INFLUENCE OF TEMPERATURE AND PACKING MATERIALS ON SHELF-LIFE OF MASS CULTURED *Trichoderma* AT STORAGE CONDITION

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ABSTRACT

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An investigation was undertaken to determine the effect of temperature (room and freezing temp/4^0C) and different colored polyethylene bags on shelf life of mass cultured antagonistic fungi, *Trichoderma* inocula at storage condition. Three *Trichoderma* strains were multiplied using cowdung, decomposed poultry refuge, sand and maize meal. The Strains-2((A03F2005) performed highest number of colony forming unit (cfu) ($1.3X10^5$) at freeing temperature(4^0C) up to 210 days after inoculation while the same strain gave $1.3X10^4$ number of cfu at room temperature. The strain-3((B07D2005) was fully unable to produce any colony at 180 days after inoculation. Transparent, Semitransparent Blue and Dark colored polyethylene bags were used to pack the *Trichoderma* inocula and kept at room temperature. Highest number of cfu ($1.0x10^6$) recorded from inocula of the strain-2 packed with Blue colored polyethylene bag stored up-to 180 days after inoculation at room temperature while the Transparent and Semitransparent polyethylene bags showed moderately effective to have viable spores among all of the tested *Trichoderma* strains.

Key words: *Trichoderma*, mass culture, influence, temperature, packing materials and storage

INTRODUCTION

The genus *Trichoderma* act as biological control agents and the antagonistic properties of them are based on the activation of multiple mechanisms. It depends on the crop plants and the environmental conditions including nutrient availability, pH, temperature, light and iron concentration (Harman 2000; Harman *et al.* 2004). A major limitation of bio-control by *Trichoderma* strains is the production of inoculum on large scale. Cowdung and decomposed poultry refuse are noted as excellent, low-cost and available substrates for growth of *Trichoderma harzianum* (Sawant and Sawant 1996). Once active strains have been identified with the *in-vitro* assays, a further selection must be done by studying other factors such as tolerance of high or low temperatures, suitability for formulation as foliar sprays and/or soil enhancement, spore viability in stored and field conditions, shelf-life and inoculum efficacy under commercial conditions etc. Formulation and shelf-life are prime important for commercial use of any biological agents. In abroad, commercial formulation of *Trichoderma* species are standardized and using widely against soil-borne pathogens. Such information is scanty in Bangladesh especially for storing and packing process of *Trichoderma* inocula. So, the present investigation was undertaken with three representatives of mono-conidial *Trichoderma* strains to determine the suitable temperature and packing materials for harvesting maximum shelf-life under storage atmosphere.

MATERIALS AND METHODS

The experiment was conducted in the laboratory of Plant Pathology, Bangladesh agricultural Research Institute, Gazipur during 2004-05 with three strains of *Trichoderma* (A 03D2004, A03F2005 and B07D2005) to determine their shelf-life under different storage temperature on the mixture of some organic substrates. The organic carrier/substrate used for colonization/multiplication of *Trichoderma* strains was consisted with mixtures of Cowdung (CD), Poultry Refuse (PR), Sand (S) and Maize Meal (MM). The ratio was CD: S: PR: MM = 8: 4: 2: 1. The carrier was kept into the heat tolerant polyethylene bag at the rate of 250g per polyethylene bag and sterilized properly. The antagonism of the three isolates of *Trichoderma* strains against *Sclerotium rolfsii* (TSr 03) and *Rizoctonia solani* (BRs 04) was tested earlier as Sundar *et al.* (1995). The carrier media preparation and inoculation process for mass culturing of the strains of *Trichoderma* were performed as described by Islam *et al*. (2002) and Prakash *et al.* (1999). The inoculated bags were placed at room temperature and freezing temperature (4^OC). Data were taken on the colony forming unit (cfu) of the *Trichoderma* colonized organic substrate (1g mixed with 100 ml sterilized water) up to 210 days after inoculation with 30 days of interval. Colony forming unit was counted using four Petri plates for each treatment containing Potato Dextrose Agar media under stereomicroscope suggested by Sawant and Sawant (1996). In another experiment, Transparent (TPB), Semitransparent (STPB), Blue and Dark (black) (DPB) colored polyethylene bags were used to record the effect of colored polyethylene bags (reflected light effect) on the shelf-life of the inocula of three *Trichodema* strains up to 180 days after inoculation at room temperature. Data on colony forming unit were taken at an interval of 30 days.

RESULTS AND DISCUSSION

The strain-2 (A03F2005) of *Trichoderma* gave highest colony forming unit (cfu) both in room and freezing temperature up to 210 days after inoculation. The maximum cfu value of 3.5 X 10^8 was calculated after 15 days of incubation in both room and freezing conditions, and 210 days after inoculation such value was harvested 1.3 X 10^4 and 1.3 X 10^5, respectively at room and freezing temperature while the *Trichoderma* strain-1 (A03D2004)) and *Trichoderma* strain -3 (B07D2005) showed poor number of shelf-life (viable spores) after same days of storage both in room and freezing conditions (Table 1). Regardless of storing temperature the shelf-life of all three tested strains declined gradually with increasing of storage period.

Table1. Effect of temperature on the spore viability of three *Trichoderma* strains at storage condition

Trichoderma strains	Temperature	Colony forming unit (cfu / g Substrate) after incubation time							
		15 DAI*	30 DAI	60 DAI	90 DAI	120 DAI	150 DAI	180 DAI	210 DAI
Trichoderma Strain-1 (A03D2004)	Room temp.	5X107	1.3X108	3.2X107	5X106	2.5X105	3.5X104	2.5X102	90
	Freezing temp. (40C)	5X107	3.3X107	5.4X106	3.5X106	4.5X105	1.7X105	2.5X104	1.2X103
Trichoderma Strain-2 (A03F2005)	Room temp.	3.5X108	1.5X109	3.9X108	3.5X108	4.5X107	5.5X105	4.8X105	1.3X104
	Freezing temp. (40C)	3.5X108	4.5X108	7.9X109	5.5X108	4.5X108	5.5X108	4.8X106	1.3X105
Trichoderma Strain-3 (B07D2005)	Room temp.	2X105	3.3X105	1.1X103	5.6X102	2.7X102	78	-	-
	Freezing temp. (40C)	2X105	1.3X105	1.1X104	5.6X103	2.7X102	1.5X102	-	-
CV(%)		1.27	1.64	2.30	2.05	2.19	2.42	2.18	2.17

DAI: Days after inoculation

Form the result, it appeared that minimum level of shelf-life of the mass produced inocula of *Trichoderma* required in keeping at the normal freezing (4^0C) temperature rather than room temperature. This finding is a partial agreement with the report stated by Sawant and Sawant (1996) who observed that lees light did not show profuse sporulation of *Trichoderma*.

Table 2. Effect of different colored polyethylene bags on the spore viability of three *Trichoderma* strains at storage condition

Trichoderma strains	Temperature	Poly Bags	Colony forming unit (cfu / g Substrate) after incubation time					
			30 DAI	60 DAI	90 DAI	120 DAI	150 DAI	180 DAI
Strain-1 (A03D2004)	Room temperature	TPB	1.3X108	3.2X107	5X106	2.5X105	3.5X104	2.5X102
		STPB	5x108	1.3x108	4.5x107	2.2x106	3.3x104	3.5x103
		BCPB	5.7x 108	7.7x108	3.3x107	2.5x107	7.5x106	5.5x105
		DPB	1.4x106	2.5x106	4.6x105	3.7x105	6.5x103	7.6x102
Strain-2 (A03F2005)	Room temperature	TPB	1.5X109	3.9X108	3.5X108	4.5X107	5.5X105	4.8X105
		STPB	5x108	1.3x109	4.5x107	2.5x107	1.6x105	3.2X104
		BCPB	7.7x 109	7.7x108	3.3x108	2.5x107	1.7x107	1.0x106
		DPB	1.4x107	2.5x106	4.6x105	4.3x105	3.2x104	2.5x103
Strain-3 (B07D2005)	Room temperature	TPB	3.3X105	1.1X105	5.6X103	2.7X102	78	-
		STPB	3.8X105	3.1X104	6.6X103	3.7X103	2.3X102	1.1x102
		BCPB	2.6x 105	4.3x105	8.5x104	3.25x104	2.5x103	5.3x102
		DPB	2.2x103	3.5x103	3.3x103	2.1x102	1.2x102	90
CV (%)			2.82	2.96	2.54	1.84	1.05	1.02

TPB: Transparent Poly Bag, STPB: Semi-Transparent Poly Bag, BCPB: Blue Colored Poly Bag DPB: Dark Poly Bag (black)

The effect of different colored polyethylene bags on the viability of *Trichoderma* strains in storage at room temperature revealed that Blue colored poly bags gave highest cfu at 180 days after inoculation followed by Semitransparent and Transparent polyethylene bags. The lower number of colony forming unit was obtained from the *Trichoderma* inocula packed with the dark polyethylene bags. Among the *Trichoderma* strains, the strain-2 showed highest performance to have shelf-life viability for 180 days after inoculation. Irrespective of polyethylene bags, cfu per gram of substrate was initially maximum in all the three strains of *Trichoderma* but its value declined gradually with the increased storing period. The *Trichoderma* strain 2 gave the highest cfu value of 1.0x10^6 at 180 DAI when stored in BCPB (Blue Colored Polyethylene Bag) followed by TPB

(Transparent Poly bag) $4.8X10^5$, STPB (Semi-Transparent Polyethylene Bag) $3.2X10^4$ and Dark Polyethylene Bag (DPB) $2.5x10^3$/g sample. Strain-3 (B07D2005) totally failed to produce any viable spore at 180days after inoculation when stored in TPB.

The study indicated that Blue colored Polyethylene bag was much better to prolong shelf-life of *Trichoderma* strains. This may be happened due to reflection of blue light which induces conidiation of organism. It has been reported that exposure of a dark-grown colony of *Trichoderma* to a brief pulse of blue light results in the formation of a ring of dark green conidia at what had been the perimeter of the colony at the time of illumination (Horwitz *et al.* 1984; Berrocal-Toto *et al.* 1999). Linden *et al.* (1997) investigated the light effect on some fungi like *Trichoderma harzianum* and *Neurospora crassa* and found tremendous impact on process in this organism.

It is concluded that for adequate shelf life of mass cultured *Trichoderma*, blue colored bags might be suitable for long term storage at room temperature which may be helpful for researchers for improving of its commercial use as bio-control agent.

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