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Assessment of Shelf Life of Trichoderma Formulations Stored in Different Packing Materials

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ABSTRACT

Trichoderma strains have become commercially available and emerging as a cheap and environmentally safe bioagent against most of the soil borne diseases. Shelf life of two different *Trichoderma* formulations, *i.e.*, Talc powder formulation and mineral oil formulation stored in different packing materials at room temperature and at refrigerated (4^0C) condition was evaluated for ten months by observing cfu once in a month on TSM. The results of this study at the end of ten months storage period *in vitro* indicated that there was more or less a gradual decline in the population of both the formulated products *i.e.*, talc powder and mineral oil formulation stored using different packing materials at different temperatures (all the packing material were assessed monthly and final population of formulation at the end of tenth month is focussed). Talc powder formulation stored in paper cover ($7.7x10^8$cfu/g) and mineral oil formulation stored in pet jar ($16.7x10^9$cfu/ml) showed longest shelf life (ten months) with highest *Trichoderma* population among all the treatments tested over both the storage temperatures.

Keywords - *Trichoderma, Shelf life, Formulations and cfu*

Biological control is proved to be a promising disease management technology against soil borne plant pathogens when applied either alone or in combination with other management practices. Among the successful bioagents, *Trichoderma* spp. is being extensively used and is available as commercial formulations to manage several soil borne plant pathogens (Papavizas, 1985).

Trichoderma spp. were the most widely used biocontrol agents since they have antifungal and anti-enduring activities (Zaidi and Singh, 2004). Though several species of *Trichoderma* are found to be effective in managing plant diseases, isolate variation existed in their antagonistic efficacy (Upadhyay and Mukhopadhyay, 1986 and Patibanda *et al.*,2002). The success of a biocontrol agent depends on its ability to produce inoculum in excess, grow and proliferate well on the plant parts whenever applied. Formulation and shelf life are of prime importance agents for commercial use of any biocontrol agent. The population of bioagent in the formulation is an important factor deciding the quantity of product necessary to apply in the field. Failure of antagonist to survive due to shorter shelf life is a major hindrance to consistent field performance.

MATERIAL AND METHODS

The present investigation was carried out in the Department of Plant Pathology, Agricultural College, Bapatla. Shelf life of the formulated product of a biocontrol agent plays a significant role in successful marketing. The shelf life study was carried out in different packing materials using effective carrier material.

Assessment of CFU from formulations using dilution plate technique **(**Elad and Chet, 1983**):** The number of colony forming units (cfu) was determined by using dilution plate technique. One gram sample of each formulation was separately mixed in nine ml of sterile distilled water in culture tube making it 10^{-1} dilution. This suspension was serially diluted in sterile distilled water. Dilution of 10^{-8} was used for estimating Th4 (*Trichoderma harzianum* 4) cfu from inoculated treatments. One hundred µl of each diluted suspension was pipetted with the help of a micropipette in to Petri

plates containing TSM medium under aseptic conditions and spread with a sterilized spreader. The inoculated plates were incubated at 29±1^{0}C for 48h. Observations on number of cfu were recorded.

The packing materials used in the present study are aluminum tin, amber coloured bottle, HDPE bottle, aluminum cover, paper cover, polythene cover and pet jar. The formulated products were stored in different packing materials at ambient temperature and at 4^{0}C (refrigerator). Viability of the formulated products was tested at 30 days interval for 10 months under laboratory conditions (each formulation replicated thrice with 100g in each pack).

RESULTS AND DISCUSSION

Shelf Life of *Trichoderma* Formulations *In Vitro*

In the present investigation, shelf life of two different *Trichoderma* formulations, *i.e*., talc powder formulation and mineral oil formulation stored in different packing materials at room temperature and at 4^{0}C was evaluated. Talc formulation of Th4 was prepared and estimated for *Trichoderma* population on TSM. The average cfu was found to be 34.0x10^8 cfu/g. Similarly, for mineral oil formulation, an average of 20x10^{11} cfu/ml was obtained. For precision, however, individual packing material was separately monitored for the presence of cfu. It may be mentioned here that, cfu just after preparation was found to differ insignificantly among the different packing material as expected as they were distributed into respective packing material after common preparation.

Storage for ten months

During first month of shelf life of Th4 formulation, highest mean cfu population of talc formulation was observed in aluminum cover and paper cover while, highest cfu of mineral oil formulation was noticed in polythene cover and aluminum tin. During second month of shelf life of Th4 formulations, highest mean cfu population of talc formulation was observed in aluminum cover, paper cover and pet jar while, highest cfu of mineral oil formulation was noticed in polythene cover and pet jar. Similar trend was also noticed during third and fourth month storage.

During fifth month of shelf life of Th4 formulations, highest mean cfu population of talc formulation was observed in paper cover, aluminum cover and pet jar while, highest cfu of mineral oil formulation was noticed in pet jar, aluminum tin, aluminum cover and polythene cover. Similar trend was also noticed during sixth month storage. Up to fourth month of shelf life of Th4 mineral oil formulation, polythene cover was noticed with highest cfu population, but from fifth month of storage, the trend was slightly changed and pet jar packing material was proved to be safe for storage of mineral oil formulation.

During seventh month of shelf life of Th4 formulations, highest mean cfu population of talc formulation was observed in paper cover, pet jar, aluminum cover and polythene cover while, highest cfu of mineral oil formulation was noticed in aluminum tin, pet jar, aluminum cover and polythene cover. During eighth month shelf life of Th4 formulations, highest mean cfu population of talc formulation was observed in pet jar and paper cover while, highest cfu of mineral oil formulation was noticed in pet jar, aluminum cover and polythene cover. Similar trend was also noticed during seventh month storage. Similar trend was also noticed during ninth month storage.

Finally, the results of this study at the end of ten months storage period *in vitro* indicated that there was more or less a gradual decline in the population of both the formulated products *i.e*., talc powder and mineral oil formulation stored using different packing materials at different temperatures. After ten months of storage of Th4 talc formulation, the population count at ambient and 4^{0}C temperature storage was statistically on par with each other with higher mean population in ambient temperature (Table 1). When the population density of talc formulated packing material was estimated, highest mean population was noticed in paper cover (7.7x10^8), pet jar (7.5x10^8), polythene cover (7.5x10^8), aluminum cover (7.3x10^8) and aluminum tin (6.2x10^8) with non significant differences among them. Least mean population was obtained from amber coloured bottle (4.5x10^8) and HDPE bottle (4.8x10^8) cfu/g which were on par with each other.

In case of talc powder formulation, aluminum tin, amber coloured bottle and HDPE bottle has a gradual declining trend of cfu count as the storage period increases. This was supported as earlier studies made by Sankar and Jeyarajan (1996) and Bheemaraya *et al*. (2011). Sankar and Jeyarajan (1996) found that the viable propagules of *Trichoderma* in talc formulation were reduced by 50%

after 120 days of storage. Bheemaraya *et al*. (2011) recorded that there was a gradual decline in cfu of *Trichoderma* from 30, 60, 90, 120, 150 days upto 180 days.

The talc carrier in all the packing materials supported maximum growth and viable propagules required for recommended concentration (x10^8 cfu/g) upto the end of storage period with gradual decline starting from initiation of shelf life **(Fig 1).** This might be due to the fact that in talc based formulation the initial Th4 population was (34.2x10^8 cfu/g) which otherwise can be converted to 3.42x10^9 cfu/g. Hence, even with a decline of 90% in population, after 10^{th} month, the formulation yielded 10^8 cfu/g which is the required dose for quality check clearance. It needs to be noted here that if the initial population is set at 1-9x10^8 cfu/g, by 10^{th} month with a decrease of 90% population, Th4 cfu would be 10^7 cfu/g which is unacceptable for trade. Hence, the present investigation revealed that the initial population should be about 10x10^8 cfu/g, or 1-9x10^9 cfu/g, instead of 1-9x10^8 cfu/g.

The mean population count of Th4 mineral oil formulation was assessed during tenth month storage and revealed that the population of ambient and 4^0C temperatures was non significantly differed between them (Table 2 & Fig 2). Among all the tested packing materials, the mean population of pet jar was highest with 16.7x10^9 cfu/ml, followed by aluminum cover (15.3x10^9) and polythene cover (14.8x10^9) with non significant differences among them. Least mean population was obtained from amber coloured bottle (10.5x10^9) and HDPE bottle (12.0x10^9) which were on par with each other.

Further, when the shelf life was assessed at ambient and 4^0C temperatures, upto certain storage period, 4^0C temperature was found effective. But thereafter, population at ambient temperature was found higher than at 4^0C temperature. These results are in agreement with Malathi and Doraisamy (2003).

Thus, the present investigation showed superiority of ambient temperature during storage of talc formulations of *Trichoderma* spp. up to ten months of storage period which is in agreement with those of previous workers, who observed the importance of ambient temperature during shelf life of talc formulations of *Trichoderma* (Hunjan *et al*., 2004 and Zaidi and Singh, 2004).

However, in the present study, addition of CMC as an additive to the talc powder formulation could be the reason for sustenance of 10^8 cfu/g upto 10^{th} month. Das *et al*. (2006) stated that the overall performance of talc in terms of cfu was found better in prolonged storage as in talc based formulation food source may be slowly available for the organism and support the population at a safe level for prolonged period.

Table 1. Effect of different packing material on the shelf life of *Trichoderma*Th4talc formulation during tenth month storage (×10^8cfu/g of formulation) *in vitro*

Packing material	Ambient temperature	4^0C	Mean
Aluminum tin	6.7	5.7	**6.2^{ab}**
Amber coloured bottle	4.7	4.3	**4.5^{c}**
HDPE bottle	5	4.7	**4.8^{bc}**
Aluminum cover	7.7	7	**7.3^{a}**
Paper cover	8	7.3	**7.7^{a}**
Polythene cover	7.7	7.3	**7.5^{a}**
Pet jar	8	7	**7.5^{a}**
Mean	**6.8**	**6.2**	
	Temperature	**Packing material**	**Temperature X Packing material**
SEm+	0.2	0.4	0.6
CD (P=0.01)	0.8	1.6	NS
CV (%)	14.8		

Table 2. Effect of different packing material on the shelf life of *Trichoderma*Th4mineral oil formulation during tenth month storage (×10^9cfu/ml of formulation) *in vitro*

Packing material	Ambient temperature	4^0C	Mean
Aluminum tin	11.7	13.7	**12.7^{bc}**
Amber coloured bottle	10	11	**10.5^{d}**
HDPE bottle	11.3	12.7	**12.0^{cd}**
Aluminum cover	14.3	16.3	**15.3^{ab}**
Polythene cover	14	15.7	**14.8^{abc}**
Pet jar	15.7	17.7	**16.7^{a}**
Mean	**12.8**	**14.5**	
	Temperature	**Packing material**	**Temperature X Packing material**
SEm±	0.4	0.7	1
CD (P=0.01)	1.7	2.9	NS
CV (%)	13.2		

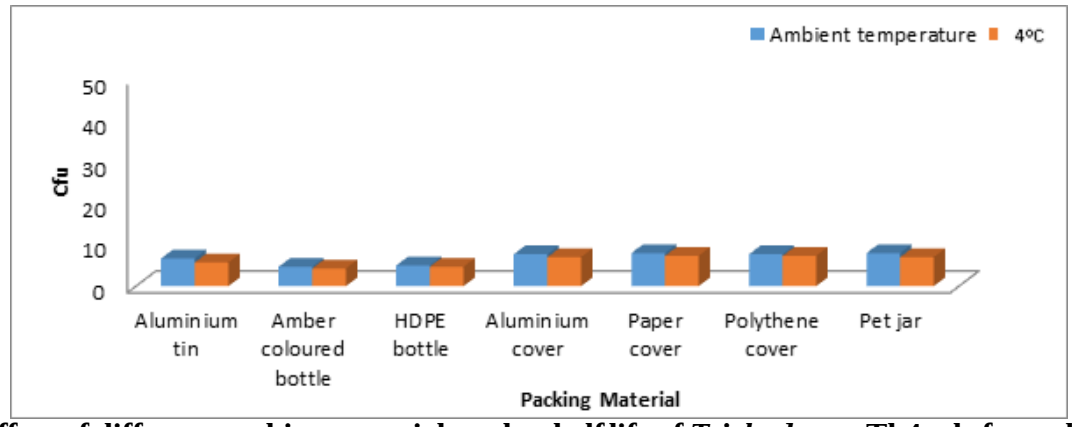

Fig. 1 Effect of different packing material on the shelf life of *Trichoderma* Th4 talc formulation during tenth month storage (×10^8 cfu/g of formulation) *in vitro*

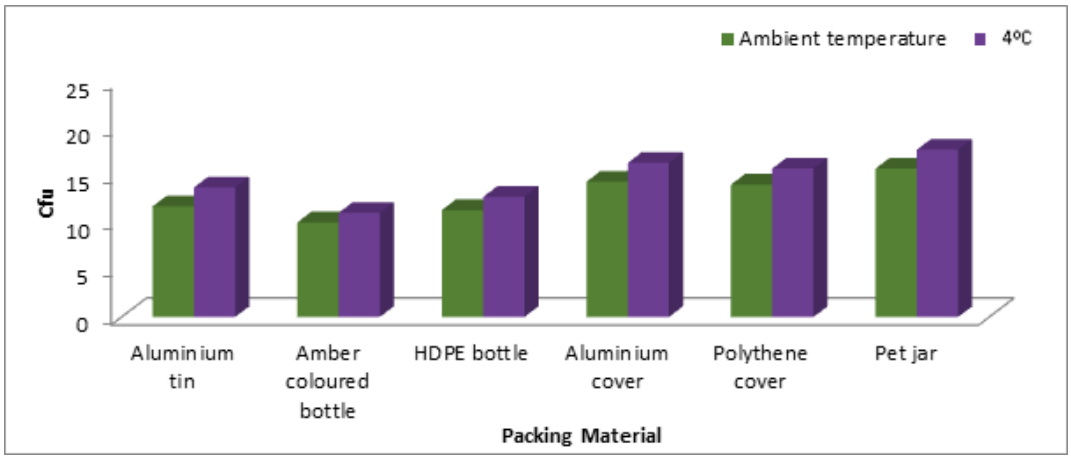

Fig. 2 Effect of different packing material on the shelf life of *Trichoderma* Th4 mineral oil formulation during tenth month storage (×10^{11}cfu/ml of formulation) *in vitro*

While working with mineral oil formulation, the results revealed that overall performance of all the packing material retained similar number of viable propagules ($x10^{11}$ cfu/ml) upto eight months of storage period and then a stiff decline of population was noticed ($x10^{9}$ cfu/ml) (Fig 2). Finally, at the end of ten months of shelf life period, pet jar was proved better with $16.7x10^{9}$ cfu/ml followed by aluminum cover and polythene cover with non significant differences among them and with pet jar. All the three performed well with survivability and maximum number of viable propagules in mineral oil upto the end of storage period. The result is in agreement with Sathiyaseelan *et al*. (2009). They confirmed that the application of paraffin oil increases 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 the phytopathogens.

However, in the present study, population of Th4 stored in mineral oil irrespective of storage temperature declined to an extent of 124 times ($20.8x10^{11}$ cfu/ml to $16.7x10^{9}$ cfu/ml). Hence, further investigation is required for finalizing a liquid formulation.

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