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Research Article

ANTAGONISTIC ACTIVITY OF *TRICHODERMA* SP AND EVALUATION OF VARIOUS AGRO WASTES FOR MASS PRODUCTION

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ABSTACT
Two species of *Trichoderma* viz. *T. viride* and *T. harzianum* have been isolated from soil. In-vitro antagonistic activities were demonstrated against plant pathogenic fungi *Colletotrichum gliosporioides* and *Fusarium* oxysporum by dual culture method. Various agricultural residues and by products such as rice husk, saw dust, maize husk and wheat bran were evaluated for mass production of *Trichoderma viride* and *Trichoderma harzianum.* Among the substrates used maize husk was found supported maximum spore production for *trichoderma viride* ($2.0x10^8$ per gram substrate) and $1.2x10^8$ per gram substrate for *Trichoderma harzianum* as compared with other substrate wheat bran, rice husk and saw dust respectively.

Key Words: *Mass Production, Agricultural Wastes Products, Trichoderma SPP*

INTRODUCTION

After green revolution people have been used chemical fertilizers and insecticides for better crop health but in recent year we have began to understand the widespread and repeated use of chemical biocide to control the host of organisms such as insect's weeds and fungi that threaten human interest. The global consensus to reduce inputs to chemical pesticides which are perceived as being hazardous by some consumer has provided opportunities for the development of novel sustainable crop protection strategies. There is a need to develop alternative control systems in the new future that is biocontrol and these must be implied.

Biological control of plant pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods (Shalini and Kotasthane, 2007; Eziashi *et al.,* 2007). *Trichoderma* species have been known since the 1930s to show antifungal activity and there have been extensive efforts to use them for plant disease control since then (Hjeljord and Tronsmo, 1998). They have been used as biological control agents (bcas) and their isolates have become commercially available (Freeman *et al.,* 2004) however, the production of effective *trichoderma* based product. In large scale to fulfillment the requirement is not yet feasible because of several regions. The quality of a microbial bio-protectant is dependent on the propagule density in the biomass and its ability to survive in nature (Harman *et al.,* 1991). Production of adequate quantities of good quality inoculum is an essential component of the biocontrol programmed. Development of simple and reliable production system for the production of spore, which having long longevity period and very effective for fungal plant pathogen can be produce in bulk is needed. Therefore the present study was aimed to find out the suitability of various agricultural residues for biomass production of selected *trichoderma* species. In present study the indigenous potent *Trichoderma* sp. Were isolated from soil and their mass multiplication on various agriculturally wastes was done.

MATERIALS AND METHODS

Isolation and Identification of Trichoderma Species

Trich*oderma* sp. were isolated using soil dilution plate technique (Johnson *et al.,* 1959) and soil washing methods (Gams *et al.,* 1987) on Potato Dextrose Agar (PDA), Malt Extract Agar (MEA) and Czepex, Dox Agar (CZA). Streptomycin 50 mg/L added in culture media for avoiding bacterial growth in

petriplate and plates were incubated at room temperature for five days. The colonies were isolated and purified on potato dextrose agar (PDA).The pure culture were identified up to species level with the help of standard monographs and reference book (Domsch *et al.*, 1980, Samuels *et al.*, 2004). The isolates were preserved in sterile soil and stored at 4°C.

Screening of Isolates for their Antagonistic Activities

The isolates of *Trichoderma* were screened against plant pathogenic fungi *Colletotrichum gliosporides* and *Fusarium oxysporum* by dual culture plate technique on PDA Medium. Ten days old cultures of respected pathogens were inoculated onto the PDA medium, 2mm away from center Inoculated plates were incubated at 28^0C for four days. After the end of four days the same plates were inoculated with *Trichoderma* sp. 2mm away from previous inoculums (plant pathogenic *Colletotrichum gliosporioides* and *Fusarium oxysporum*) and again keep it for incubation at 28^0C for four days. The antagonistic activity of *Trichoderma* sp. were observed and calculated.

Solid Substrate Fermentation for Mass Production of Trichoderma SP

Various agricultural wastes viz. Rice husk, Saw dust, Maize husk and wheat bran were evaluated for growth of test organisms.300g of dried powder of above waste materials were taken in autoclavable plastic bags(1kg). The material is subjected to moist (45%) with basal media and sterilized twice at 121^0C for 20 minutes. Ten days old cultures of respected *Trichoderma* sp. were used as source on inoculums. The spore suspensions of isolates were made in distilled water ($1x10^6$ conidia per ml). The bags were inoculated with respected isolates approximate 10 ml of spore suspension per bags and kept it for incubation at 25^0C, observe the growth pattern of *Trichoderma* sp. on respected substrates. After four days of incubation the inoculated bags were mixed by shaking and transfer the material in clean sterilized plastic trays (1.5x1x0.3ft), cover the tray with autoclaved transparent plastic sheets. After end of 10 days of incubation of trays dry spore were harvested by sieving. The Colony forming units of *Trichoderma viride* and *T. harzianum* on various substrates were calculated.

RESULTS AND DISCUSSION

Evaluation for in-vitro antagonistic potential against two very serious fungal plant pathogen viz. *Colletotrichum gliosporioides* and *Fusarium oxysporum* by dual culture techniques was done. *Trichoderma viride* and *T.harzianum* inhibited mycelia growth of both pathogens which were well stabilized in plate (Table-1). In dual culture plates *Trichoderma viride* and *T. harzianum* completely colonized *Fusarium oxysporum* (over growth 71.6 and 66.66 cm). The dual culture plates of both antagonistic fungi against *Colletotrichum gliosporiodes* showed completely restricted the mycellial growth of pathogen in plates (68.4 and 66.66 cm over growth). It might be secretion of

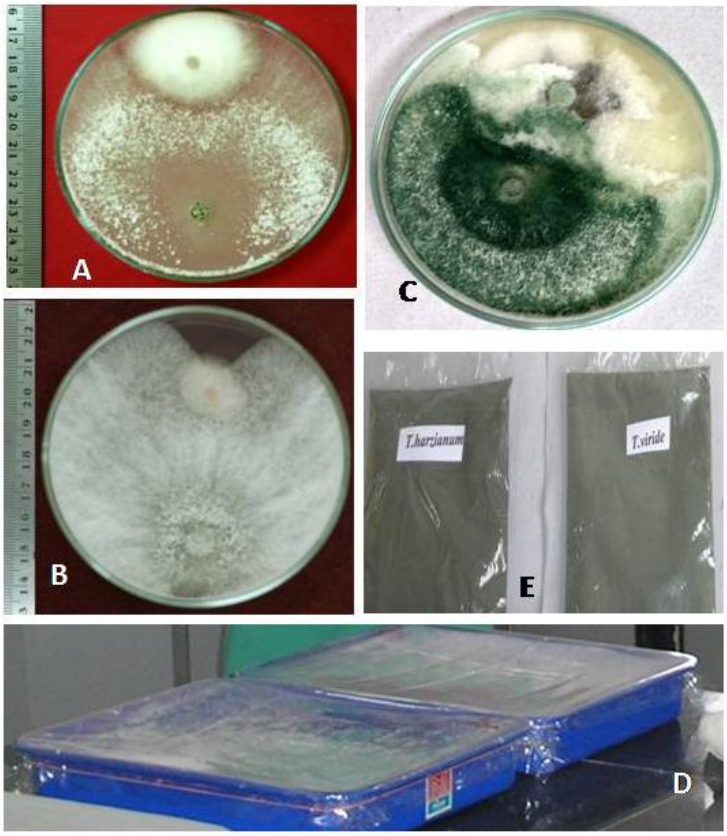

Figure 1: A - *T.harzianum + Colletotrichum gliosporiodes*. ***B*** **-** *Harzianum + Fusarium sp.* ***C*** **-** *Trichoderma Viride + Colletotrichum gliosporiodes*. ***D*** **-** Growth of ***Trichoderma Viride sp.*** in trays. ***E*** **-** Packet of *Trichoderma* dry spores.

some secondary metabolites which diffused in the culture medium and inhibited the growth of pathogen. It is thus evident from result that *T. viride* and *T. harzianum* can effectively colonize *C.gliosporiodes* and *F. oxysporum* and can therefore be used as a biocontrol agent effectively against these two fungal pathogens. Both *T. viride* and *T. harzianum* is well known biocontrol agents and used for managing various plant diseases (Butt *et al.*, 2001, Tiwari and Mukhopadhyay, 2001; Rini and Sulochana, 2007). For effective management there is needed to be screened potent strains of these antagonistic fungal species (Figure 1).

For selection of suitable, cheaper and easily available substrate for mass multiplication of the biocontrol agents, four different agro wastes, viz. Rice husk, Saw dust, Maize husk and Wheat bran were evaluated for conidial count. The data regarding growth rate and sporulation pattern of *Trichoderma viride* and *Trichoderma harzianum* are summarized in table no. 2.

Table: 1 Antagonistic activities of *Trichoderma* sp

Pathogenic Fungi	**Percent Inhibition in Radial Growth in (cm)**	
	Trichoderma Viride	***Trichoderma Harzianum***
Colletotrichum gliosporiodes	68.4±1.44	61.66±0.88
Fusarium oxysporum	71.6±1.25	66.66±2.2

Table 2: Growth and sporulation of *Trichoderma* isolates on agro-wasted after 15 days of incubation

Substrate	***Trichoderma Viride***			***Trichodema Harzianum***		
	GR	**SP**	**Spore Density (Cfu/g)**	**GR**	**SP**	**Spore Density (Cfu/g)**
Rice husk	R	++	$1X10^6$	R	++	$0.5X10^6$
Saw dust	M	+++	$1.2x10^7$	M	+++	$1.5X10^7$
Maize husk	F	+++	$2X10^8$	F	+++	$1.2X10^8$
Wheat bran	F	++	$1.8X10^6$	F	++	$1X10^6$

GR= Growth Rate, SP. = Sporulation Pattern, ++ Moderate (M), +++ Fast (F), R= Restricted

Among the different substrates tested, Maize husk showed the highest growth of *T. viride* .White mycelial growth was observed on maize husk on the third day of incubation and it covered the entire surface of the substrate with profuse green sporulation in 6 days. Maximum spore production of *T. viride* ($2.0X10^8$ cfu g-1) at 10 days of incubation also was noticed on this substrate, which was significantly superior to others (Table 2). On the Wheat bran mycelial growth was visible over the surface on second day and it took 5 days to cover the whole substrate but spore formation was very less at the end of 10 days. A population of $1.8X10^6$cfu g-1 was recorded on this substrate 15 days after incubation. However, moderate growth was observed on sawdust but it exhibited good spore population ($1.2x10^7$ cfu g-1) after end of 10 days of incubation. Rice husk exhibited restricted mycellial growth as well as low sporulation ($1X10^6$ cfu g-1). Growth rate of *T. harzianum* on different organic substrates was similar to that of *T. viride* Maize husk maintained the maximum growth and spore count ($1.2X10^8$ cfu g-1) followed by sawdust ($1.5x10^7$ cfu g-1). Wheat bran and Rice husk recorded lower spore counts (1.0X106 and $0.5X10^6$ cfu g-1 respectively).

In the present study agricultural wastes viz. Maize husk and sawdust was found best for the growth of *Trichoderma viride* and *Trichoderma harzianum* isolates in terms of spore production while Wheat bran produced more biomass. Suitability of the agricultural residues for the growth of *Trichoderma* has been reported by various workers (Pramod kumar *et al.*, 2009, Chaudhari *et al.*, 2011, Sobita, 2011). The finding of this study clearly indicate that *Trichoderma* are very effective against various pathogens and are able to grow on a wide variety of agriculture by products, this can be useful to farmers to cultivate these fungi very easily.

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