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In vitro assessment of antagonistic activity of *Trichoderma viride* against post harvest pathogens

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Trichoderma viride isolated from partially decomposed fruits and vegetable waste which collected from local market of Namakkal District, Tamil Nadu. It showed inhibitory effects on some common post harvest pathogens of fruits and vegetables namely *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Fusarium* sp. and *Penicillium* sp. *in vitro*. *T. viride* inhibited the radial growth of *A. niger* (55%), *A. flavus* (51%), *A. fumigatus* (52%), *Fusarium* sp. (64%) and *Penicillium* sp. (54%) in dual culture. Maximum growth inhibition of *A. niger* (64%), *A. fumigatus* (49%) and *A. flavus* (48%) were found with the use of 50% culture filtrate in medium.

Key words: Antagonistic activity, *Trichoderma viride*, post harvest pathogens

Introduction

Fruits and vegetables are highly perishable products, especially during the postharvest phase, when considerable losses due to microbiological diseases, disorders, transpiration and senescence can occur. A number of microorganisms, which effectively control postharvest pathogens, have been identified for post harvest control (Wilson and Wisniewski, 1989). Biological control of plant pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods (Baker and Paulitz, 1996). *Trichoderma* spp. are now the most common fungal biological control agents that have been comprehensively researched and deployed throughout the world. Several fungal cell wall degrading enzymes, amongst them chitinase and glucanase, which seem to play an important role in the antagonistic action of Trichoderma against a wide range

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of fungal plant pathogens (Kucuk and Kivanc, 2008). *T. harzianum* had antagonistic effects against *Botryodiplodia theobromae*, *Colletotrichum gloeosporioides* and *Gliocephalotrichum microchlamydosporum*, causative fungi of stem end rot, anthracnose and brown spot of rambutan respectively and also retained the overall quality and color of the fruits (Sivakumar *et al.*, 2000).The present study aimed to find out the efficiency of *T. viride* against some post harvest pathogens.

Materials and methods

Organisms

Trichoderma viride and Post harvest pathogens, such as *Aspergillus niger, A. flavus, A. fumigatus*, *Fusarium* sp. and *Penicillium* sp. which were isolated from partially decomposed fruits and vegetables waste using the standard dilution plate method on the Trichoderma Selective Medium (TSM) developed by Papavizas and Lumsden (1980) and Czapek Dox Agar medium respectively. Both the cultures of the pathogens and the antagonist were maintained on Czapek Dox Agar at 4 °C.

Examination of antagonism of Trichoderma viride

Dual culture method

Antagonism of *Trichoderma viride* on post harvest pathogens was studied by dual culture technique (Rama Bhadra Raju *et al*., 2000). A mycelial disc (9 mm diameter), obtained from the peripheral region of 5 days old cultures tested fungi and *T. viride* were placed simultaneously on the periphery, about 1 cm from the edges of the Petri-dishes (9 cm diameter) at opposite sides. The Petri dishes containing the Czapek Dox Agar medium inoculated with the tested pathogen alone served as control. All the plates were incubated at 28 °C and measurements were taken after 5 days. At the end of incubation period, radial growth was measured. The percentage inhibition growth of tested pathogens in presence of *T. viride* was calculated over control. The growth inhibition was calculated by using the formula: 100 X C- T / C, Where C= growth in control and T = growth in treatment.

Effect of culture filtrate

The effect of culture filtrate of *T. viride* on tested pathogens was studied by following methods, developed by Maheswari *et al*. (2001). Five discs (9 mm diameter) of *T. viride* were cut from vigorously growing margin of 5 days old

cultures and inoculated separately into 100 ml sterile Czapek Dox broth (pH 5.6). Flasks were incubated at 28°C with 100 rpm on a mechanical shaker for 10 days so as to fragment the hyphal mats and to maintain homogeneous growth in liquid medium. After incubation, the cultures were filtered first through Whatmann filter paper No.4 and finally through Millipore filter (0.45 μm) to obtain sterile culture filtrate. The culture filtrate was adjusted to pH 5.6 by using 0.1N HCl and / or 0.1N NaOH before use. Different concentrations viz., 10, 20, 30, 40 and 50% of the culture filtrate were mixed with cooled Czapek Dox Agar before plating. The medium devoid of culture filtrate served as controlled. Petri dishes were inoculated separately with a 9 mm agar disc of the tested pathogens, cut from actively growing colony of 5 days old culture, and incubated at 28°C. The radial growth of tested pathogens was measured after 24 hours intervals.

Results and discussion

Result showed that *Trichoderma viride* could restrict growth of post harvest pathogens on Czapek Dox Agar medium in the dual culture (Table 1). The per cent inhibition of radial growth of tested fungi viz., *A. niger* (55%), *A. flavus* (51%), *A. fumigatus* (52%), *Fusarium* sp. (64%) and *Penicillium* sp. (54%) were reduced by *T. viride* which grown over the colonies of all five pathogens, with greatest reduction occurring in *Fusarium* sp. and *A. niger*. The growth inhibition of postharvest fungi by dual culture in this study could be due to its fast growing nature, secretions of harmful extra-cellular compounds like antibiotics, cell wall degrading enzymes such as gluconases, endochitinases and chitinases and mycoparasitism in dual culture as found with other fungi (Ramesh Sundar *et al*., 1995; Thirumala Rao and Sitaramaiah, 2000; Nakkeeran *et al.,* 2002).

Table 1. Antagonistic activity of *Trichoderma viride* against post harvest pathogens by dual culture method.

Post harvest Pathogens	Growth in control (mm) ± SEM*	Growth with *T. viride* (mm) ± SEM*
A. niger	50.42±0.2995	22.60±0.3262
A. flavus	41.72 ±0.4687	20.64 ± 0.3288
A. fumigatus	40.73 ±0.4615	19.67±0.3288
Fusarium sp.	57.57 ±0.3235	20.51±0.2443
Penicillium sp.	35.33 ±0.2962	16.28 ±0.1677

*Values are mean of three-relicate ± standard error.

Culture filtrate of *T. viride* inhibited the growth of tested pathogens. The growth inhibition increased with increase in the concentration of culture filtrate (Fig. 1). A culture filtrates with 50% medium showed the highest percentage of inhibition against *A. niger* (64%), *A. fumigatus* (50%) and *A. flavus* (48%).

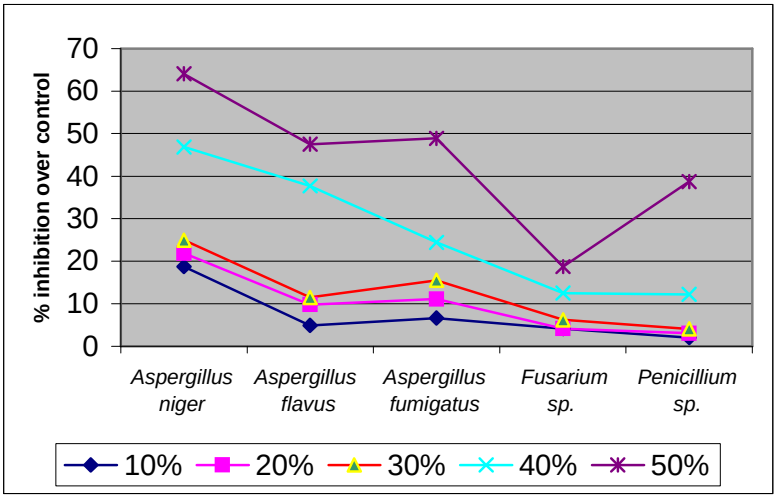

Fig. 1. Effect of culture filtrate of *Trichoderma viride* against post harvest pathogens on the volume of culture filtrate used.

The growth inhibition of tested pathogens may be due to antibiotic secretion of like trichodermin, dermadin, trichovirdin and sesquiterpene heptalic acid (Nakkeeran *et al*., 2002), nutrient impoverishment and pH alteration in the medium (Maheshwari *et al.*, 2001). Hence, *T. viride* has a potential to develop as a biological agent to control the common post harvest diseases.

The *in vitro* screening for microbial bio-control agents effective against post harvest pathogens is a simplistic approach to understand the biological system in the control of post harvest diseases. Our result explains that significant success in bio-control is achieved under *in vitro* conditions. Even though more research is needed to understand the antagonistic mechanism, improvement of strains and development of supplementary products of biocontrol agent for restraint of post harvest pathogens. Thus, it is noticeable that a microbial biocontrol agent offers harmless to the animals and human beings, cheaper than chemicals and highly effective. There is no risk of the pathogens develop resistance, fungicide residues in food and ground water.

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