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Shelf Life Evaluation of *Trichoderma harzianum* on Different Organic Urban Waste

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A B S T R A C T

The experiment was established for evaluate the self life period and biological efficacy of *Trichoderma harzianum* of different urban waste materials i.e. Sugarcane baggage, Peeled potato, Cow dung, Waste tea leaf and Farm Yard Manures. These all materials are easily available in local area. Mycelial growth, conidial production and biomass yield of *Trichoderma harzianum* was examined the substrate had gives significant difference in growth rate, conidial population and viability. Waste tea leaf in solid state (100g) inoculated with 50 ml of *Trichoderma harzianum* (developed on Potato Dextrose medium) was found significantly best in comparison to others substrate in taking minimum days for growth of mycelia (9.2 days), maximum number of spore production (156.3) and maximum days self life @ 30ºC (18.5 days). Present study deals with use of household waste materials for assessment and production of mass quantity of bio-control agent on cheap substrates and develops effective production methodology which can be easily adopted by the farmers.

Introduction

Trichoderma harzianum is one of the common fungal bio-agent belongs to sub-division deuteromycotina and worldwide used for sustainable management of various foliar, seed borne and soil borne pathogens due to antagonistic nature of the fungi. The members of genus *Trichoderma* are free-living, opportunist, a virulent, symbiotic fungus that are common in soil and root ecosystem (Harman *et al.,* 2004). Antifungal metabolites of *Trichoderma* have been grouped by Ghisalberti and Sivasithamparam (1991). *Trichoderma* spp. is known to produce mycolytic enzymes such as β-1, 3, glucanasa, β -1, 4 endo-glucanase, chitinase and protease. These enzymes play an important role in the degradation of chitin which is the structural component of the target pathogens and herbivorous insects and consequent myco-parasitism (Harman *et al.,* 1993). *Trichoderma* has gained maximum attention as bio-control agent due to the fact that it is effective against a large number of soil-borne plant pathogenic fungi, suppressive effects on some root nematodes without adversely affecting beneficial microbes like *Rhizobium* and capable of promoting growth of certain crops. Bio-control technologies have gained

momentum in disease control of crop plants in recent times as these technologies not only minimize or replace the usage of harmful chemical pesticides but also found to be cheaper control of some of the diseases like, foot rots, root rots, damping off, collar rots, wilts and many soil borne diseases. Mass production of *Trichoderma* required to find out suitable media on large amount of *Trichoderma* biomass is required therefore, the first step for the mass production of any bio-control agent is to identify the suitable substrates, which should be comparatively cheap, stable and easily available within a short period of time. The type and form of substrate i.e. broth and solid may also vary according to the specific purpose for which bio-control agent biomass is required. In India, talc based formulations of *T. viride* was developed at Tamil Nadu Agricultural University, Coimbatore for seed treatment of pulse crops and rice (Jeyarajan *et al.,* 1994).

Materials and Methods

Standard pure culture of *Trichoderma* was brought from Department of Plant Pathology, Birsa Agriculture University, Kanke, Ranchi (Jharkhand). It has a shorter life period, so periodical sub culturing was done after fifteen days. The experiment was incepted at Krishi Vigyan Kendra, Koderma (Jharkhand). Sterilization of glass ware was done in Autoclave (at 121ºC for 30 min). All the selected substrate urban waste materials i.e. Sugarcane baggage, Peeled potato, Cow dung, Waste tea leaf and Farm Yard Manures have been collected from local area and after washing with clean tap running water substrate were placed in shade for 72 hours. The moisture level of each substrate was adjusted to 60%, using oven dry method by adding known quantity of water considering the initial moisture content. After drying in shaded place first step of sanitization of substrate was done with Formaldehyde @3%. Then the materials were filled in conical flask with the weight of 100g of substrate in each flask. After filling of conical flask the substrate were autoclaved for 30 minutes at 121ºC. The experiment was designed according to CRD; each treatment combination was replicated five times. Initial multiplication of *Trichoderma* sp. was done on Potato Dextrose Agar medium and inoculation on substrate was done in broth. The all replicates of experiment were left for 7 days under dark shade on 25ºC. After that an aliquot of 10 ml of distilled sterile water (DSW) was added to each flask and the mycelium was scraped with a spatula until the culture surface was free from mycelia and the suspension was collected in a 100 ml conical flask (Nampoothiri *et al.,* 2004). Serial dilution technique was used for reducing conidial strength in suspension. The number of conidia was determined by a Haemocytometer (Pandey *et al.,* 2001) and calculated as conidia/g of substrate. One gram of conidial substrates was mixed with 9 ml of distilled and sterilized water. The mixtures of conidial substrates were filtered through three layers of cheese cloth. One ml of collected suspension of *Trichoderma* sp. was poured on PDA containing Petri Plates under Laminar Air Flow chamber and leave for five days at 25ºC room temperature (Rahman *et. al*., 2011). After five days of inoculation circular white patches of mycelium was observed on PDA medium. Colony counter meter was used for counting number of spores per ml of suspension in each replication of substrate. Different agro industrial wastes were evaluated for maximum conidia production of *Trichoderma* under solid state fermentation (Cavalcante *et al.,* 2008). The calculation of conidial spore on substrate was done periodically after seven days of interval.

Results and Discussion

Among the substrates tested, waste tea leaf produced maximum number of conidia 156.3 in 10^{-5} dilution point moreover maximum days

survival 18.5 @30ºC have been recorded which was followed by Farm Yard Manures 120.1 (16.3 days), Cow dung 98.2 (16.5 days), Peeled potato 82.8 (15.6 days) and Sugarcane Baggage Solid 46.1 (12.3 days). Moreover the maximum radial growth and minimum days took place to whole mycelium run observed with waste tea leaf (9.2 days) followed by cow dung (12.5), Sugarcane Baggage (14.2), Farm Yard Manures (15.6 days) and peeled potato (18.3 days) (Fig. 1 and 2; Table 1 and 2).

Table.1 The periodical conidia yield of *Trichoderma harzianum* using different

Substrate Type and weight	Development of Spores periodically @10^{-5}		
	7^{th} day	15^{th} day	21^{st} days
Sugarcane Baggage Solid (100g) × 50 ml	10.2	23.7	46.1
Peeled Potato Solid (100g) × 50 ml	21.3	45.8	82.8
Waste Tea Leaf Solid (100g) × 50 ml	36.8	72.1	156.3
Cow Dung Solid (100g) × 50 ml	28.7	46.9	98.2
Farm Yard Manures Solid (100g) × 50 ml	32.4	69.4	120.1

Table.2 The periodical mycelia growth, number of spore/conidia yield and self life of *Trichoderma harzianum* using different

Substrate Type and weight	Mycelia growth	Spore (10^{-5})	Self life Days@ 30ºC
Sugarcane Baggage Solid (100g) × 50 ml	14.2	46.1	12.3
Peeled Potato Solid (100g) × 50 ml	18.3	82.8	15.6
Waste Tea Leaf Solid (100g) × 50 ml	9.2	156.3	18.5
Cow Dung Solid (100g) × 50 ml	12.5	98.2	16.5
Farm Yard Manures Solid (100g) × 50 ml	15.6	120.1	16.3

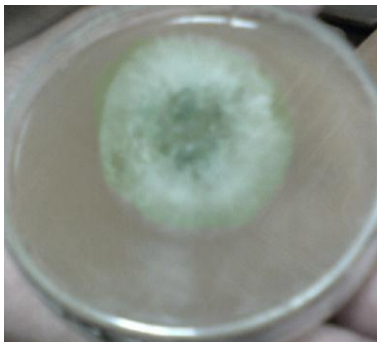

Fig.1 Colony development on PDA

Fig.2 Mass multiplication of *Trichoderma*

The most probable reason according to limited information available regarding the usage of different urban waste materials for mass multiplication of *Trichoderma harzianum* may have been the C/N ratio, availability of micro and macro nutrients, growth region, structure of species and physical condition of substrate i.e. bulk

density, porosity, water holding capacity accelerate the development of spore (Gupta *et al.,* 1997 and Irshad *et. al.,* 2011). The other microbiologist have been also support the possible reasons for the waste tea leaf substrate may be useful for mass multiplication of *Trichoderma* sp. on low cost substrate.

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