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CONTROL OF POSTHARVEST PATHOGENS OF FRUITS BY CULTURE FILTRATE FROM ANTAGONISTIC FUNGI

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Abstract: *In vitro* test of antagonistic activity of culture filtrates from *Trichoderma harzianum* Rifai and *Trichoderma pseudo-koningii* Rifai strains against post-harvest pathogens of some fruits were investigated. The undiluted culture filtrates of the two *Trichoderma* species completely inhibited germination of conidia/spores of all the rot pathogens, but 50% dilution showed varying degree of inhibition of spore germination. *T. pseudo-koningii* culture filtrate had a rather moderate to strong inhibitory effect on mycelia of the pathogenic fungi. The highest per cent inhibition of 45.6% of mycelial growth was recorded for *Aspergillus niger* Tiegh.

Key words: antagonistic fungi, culture filtrate, pathogens, post harvest

INTRODUCTION

Trichoderma species are known as bio-control agents against plant pathogenic fungi. Despite the extensive research on capability of *Trichoderma* spp. to reduce the incidence of disease caused by soilborne plant pathogens, the mechanism by which disease control is achieved is not clearly understood. The mechanisms suggested to be involved in bio-control by these fungi are antibiosis, lysis, competition, mycoparasitism and promotion of plant growth (Henis 1984; Papavizas 1985; Chet 1987 and Lynch 1990).

It seems reasonable to assume that successful antagonism may rely on a combination of these modes of action. Specifically mycoparasitism, which may require production of cell wall degrading enzymes has been indicated as a major mechanism of action. The role of enzymes in bio-control may be assigned to the mechanism of parasitism and antibiosis. In particular, cell wall degrading enzymes such as chitinases, 1-3 glucanases and cellulases are not the only important features of mycoparasitism for colonization of their own (Di Pietro et al. 1993). Lorito et al. (1993) in their work on the antifungal activity of purified endochitinase and chitobiosidase produced by *T. harzianum* found out that endochitinase

purified to homogeneity, strongly inhibited the growth and development of all the chitin-containing fungi tested *in vitro*.

The objective of this study therefore is to investigate the effect of culture filtrates of some *Trichoderma* species isolated from the soil and rhizosphere of maize on the fungi associated with rot of some commonly consumed fruits in Oyo State of Nigeria.

MATERIALS AND METHODS

Fungal isolates

Trichoderma species viz *T. harzianum* and *T. pseudo-koningii* used in this investigation were obtained from Dr. Ayodele Sobowale of Maize Pathology Unit of International Institute of Tropical Agriculture, Ibadan, Oyo State, Nigeria. They were maintained on acidified potato dextrose agar (PDA) stants in McCartney bottles and kept in a cold room.

Isolation of pathogens

Pathogens were isolated from naturally infected fruits, sectioned tissues were soaked in 0.1% sodium hypochlorite for 5 min for surface sterilization and then rinsed in changes of sterile distilled water. The infected tissues were later picked onto sterile filter paper using a sterile forceps and then wrapped with filter paper for 3–5 minutes. The dried infected tissues were placed onto several prepared sterile plates of acidified potato dextrose agar (PDA) and the plates were incubated at 28–30°C in an incubator for 3–5 days. The different fungi that grew from infected tissues were sub-cultured on separate sterile acidified PDA plates and incubated to obtain pure cultures of pathogens. The identification of fungi was done at International Institute for Tropical Agriculture, Ibadan Nigeria.

Culture conditions

The culture filtrates of *Trichoderma* spp. were obtained by growing the fungi in flasks containing 100 ml modified Richards medium as used by Harman et al. (1993) containing KNO_3, 10g; KH_2PO_4, 5g; anhydrous $MgSO_4$, 1.5 g; $FeCl_3$, 20 mg; sucrose, 8 g and 150 ml of tomato juice in 1 litre of sterile distilled water. The flasks were inoculated with 5 mm agar plug from 7 day-old cultures on acidified PDA and incubated for 5 days at approximately 28°C. The biomass was removed by filtration and the culture filtrate was used for anti-fungal tests.

The inhibitory activity of culture filtrates of *Trichoderma* species was determined by spore germination test and agar method for mycelial growth.

Effect of culture filtrates on spore germination

Conidia obtained from 5-day-old acidified PDA cultures of *Macrophomina phaseolina* (Schw) Andrus and More, *Atternaria* sp., *Fusanum solani* Mart. and *Aspergillus niger* were separately suspended in a full strength (undiluted) culture filtrate, and also in 50% dilution of the filtrate. The spore/conidia concentration was readjusted with haemocytometer to 10^{-2} spores/ml. Four replicate drops of the suspension were incubated on microscope slides placed in Petri dishes for 24 hrs during which the percentage spore germination was determined.

Spore/conidia suspension of each of the fungi incubated in sterile potato dextrose broth served as control.

Determination of inhibitory activity of culture filtrates using agar method

Agar method described by Turhan and Grossmann (1994) was used with modification consisting of mixing in culture filtrates obtained from 5 day-old liquid cultures of the Trichoderma species with melted sterilized potato agar at the ratio 1:3. The amended media were adjusted to pH7 and poured into 9 cm Petri dishes to solidify. A 5 mm agar disc of the test fungus was placed at the centre of a Petri dish; the control was plain potato dextrose agar. The plates were incubated at 28±2°C. Diameter of developing mycelium was measured daily for 5 days and the average was calculated. Percentage inhibition was calculated according to the method described by Whipps (1987).

RESULTS

Effect of culture filtrate of *Trichoderma* species on germination of spores of pathogenic fungi

The undiluted culture filtrate of *T. harzianum* and *T. pseudo-koningii* completely inhibited germination of spores of all the tested pathogenic fungi, but 50% dilution had varying effect on the degree of spore germination, which ranged from 5% to 10% for *T. harzianum* and 5% to 19% for *T. pseudo-koningii* (Table 1).

Table 1. Percentage germination of conidia/spores of pathogenic fungi isolated from rotten fruits in culture filtrate of antagonistic *Trichoderma* spp.

T. harzianum				
Culture filtrate concentration (%)	*M. phaseolina*	*F. solani*	*Alternaria* sp.	*A. niger*
Concentration				
100	0	0	0	0
50	15.0	13.1	12.0	10.0
Potato dextrose broth (control)	75.3	80.0	73.2	88.0
T. pseudo-koningii				
Culture filtrate concentration (%)	*M. phaseolina*	*F. solani*	*Alternaria* sp.	*A. niger*
100	0	0	0	0
50	19.2	12.1	15.0	5.0
Potato dextrose broth (control)	75.3	80.0	73.0	88.0

Germination was assessed after 12 hours
Four replicate drops were evaluated

Effect of culture filtrate of *Trichoderma* species on mycelial growth on agar

Culture filtrate of *T. harzianum* had a minimum inhibitory effect on most of the pathogens, while *T. pseudo-koningii* showed a rather moderate to strong inhibitory effect. *T. pseudo-koningii* showed the highest per cent inhibition on *A. niger* followed by *M. phaseolina* 30.0%, *F. solani* 23.2% and the lowest inhibition on *Alterneria* sp. 22.6%.

T. harzianum inhibition of *M. phaseolina* was 11.1% *Alternaria* sp. 16.7% and *A. niger* 24.4% (Table 2).

Table 2. Percentage of mycelium inhibition of pathogenic fungi isolated from rotten fruits in culture filtrate of antagonistic *Trichoderma* spp.

Trichoderma spp.	*M. phaseolina*	*F. solani*	*Alternaria* sp.	*A. niger*
T. harzianum	11.1	15.0	16.7	24.4
T. pseudo-koningii	30.0	23.2	22.6	45.6
Control (PDA)	0	0	0	0

$$\text{Percentage inhibition} = \frac{R_1 - R_2}{R_1} \text{x} \frac{100}{1}$$

Where R_1 is the value of radial growth of pathogen on control plates and R_2 is the value of radial growth of pathogen in culture filtrate incorporated into agar plate.

DISCUSSION

The results obtained suggested that *Trichoderma* spp. are capable of producing a range of metabolites, which have antifungal activity in liquid cultures. These extracellular metabolites in the culture filtrate inhibited spore germination and growth of all the tested post-harvest pathogens on agar media. One of possible mechanisms by which pathogenic fungi are biologically controlled is production of antibiotics which arrest fungal growth. Such antibiotics had indeed been reported by workers like Jayawal et al. (1990) and Mcloughlin et al. (1992).

Trichoderma spp. have been found to control post harvest *Botrytis* rot of strawberry by antibiotic production (Tronsomo and Dennis 1977) whereas Dennis and Webster (1971a, b) reported production of both non-volatile and volatile antibiotics by species of *Trichoderma* including *T. viride*. The growth inhibition of pathogenic fungi by culture filtrates in this study could be due to antibiotics or specific enzymes. Cell-wall degrading enzymes may be required in mycoparasitism as some workers suggested (Lorito et al. 1993; Harman et al. 1993).

Trichoderma spp. are known to be efficient producers of polysaccharide lyases, proteases, and lipases, all of which may be used for degradation of cell walls of target fungi (Cherif and Benhamou 1990). Chitinolytic and glucanolytic enzymes from *T. harziamum* have also been found to be inhibitory to other fungi and to interact synergistically to achieve a high level of antifungal activity (Lorito et al. 1993). This study has therefore demonstrated the presence of antifungal substances in the culture filtrate of *T. harzianum* and *T. pseudo-koningii* against the postharvest pathogens of fruits.

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POLISH SUMMARY

ZWALCZANIE PATOGENÓW WYSTĘPUJĄCYCH PO ZBIORZE OWOCÓW FILTRATEM KULTUR GRZYBÓW ANTAGONISTYCZNYCH

Badano in vitro aktywność antagonistyczną filtratów kultur *Trichoderma harzianum* Rifai i *Trichoderma pseudo-koningi* Rifai przeciwko patogenom niektórych owoców. Nierozcieńczone filtraty kultur obydwóch gatunków *Trichoderma* całkowicie inhibowały kiełkowanie konidiów/zarodników wszystkich badanych patogenów wywołujących zgnilizny owoców, ale 50% roztwór filtratów wykazywał różny stopień inhibicji kiełkowania zarodników. Filtrat kultury *T. pseudo-koningi* wywoływał raczej umiarkowany do silnego efekt inhibicyjny w stosunku do grzybni patogenów.

Najwyższy efekt inhibicyjny wynoszący 45,6% zahamowania wzrostu grzybni odnotowano w przypadku grzyba *Aspergillus niger* Tiegh.