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MASS MULTIPLICATION AND SELF LIFE OF *TRICHODERMA* SPECIES USING VARIOUS AGROPRODUCTS

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ABSTRACT
Six agro based substrates such as sorghum grain, wheat grain, rice bran, rice husk, maize flour and saw dust were evaluated for mass production of three *Trichoderma* spp. Amongst, highest population was recorded in sorghum grain after 30 days of storage period of all three species i.e. *T. harzianum* (58.68 $\times 10^6$ cfu/g) *T. viride* (54.24 $\times 10^6$ cfu/g) and *T. hamatum* (52.51 $\times 10^6$ cfu/g). The population of different species remained constant for a period of 60 days after which it started to decline slowly, the rate of decline varied with different substrates. The lowest population was observed in saw dust on *T. harzianum* (2.32 $\times 10^6$ cfu/g). Sorghum based substrate could be useful for commercial production of *Trichoderma* spp with quality number of cfu which is essential for application of any bio- agent for management of plant diseases.

INTRODUCTION

There is an increasing awareness on deleterious effects of chemical pesticides on crop ecosystem. Biological control of plant diseases has got momentum as it offers many advantages over the conventional methods of control (Mukhopadhayay, 1994). Use of Trichoderma fungi in biological control has advantages such as reducing pesticides entering the environment and the changing conditions favourable for plants. Several beneficial activities were reported with *Trichoderma* spp. such as increase nutrient uptake from soil (Harman *et al.,* 2001); to stimulate plants for producing chemical defences compounds and induces resistance in the plants (Hawell *et al*, 2000 and Dewit *et al.,* 2002); mycoparasitism or directly attack to other pathogenic fungi (Viterbo *et al.,* 2002) and improve root system and plant growth (Harman, 2000).

Trichoderma as a potent fungal biocontrol agent against a range of plant pathogens has attracted considerable scientific attention (Rini and Sulochana, 2007).Commercial success of a biocontrol agent depends not only on its bioefficacy or shelf life but also its suitable substrate for multiplication, which is easily available and relatively inexpensive. *Trichoderma* spp. have been grown on wide range of grains *viz.* maize, sorghum, pearl millet, wheat, Jhangora weed *(Echinocloa frumantacea),* wheat bran, wheat straw, waste tea leaves, banana fruit bark, coffee husk, paddy-straw, Diatomaceous, earth granule impregnated with molasses (Zaidi and Singh 2004; Lewis and Papawizas, 1984). Successful use of fungal biocontrol agents like *Trichoderma* spp. for the control of soil borne diseases caused by pathogens like, *Rhizoctonia, Sclerotium, Fusarium, Pythium,* and *Phytophthora* in several crops have been reported (Cook and Baker, 1983). *Trichoderma* spp. are under intensive research because of their abundant natural occurrence, biocontrol potential against fungal and nematode diseases as well as host defense inducing ability (Haraman & Kubicek, 1998). Bioagents like, *T. harzianum, T. viride* and *T. hamatum* are being successfully used for the control of some of the dreaded diseases like, footrot of black pepper, root rots and wilt of pulses, damping off, collar rots and *Fusarium* wilt of horticultural crops. Economic mass production of such antagonist can be achieved by using readily available inexpensive substrates. Most of the commercial formulations of *Trichoderma* are based on inert material (talc), act only as carrier and bulking material but do not support the growth and survival of antagonists under storage condition. Formulation of biological control agents depend upon biomass production and maintaining viability at the end of the process (Adekunel *et al.*, 2001).

Therefore, a study was conducted to evaluate some locally available organic substrates for mass multiplication and long-term survival of *Trichoderma* spp.

MATERIALS AND METHODS

Fungal species *T. harzianum, T. viride* and *T. hamatum* was isolated from soil samples by using potato dextrose agar (PDA) medium. Samples were inoculated over plates by multiple

tube dilution technique (MTDT) and the plates were incubated at 26°C for 4 days. The fungal colonies which were picked up and purified by streaking and incubated at 26°C for 7-8 days. Green conidia forming fungal bodies were selected and microscopic observation was identified to be *Trichoderma viride*. The culture was maintained on PDA slants.

Maintenance of culture

The culture of native *T. harzianum* Rafai, *T. viride* Pers. Ex Gray and *T. hamatum* were collected from Department of Plant Pathology, G. B. Pant University of Agriculture and Technology, Pantnagar. A loopful of inoculum from cultured plates of all three *Trichoderma* spp. were transferred to Potato Dextrose Agar (PDA) slants and maintained as pure culture. For laboratory studies, the fungal species were cultured on PDA medium. The medium was sterilized at 15 psi for 30 min in autoclave, poured to sterilized plates, cooled and inoculated with pure culture of the fungus under aseptic conditions. The plates were then incubated at room temperature (26 ± 2°C) for ten days. After complete sporulation, conidia from the medium were harvested by washing them thoroughly with sterilized water containing Tween-20 (0.2%) for immediate use. Otherwise, spores were harvested with the help of a small sterile metal spatula. Harvested conidia were air dried under laminar air flow and stored in a small air tight screw cap vials (10 cm with 2.5 cm diameter) in refrigerator at 4°C before using for further studies.

The substrates *viz*. sorghum grain, wheat grain, rice bran, rice husk, maize flour and saw dust were screened for production of mass inoculums of *Trichoderma* spp. Triplicate sample of moistened substrate were transferred to 250 ml conical flasks and autoclaved twice at 15 psi at 120°C for 30 minutes. The flasks were allowed to cool down to room temperature prior to inoculation. The culture of native *T. harzianum* Rafai, *T. viride* Pers. Ex Gray and *T. hamatum* were collected from Department of Plant Pathology, G. B. Pant University of Agriculture and Technology, Pantnagar. The spore suspension was prepared by harvesting the spores from 1 week old culture of *Trichoderma* spp. 5 ml (10^6spore/ml) spore suspension was injected into the autoclaved flasks containing sterilized water with different substrates. After 15 days of growth, the colonized substrate were dried at 30° C and ground to powder using a laboratory blender. The powdered formulations were stored in room temperature. The estimation of colony forming units (cfu) of *Trichoderma* spp. in different formulations was done by suspending 1 g of dried product prepared on substrates by serially diluting the powder and finally plating suspension on fresh *Trichoderma* selective medium (Elad and Chet 1983). The plates were incubated at 28 ± 1° C. Number of colonies was counted as colony forming units (cfu) per gram bio-formulation. The evaluation of *Trichoderma* spp. in substrates was done at every 30 days interval and continued up to 120 days.

RESULTS AND DISCUSSION

Six substrates viz. sorghum grain, wheat grain, rice bran, rice husk, maize flour and saw dust were used for their mass production studies of *T. harzianum, T. viride* and *T. hamatum*. Among the different substrates evaluated significantly highest population was recorded in sorghum grain after 30 days of storage period on both the species i.e. *T. harzianum and T. viride* (Table 1). However, in different substrates, the population counts remained constant for a period of 60 days after which it started to decline slowly, the rate of decline varied with different substrates. Significantly the lowest population of *T. harzianum* was recorded in saw dust (2.32 × 10^6 cfu/g) which was on par with maize flour (26.22 × 10^6 cfu/g) at 120 days after storage period while lowest population *T. viride* was recorded in saw dust (2.38 × 10^6 cfu/g) which was on par with wheat grain (22.40 × 10^6 cfu/g) at 120 days after storage period (Table 1). In *T. hamatum,* significantly highest population was recorded in sorghum grain after 30 days of storage period (52.51 × 10^6 cfu/g) (Fig. 1). However, in different substrates, the population counts remained constant for a period of 60 days after which it started to decline varying with different substrates. Significantly the lowest population was recorded in case of saw dust (3.38 × 10^6 cfu/g) which was on par with Maize flour (22.33 × 10^6 cfu/g) at 120 days after storage period. Rini and Sulochana (2007) evaluated eight different subtracts for multiplication of *T. viride* and *T.*

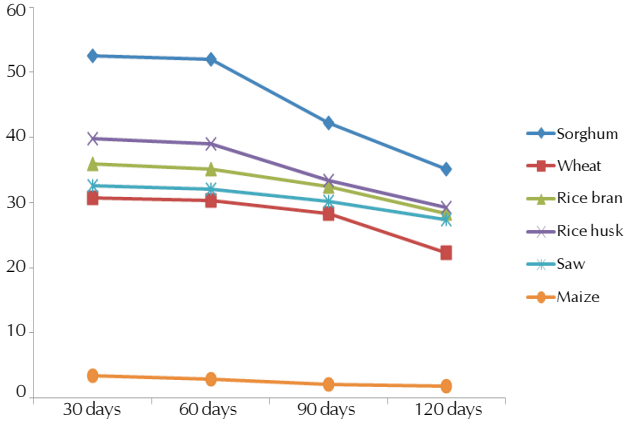

Fig.1. Effect of different substrates on shelf life of *Trichoderma hamatum* (1 × 10^6 cfu/g) in various agro products

Table1: Effect of different substrate on shelf life of *Trichoderma harzianum* and *T. viride* in various agro products

Agro products	*Trichoderma harzianum* (1 × 10^6 cfu/g)				*Trichoderma viride* (1 × 10^6 cfu/g)			
	30d	60d	90d	120d	30d	60d	90d	120d
Sorghum grain	58.68	58.38	52.16	47.26	54.24	53.36	48.59	42.53
Wheat grain	30.62	30.21	28.40	24.367	31.68	31.66	26.28	22.40
Rice bran	39.32	38.91	32.28	30.32	37.55	37.27	34.51	30.35
Rice husk	45.56	45.00	41.25	37.33	43.62	43.11	39.59	32.69
Maize flour	34.73	34.20	30.49	26.22	33.77	33.33	30.60	26.64
Saw dust	4.15	4.11	3.16	2.32	4.43	4.07	3.27	2.38
CD at 0.5%	0.42				0.45			

harzianum and reported that highest population of both the species of Trichoderma was observed in sorghum grain and lowest was on saw dust. Similar type of observation also observed at present investigation.

Population density and shelf life of *T. harzianum* (Th-2) was significantly superior over other *Trichoderma* isolates tested (Gutierrez-Correa *et al.,* 1999) established that strain compatibility and nutritional status of the substrate were determining factors for successful mixed culture formulation of *Trichoderma* and *Aspergillus*. *Trichderma* spp. is sole bio-agents in class of fungal bio- agent as commercially available which cover about three-fourth of the total application of fungal antagonist. Above six agro-based tested substrates are locally available and cheap, sorghum based substrate could be useful for commercial scale of production with quality number of cfu which is essential for application of any bio- agent for management of plant diseases.

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