2	35.60^{cd}	34.60^{cd}	32.10^{cd}	28.20^{cd}	79.21	20.00^d	14.60^{cd}	3.67^c	10.30
TF.WP3	32.40^c	31.10^c	28.30^c	24.20^{bc}	74.69	16.20^c	10.40^b	2.50^b	7.72
2. Oil based (*Trichoderma* filtrate/ spore based)									
TF.LQ4	37.20^d	36.30^d	32.17^{cd}	29.17^{cde}	78.41	21.00^d	16.20^{cd}	5.33^d	14.34
TF.LQ5	37.60^e	36.57^d	33.17^d	31..13^{def}	82.79	21.07^d	17.90^{de}	5.48^d	14.58
TF.LQ6	43.10^e	42.43^e	39.40^e	35.13^f	81.51	28.23^e	23.67^f	9.67^f	22.43
TF.LQ7	42.40^e	41.30^e	38.70^e	34.60^{ef}	81.60	26.20^e	21.40^{ef}	8.00^e	18.87
3. TF.WP1+oil (1:1)									
TF.Paste8	16.60^a	16.20^a	15.60^a	14.20^a	85.54	10.20^b	10.90^b	4.33^c	26.10
TF.Paste9	16.70^a	16.10^a	15.00^a	13.80^a	82.63	10.70^b	9.20^b	4.00^c	23.95
4.*Trichoderma* powder +Talc (1:1)									
Commercial formulations (standard check)	20.60^{ab}	19.10^a	16.70^a	10.27^a	49.85	6.30^a	2.10^a	0.33^a	1.62
CD(0.05)	(A)	(B)	(AxB)						
	1.63	1.26	4.00						
CV (%)	10.37								

*A-Trichoderma formulation; B-Month; *Mean of three replicates; Values in each vertical column followed by same letter do not differ significantly*

Table 2a : Shelf life of different formulation of *T. harzianum* at 4^0 C (refrigerator)

Trichoderma formulation	CFU (x 10^7 g or ml)*								
	Months								
	0	1	2	3	Viability (%)	4	5	6	Viability (%)
4. WP based (*Trichoderma* filtrate)									
TF.WP1	24.50^{b}	24.30^{c}	24.10	23.90^{c}	97.55	23.40^{b}	22.60^{b}	21.30^{b}	86.94
TF.WP2	35.60^{cd}	35.30^{e}	35.00	34.60^{e}	97.19	33.70^{d}	32.47^{d}	29.87^{d}	83.90
TF.WP3	32.40^{c}	32.00^{d}	31.60	31.20^{d}	96.30	30.20^{c}	28.83^{c}	26.30^{c}	81.17
5. Oil based (*Trichoderma* filtrate/ spore based)									
TF.LQ4	37.20^{d}	36.67e	36.23	35.80^{e}	96.24	34.83^{d}	33.80^{d}	31.67^{d}	85.13
TF.LQ5	37.60^{d}	37.00^{e}	36.60	36.10^{e}	96.01	35.17^{d}	33.83^{d}	31.93^{d}	84.92
TF.LQ6	43.10^{e}	42.53^{f}	42.10	41.30^{f}	95.82	40.30^{e}	38.90^{e}	36.50^{e}	84.69
TF.LQ7	42.40^{e}	42.10^{f}	41.67	41.20^{f}	97.17	40.40^{e}	38.70^{e}	36.20^{e}	85.38
6. TF.WP1+oil (1:1)									
TF.Paste8	16.60^{a}	16.50^{a}	16.30	16.20^{a}	97.59	15.57^{a}	15.07^{a}	14.60^{a}	87.95
TF.Paste9	16.70^{a}	16.20^{a}	16.00	15.90^{a}	95.21	15.60^{a}	14.77^{a}	13.10^{a}	78.44
4.*Trichoderma* powder +Talc (1:1)									
Commercial formulations (standard check)	20.60^{ab}	20.20^{b}	19.80	19.33^{b}	93.83	18.50^{a}	15.80^{a}	11.50^{a}	55.83

*A- Trichoderma formulation; B Month; *Mean of three replicates*
Values in each vertical column followed by same letter do not differ significantly

Table 2b : Shelflife of different formulation of *T. harzianum* at 4^0 C (refrigerator)

Trichoderma formulation	CFU (x 10^7 g or ml)*						
	Months						
	7	8	9	Viability (%)	10	11	Viability (%)
7. WP based (*Trichoderma* filtrate)							
TF.WP1	19.40^{c}	17.00^{c}	14.40^{c}	58.78	6.83^{bc}	2.00^{cd}	8.16
TF.WP2	25.80^{d}	21.50^{d}	15.50^{c}	43.54	7.87^{c}	1.67^{bcd}	4.68
TF.WP3	21.20^{c}	14.10^{b}	9.80^{b}	30.25	4.50^{b}	0.67^{ab}	2.06
8. Oil based (*Trichoderma* filtrate/ spore based)							
TF.LQ4	28.33^{e}	24.63^{e}	18.70^{d}	50.27	8.67^{cd}	1.33^{abc}	3.58
TF.LQ5	28.10^{de}	24.60^{e}	19.20^{d}	51.06	11.20^{d}	1.67^{bcs}	4.43
TF.LQ6	32.80^{f}	29.10^{f}	23.20^{f}	53.83	15.70^{e}	3.83^{f}	8.89
TF.LQ7	32.30^{f}	28.50^{f}	21.10^{e}	49.76	14.00^{e}	3.33^{ef}	7.86
9. TF.WP1+oil (1:1)							
TF.Paste8	13.40^{b}	12.20^{b}	10.40^{b}	62.65	6.70^{bc}	2.67^{de}	16.06
TF.Paste9	12.70^{b}	11.30^{b}	9.70^{b}	58.08	6.10^{bc}	2.00^{cd}	11.98
4.*Trichoderma* powder +Talc (1:1)							
Commercial formulations (standard check)	5.60^{a}	2.30^{a}	0.67^{a}	3.25	0.67^{a}	0.33^{a}	1.62
CD(0.05)	(A)	(B)	(AxB)				
	0.83	0.87	2.75				
CV (%)	7.09						

*A- Trichoderma formulation; B Month; *Mean of three replicates; Values in each vertical column followed by same letter do not differ significantly*

CONSLUSION

All the prepared formulations retained optimum viability. Paste and liquid based formulations gave higher shelf life of *Trichoderma* compare to wettable based formulations. Application of paste and liquid formulation of bicontrol agents in orchards and in the field would help the farmer in promising better yield.

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