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Full Length Research Paper

Antagonistic properties of *Trichoderma viride* on post harvest cassava root rot pathogens

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The effective *in vitro* screening tests of *Trichoderma viride* for antagonism against post harvest pathogens of cassava roots (*Manihot esculenta* Crantz) rot together with its competitive and mycoparasitic abilities informs its selection as the most promising candidate for the biocontrol of post harvest cassava root rot pathogens. While the percentage germination of the spores were observed to increase with increasing dilution of the culture filtrate (100, 50, 25, and 10%) at a concentration of 20,000 spores/ml, the percentage rot recorded amongst the untreated cassava roots ranged from 4% (*Rhizopus oryzae)* to 44% (*Aspergillus flavus*). Treatment with the antagonist and the subsequent storage resulted in a remarkable reduction in the frequency of occurrence of the normal root surface mycoflora and the pathogens over a 3 weeks storage period. *Botryodiplodia theobromae* and *R. oryzae* were isolated only in the first week of storage and at a frequency of 3 and 2% rot, respectively, after treatment whereas *A. flavus* and *Fusarium solani* persisted throughout the whole storage period with 2 and 3% rot on the third week, respectively. The observed results suggest that *T. viride* is root surface competent and highly antagonistic.

Key words: *Trichoderma viride*, Biocontrol, Cassava root rot pathogens.

INTRODUCTION

Trichoderma viride is a filamentous soil fungus known to be an effective biocontrol agent of a range of important airborne and soil borne pathogens. *Trichoderma* spp. is the most widely studied biocontrol agents (BCAs) against plant pathogens. Weindling and Emerson (1936) observed that they could excrete extracellular compound which was named gliotoxin. Many antibiotics and extra-cellular enzymes were isolated and characterized later, and the biocontrol mechanisms became clearer (Haran et al., 1996; Zhihe et al., 1998).

Fresh cassava roots (*Manihot esculenta* Crantz) are prone to biodeterioration. The first to appear, named "Primary deterioration" consist of physiological changes characterized by an internal root discoloration called vascular streaking. Wounds and bruises are the major points of entry for micro-organisms after harvest leading to the second stage of cassava root spoilage, known as "Secondary deterioration"

Integrated disease management incorporating cultural and biological control methods with reduced chemical inputs seems to be a promising approach. Biological control of soil borne disease by microbial antagonists has been widely reported (Deacon, 1988; Weller, 1988; Hornby, 1990). *T. viride* is a fast growing, soil fungus that parasitizes the mycelia of other fungi. The parasitic activity of *T. viride* is mediated by its excretion of a variety of enzymes including cellulases and chitinases and antibiotics such as gliotoxin (Haran et al., 1996; Zhihe et al., 1998). *Trichoderma* spp. is economically important, in part because of their mycoparasitic ability, which makes them suitable for application as biocontrol agents against soil-borne plant-pathogenic fungi (Benitez et al., 1998; Manczinger et al., 2000). The present study addresses the biocotrol mechanisms and application of *Trichoderma*

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Table 1. Percentage germination of spores of the pathogens (*B. theobromae, A. flavus, F. solani, and Rhizopus* sp.) in the culture filtrate of *T.viride*.

Culture filtrate concentration (%)	*Botryodiplodia theobromae*	*Aspergillus flavus*	*Fusarium solani*	*Rhizopus* sp.
100	0	0	0	0
50	6.2	7.2	9.0	14.3
25	18.6	25.4	17.4	12.4
10	48.4	51.3	39.6	65.2
Potato dextrose broth (PDB)	59.8	86.7	69.6	81.8

Three replicate drops were evaluated.

Table 2. Cumulative percentage incidence of rot during storage of cassava roots inoculated with *T. viride* and the postharvest pathogens.

Treatment	1 week	2 weeks	3 weeks
Uninoculated (control)	4	12	28
B. theobromae	8	16	20
Aspergillus flavus	6	20	44
Fusarium solani	9	15	35
Rhizopus oryzae	4	24	30
Trichoderma viride (antagonist)	0	0	0
Trichoderma viride + *B.theobromae*	3	0	0
Trichoderma viride + *Aspergillus flavus*	1	1	2
Trichoderma viride + *Rhizopus oryzae*	2	0	0
Trichoderma viride + *Fusarium solani*	1	1	3

Figures are expressed in percentage.
Two hundred and fifity cassava roots were observed.

spp. with particular emphasis on *T. viride*.

MATERIALS AND METHODS

Isolation of the antagonist

T. viride was isolated from soil samples collected from NRCRI cassava farm, Umudike-Umuahia, Abia state, Nigeria. Serial dilutions of the soil samples were prepared and plated on potato dextrose agar with antibiotics (30 mg/l penicillin and 50 mg/l streptomycin) and incubated for 5 days. Colonies were sub-cultured to obtain pure cultures. The isolated fungi were identified according to their micro-morphology as well as the colour and morphology of their sporulating structures and conidia. Glass slides preparation was done using lactophenol as mutant and cotton blue as stain (Harrican and McCance, 1976; Barnett, 1992). Microscopic examination of the prepared slides was done using the low power objective followed by the 40 magnification objective lens.

In vitro screening of the antagonist on the germinating spores of post harvest pathogens of cassava roots

Culture filtrate of *T. viride* was obtained by passing a 5 day old potato dextrose broth (PDB) culture of the fungus through a membrane filter (Sartorius, Germany) of pore size, 0.5 µm. The harvested spores of the 5 day old PDA cultures of *Botryodiplodia theobromae, Aspergillus flavus***,** *Fusarium solani* and *Rhizopus oryzae* were separately suspended in the full strength filtrate and also in the 50, 25, and 10% dilutions of the *T. viride* filtrate (Table 1). The concentration was initially readjusted to 20,000 spores/ml. Three replicate drops of the suspensions were incubated on microscope slides in humidified Petri dishes for up to 72 h during which the percentage spore germination was determined hourly. The spore suspension of each of the fungi incubated in the PDB was used for comparative studies.

In vitro effect of the antagonist on cassava root rot pathogens in storage.

Thirty six healthy and wholesome cassava roots were first washed with tap water before sterilizing with 70% ethanol. The roots were grouped into two: the first three groups were inoculated with *T. viride* (the antagonist) whereas the remaining three groups were sprayed with equivalent volume of blank sterile distilled water. Each group were covered separately with sterile polyethylene sheets and incubated on raised plat forms for 5 days. The first groups inoculated with *T. viride* were further inoculated with *B. theobromae, A. flavus, F. solani, R. oryzae* and blank sterile water after 24 h. The concentration was 4.3 x 10^9 spores/ml at the rate of 10 ml of suspension per root according to Okigbo and Ikediagwu (2000). The second groups earlier treated with sterile distilled water were again inoculated with *B. theobromae, A. flavus, F. solani, R. oryzae* and blank sterile distilled water respectively (Table 2). All the treatments were covered with polyethylene sheets and further incubated for additional 5 days. The sheets were later removed to expose all the roots fully to the ambient temperature for 3 weeks period. The roots were examined daily for the occurrence of rot and determination of the root surface mycoflora.

RESULTS

The antagonistic suppression of *T. viride* against the post harvest pathogens of cassava roots was observed. The undiluted culture filtrate of *T. viride* demonstrated a remarkable inhibition against the germinating spores of all the tested pathogens. Though the percentage germination of all the spores increased with increasing dilution of the culture filtrate, its effect remained considerably even at 10% dilution (Table 1). Strikingly, the respective populations of the inoculated pathogens rose appreciably early in storage but only *A. flavus* and *F. solani* maintained a high frequency of occurrence throughout the duration of storage. *B. theobromae* and *R. oryzae* were isolated only in the first week of storage and at a frequency of 3 and 2% rot, respectively (Table 2). The result suggests that the inoculated *B. theobromae* and *R. oryzae* did not survive beyond 1 week of storage whereas *A. flavus and F. solani* persisted throughout the whole period (Table 2). It was observed that all the roots inoculated with *T. Viride* showed a drastic reduction in the range and number of fungi for a period of 3 weeks. The result suggests that *T. Viride* is root surface competent and highly antagonistic.

Biodeterioration was observed to progress faster among injured roots which were inoculated with the pathogens than in the wholesome roots that were not experimentally inoculated. The percentage rot among the different groups, ranged from 4% (*R. oryzae*) to 44% (*A. flavus*) (Table 2). The group of roots that had *T. viride* inoculation in contrast, recorded a low percentage of rot.

DISCUSSION

The idea of a sustainable agricultural practice and environmental protection enhances the importance of biocontrol. The adoption of a sustainable agricultural practice, using strategies that are environmentally friendly, less dependent on agricultural chemicals is gaining world wide recognition. One of the key elements of such sustainable agriculture is the application of biocontrol agents. *Trichoderma* spp. is antagonistic by nature with rich resource and a broad action scope. The present study addresses the effective control mechanisms of *T. viride* against the cassava root rot pathogens. Interestingly, the result obtained in the inhibition of *T. viride* against the post harvest pathogens, suggests that the organism is strongly antagonistic to the pathogens and has potential in post harvest (secondary deterioration) control of cassava root rot pathogens. *A. flavus* and *F. solani* were observed to persist over a more prolonged duration with 2 and 3% rot, respectively (Table 2). The observation is similar to the findings of Tronsmo and Dennis (1977) and Okigbo and Ikediagwu (2000) in their investigation on the effects of *T. viride* on post harvest *Botrytis* rot of strawberry and yam rot, respectively.

The additional contribution of the extracellular metabolites of *T. viride* as a means of biocotrol is observed not only by the *in vitro* inhibition of spore germination of all the tested rot pathogens by the culture filtrate of the antagonist (Table 1), but also by the induction of a zone of inhibition by the antagonist against the colonies of the pathogens. The principal mode of action of this organism appeared to be antagonism by production of pyrrolnitrin, a powerful antifungal compound. The production of volatile and non-volatile antibiotics by the species of *Trichoderma,* including *T. viride* has been reported by Dennis and Webster (1971a,b). It is therefore probable that such metabolites might have been involved in the biocontrol of cassava root rot pathogens as well as in yam tubers and in the other situations (Hartman et al., 1980; Chet and Baker, 1981; Wilson and Wisniewski, 1989), where species of *Trichoderma* have been exploited in the control of rot in fruits and vegetable diseases. Furthermore, Lei and Huaying (1998) and Liansheng and Wweihua, (2000) reported that strong mycoparasitism is part of biocontrol mechanism of *T. viride* against *Botrytis cinerea*. In addition to its well recognized mycoparasitic nature, it is suggested that *Trichoderma's* association with roots reduce root disease through activation of the plant's defence response (Yedidia et al., 2000).

Appreciably, the results of the storage studies, demonstrated clearly that a single application of the spore suspension of *T. viride* protected the cassava roots remarkably well from subsequent secondary deterioration caused by microorganisms. The observed drastic suppression of the normal root surface mycoflora and the pathogens may indicate that the control of post harvest rot pathogens is related to an *in vitro* antagonism by *T. viride.* The capacity shown by *T. viride* to displace the colonies of the tested pathogens *in vitro* and to coil round the hyphae of *B. theobromae*, suggests that the phenomenon of hyphal interaction described by Dennis and Webster (1971c) for *T. viride* and some other species of *Trichoderma* is implicated in the biocontrol mechanism.

Economically, primary deterioration is more significant than secondary deterioration as it causes a distinct decline in the value and appeal of the roots. According to Booth et al. (1976) extended storage can have two adverse effects on quality as starches are converted to sugars and roots becoming fibrous thereby lengthening cooking time. To achieve a maximum period of storage for cassava roots, it must be made sure that they are not injured or squashed during harvesting, transport and storage as injuries accelerate the physiological deterioration of the tissue. The most serious injuries occur at the shoulder of the root where it is connected to the plant by the root collar. Ingram et al (1972) reported that this can be avoided by harvesting the whole plant or by leaving a short piece of stalk on the root. It therefore appears that roots harvested in this way discolour far more slowly than those harvested in a conventional fashion.

In most plants tissue damage results in a cascade of wound responses that quickly result in the defence of the wounded tissue and the subsequent sealing of exposed tissue by regeneration of a protective barrier (periderm formation). This apparent inability of cassava plant for such wound healing responses which is responsible for primary or physiological deterioration remains an area of further research which biotechnology may offer a promising solution.

However, other storage methods have been investigated by other scientists and they includes: the use of sawdust, plastic films, paraffin wax, waxing and holding at 0 to 5°C but physiological deterioration remains a scientific problem that requires solution.

In conclusion, the excellent root surface competence of *T. viride*, distinctly demonstrated by its persistence at a high frequency of occurrence on the surface of cassava roots in storage for weeks even in the ambient environmental conditions renders the repeated re-spraying that is required for chemical pesticides unnecessary.

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