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Research Article

VIABILITY STUDY OF *TRICHODERMA VIRIDE* IN DIFFERENT FORMULATIONS AT DIFFERENT TEMPERATURES

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ABSTRACT

Trichoderma is a well documented biocontrol agent of fungus origin being used for the control of various plant pathogens as people are being aware of the various detrimental effects of chemical pesticides. In Nepal also, the biocontrol agents are gaining popularity and there are several *Trichoderma* products in different trade names available in the market with label that they can be used within six months from the date of manufacture. However, it is very essential to know the fact that these microbial products contain living micro-organisms and therefore the conditions of storage for long term viability of the product should be given prior importance. This study was performed to find out the shelf life of *Trichoderma viride* based on various formulations and to know the effect of temperature on its viability. It was found that liquid based formulation and organic formulation had greater shelf life compared to talc based powder formulation. Also low temperature (4ºC) significantly increased the shelf life of the product (for over a year) while at ambient temperature conditions (15-25ºC), optimum viability of the biocontrol agent could be achieved only for a maximum of 3 months. Therefore it is important that farmers use fresh products and/ or store the products at cool and dark place to exploit the benefits of biopesticides.

Key words: Biopesticide, biocontrol agent, shelf life, Trichoderma, viability

INTRODUCTION

Trichoderma is well established as a biopesticide, biofertilizer in agriculture. It is mainly used for the control of soil borne micro-organisms as it colonizes the rhizosphere and various mechanisms have been explained for the management of plant pathogens with this organism (Kumar *et al.*, 2017). More than 100 species of *Trichoderma* have been recorded worldwide (Pandya *et al.*, 2011), many of which are potential biocontrol agents (Benitez *et al.*, 2004). *Trichoderma* spp. is used in more than 60% of the commercial biopesticides in various forms such as spore suspension, chlamydospores, mycelium (Verma *et al.*, 2007). It can be used as seed treatment, seed biopriming, seedling treatment, soil applications and

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foliar applications. Although the benefits of biocontrol agents have been realized from as early as 1930's (Weindling, 1934), the commercialization of biopesticides is still slow in progress, with just 2% of the pesticides being based on biological entities (Kumar *et al.*, 2014). This is primarily because of the poor/inconsistent shelf life of products (Navaneetha *et al.*, 2014). As the biopesticides contain living micro-organisms, if conditions are not favorable, they lose their viability and the product is no more effective. Thermotolerant *Trichoderma* have also been identified which can be stored without loss of viability at higher ambient temperature conditions (Poosapati *et al.*, 2014).

Biopesticide production is Nepal is at initial stage. There are a few companies which produce *Trichoderma* based biopesticides. Those are available in talc based formulations or paraffin oil based formulations. Generally colony forming units (CFU) greater than $1X10^8$ is labeled on the products and the companies claim those can be used for as long as 6 months from the date of manufacture. In agro-vets supplying the products, they are kept at ambient conditions with no facility of cold storage. Farmers also do not keep the products in cool and dark place and this hinders the shelf life of products. The objective of the study was to find out the viability of *Trichoderma* in various formulations and to compare the shelf life of *Trichoderma* in ambient temperature conditions compared to refrigerated conditions.

MATERIALS AND METHODS

Products used for the study

The experiment was conducted using *Trichoderma viride* as test isolate. Three formulations were chosen for the study, two of which were commercial products and the other was prepared in laboratory at Plant Pathology Division using rice husk mixed with rice bran at the ratio of 5:1 vol/vol. Two hundred gram of this medium was put in plastic bags (sterilisable) which were then autoclaved at 121ºC for 20 minutes. After cooling of the media, one week old culture of *T. viride* grown on Potato Dextrose Agar (PDA) was used to multiply in the media. Ten circular PDA discs (cultured with *Trichoderma*) of 1 cm diameter were excised with sterile cork borer and inoculated in the plastic bags with media. This media was allowed for *Trichoderma* multiplication by incubating at 25ºC for 15 days. This period of time was considered as 0 months for rice husk media. Of the commercial biopesticides, one was liquid formulation using paraffin oil and another product was talc based powder formulation.

Shelf life study of *T. viride*

Viability of *Trichoderma* in the mentioned products was tested at two different temperature conditions, one at 4ºC and another at ambient temperature (15 to 25ºC). For the products stored at room temperature, viability test of spores was performed for a period of 6 months at one month interval as they lost their viability before 5 months of storage. For the products kept in refrigerator at 4ºC, shelf life was observed for one year. For examining the shelf life of the bioproducts, serial dilutions of the products were performed from 10^1 to 10^8 times and

1 ml of the suspension from each dilution was poured onto PDA plates under laminar flow and spread with sterile L-shaped bent glass rod. The plates were then left for some time to dry completely. For each dilution, three plates were used as replicates, one plate as one replication, The plates were incubated at 25ºC for two to three days, then CFU of *Trichoderma* were counted in plates of different dilution factors with ease in counting. Total CFU/g or ml was calculated as the no. of colonies observed per plate multiplied by the dilution factor.

RESULTS AND DISCUSSION

Viability of *Trichoderma* was greatly affected by temperature conditions. This was evident from the differences in viable CFU observed at two different temperatures of storage. The products kept at ambient temperature conditions were found to lose their viability rapidly and viable CFU could only be recorded for a maximum of 6 months (Table 1). The products stored in refrigerator showed higher viability percentages over the time frame and satisfactory level of CFU was observed for until six months (Table 2a). However, after this period of storage, the viability reduced significantly and by the end of one year, only a maximum of 7.23% of viability was achieved (Table 2b). Rai and Tewari (2016) also observed in their study that lower temperatures could increase the shelf life of *Trichoderma harzianum* bioproducts. Daryaei *et al*.(2016) also suggested that *T. atroviridae* stored at lower temperature had greater shelf life than those stored high temperatures suggesting that this decline in viability could be due to increased metabolism at higher temperature. Therefore, higher metabolism rate could have an adverse effect on the shelf life of the biocontrol agent.

It was observed that the type of formulation tested for storage also affected the shelf life of the products. The initial CFU count was highest for talc formulation ($28.6X10^8$) and least for the rice husk ($17X10^7$). Lower concentration of *Trichoderma* in rice husk could have resulted because the rice husk was relatively larger in size and hence provided much less surface area for *Trichoderma* growth as compared to talc or paraffin. If the same organic base were used in smaller size or powder form, it could have favoured more *Trichoderma* multiplication in the product. Nevertheless, it was interesting to see that viability percentage of *T. viride* recorded in the following months at room temperature showed the greatest viability percentage in rice husk and least in talc. After 3 months of storage, the viability observed in rice husk, paraffin oil and talc were 73.3%, 26.53% and 0.3% respectively. At the end of six months, the CFU count in rice husk was also the greatest ($21.6X10^5$) followed by paraffin ($6.6X10^2$) and no CFU could be observed in talc. There are several reports which suggest that *Trichoderma* grown in organic food base tends to have longer viability than those multiplied in inorganic food bases (Khan *et al.*, 2011; Kumar *et al.*, 2014). Khan *et al* (2011) also observed that talc and gypsum based products had poor growth and shorter shelf life. Rice husk is an organic food base and hence provided food supplement for the biocontrol agent and consequently the product could be stored for a longer time. Greater

viability percent of *T. harzianum* in liquid formulation was also previously reported by Rai and Tewari (2016). Pesta granules based formulation had 8 months of shelf life of *Trichoderma* at ambient conditions (Kumar *et al.*, 2014). The talc based formulation in India reports an average of 3-4 months of viability of *Trichoderma* (Khan *et al.*, 2011) and similar shelf lives are also reported in peat, lignite and kaolin based formulations. With regard to viability of different formulations at 4ºC, rice husk and paraffin oil showed higher shelf lives compared to talc. The viability of *T. viride* observed at 6 months of storage at refrigerated conditions for paraffin oil, talc and rice husk were 76.87%, 69.93% and 72.35% respectively. So, it was observed that the type of formulation did not affect viability of *T. viride* at 4ºC until this period of storage. This would mean that at lower temperatures, the metabolic rates were reduced and rice husk as organic food supplement might not be necessary for the microorganism in that condition. However, after one year of storage, rice husk showed highest viability percentage (7.23%) followed by paraffin (3.31%) and least in talc (0.06%). As for the CFU count, highest count was observed in paraffin oil ($5.3X10^7$) followed by rice husk ($12.3X10^6$) and least in talc ($17.66X10^5$). The optimum CFU in biopesticides is considered to be above 10^6 per g or ml (Shukla *et al.*, 2016) and hence these formulations showed optimum CFU counts even after one year of storage at refrigerated conditions. This explains that storage of *Trichoderma* biopesticides at lower temperature is a crucial factor in increasing shelf life of the microorganism contained in biopesticides.

Table 1. Shelf life of *T. viride* in different formulations at room temperature (15°-25°C)

Trichoderma formulation	CFU count of *T. viride* (per g or ml)								
	Months (Jan to Jul 2016)								
	0	1	2	3	Viabillity (%)	4	5	6	Viability (%)
Paraffin oil	$16X10^8$	$13.3X10^8$	$8.6X10^8$	$23X10^7$	14.37	$13.3X10^6$	$20.6X10^4$	$6.6X10^2$	~0
Talc powder	$28.6X10^8$	$20X10^8$	$6X10^8$	$18X10^5$	0.06	$10.3X10^3$	26.6X10	0	0
Rice husk	$17X10^7$	$17.3X10^7$	$15X10^7$	$11X10^7$	64.7	$7.6X10^7$	$36.6X10^6$	$21.6X10^5$	1.27

Table 2a. Shelf life of *T. viride* in different formulations at refrigerated condition (4°C)

Trichoderma formulation	CFU count of *T. viride* (per g or ml)								
	Months (Jan to Jul 2016)								
	0	1	2	3	Viabillity (%)	4	5	6	Viability (%)
Paraffin oil	$16X10^8$	$15.6X10^8$	$15X10^8$	$14.6X10^8$	91.25	$13.6X10^8$	$13X10^8$	$12.3X10^8$	76.87
Talc powder	$28.6X10^8$	$28.3X10^8$	$28.3X10^8$	$25.6X10^8$	89.51	$24.3X10^8$	$22.6X10^8$	$20X10^8$	69.93
Rice husk	$17X10^7$	$17X10^7$	$16.3X10^7$	$16X10^7$	94.11	$15.3X10^7$	$14X10^7$	$12.3X10^7$	72.35

Table 2b. Shelf life of *T. viride* in different formulations at refrigerated condition (4°C)

Trichoderma formulation	CFU count of *T. viride* (per g or ml)							
	Months (Aug 2016 to Jan 2017)							
	7	8	9	Viabillity (%)	10	11	12	Viability (%)
Paraffin oil	$10X10^8$	$7.6X10^8$	$5.6X10^8$	35.00	$4X10^8$	$19.3X10^7$	$5.3X10^7$	3.31
Talc powder	$16X10^8$	$10.6X10^8$	$4.3X10^8$	15.03	$8.8X10^7$	$15.3X10^6$	$17.66X10^5$	0.06
Rice husk	$10.3X10^7$	$7.6X10^7$	$5.6X10^7$	32.94	$4X10^7$	$25.3X10^6$	$12.3X10^6$	7.23

CONCLUSION

It was observed from the study that the shelf life of biopesticides (*T.viride*) is rapidly declined at room temperatures and the products lost viability within 5 months while at refrigerated conditions, the viability decreased gradually and satisfactory percentage of viability could be observed for 6-8 months. Different formulation type also influenced the viability of *Trichoderma* at room temperatures but at refrigerated conditions (lower temperature), the formulation type did not have much effect on the viability at least for until 6 months of storage. Hence, biopesticides should be kept at cool and dark place to expand their shelf lives. Products older than 6 months of age should be discouraged as agro-vets do not keep the products in cold storage.

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