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Growth Inhibitory Effect of Different Concentrations of Native *Trichoderma harzianum* Metabolite on some Postharvest Pathogens of Cassava.

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Abstract

The growth inhibitory potential of native Trichoderma harzianum metabolite at different concentrations against some post harvest fungi of cassava (M. esculenta) roots was examined in vitro. Rotting and fresh cassava roots were obtained from three vendors each from three different markets in Ibadan. Cultures of isolated fungi were prepared and severally sub-cultured to obtain a pure cultures maintained in Agar medium. The fungal isolates were Botryodoplodia theobromae, Aspergillus flavus and Penicillium notatum. Selected fungi were inoculated onto sterile PDA plates containing different concentrations (10%, 25%, 50% and 100%) of T. harzianum metabolite and incubated at 28±2^{0}C for 7 days and radial growths of the fungi were measured daily. T. harzianum metabolite exhibited significant at (P<0.05) inhibitory potential against the selected fungi at all concentrations. T. harzianum metabolite had higher inhibition impact on the test fungi at 100% concentration. Inhibitory activity of the metabolite was concentration dependent. A. flavus (1.50) was mostly inhibited followed by P. notatum (2.88) and B. theobromae (3.38) at 100% concentration. T. harzianum metabolite is therefore said to possess effective antifungal potential against fungi associated rotting of cassava.

Key words: Postharvest Pathogens, *Trichoderma harzianum*, Cassava roots, Inhibition and Concentration

Introduction

Cassava is one of the leading feeds of the world, and it is the fourth amongst the staple crops with a global production of about 160 million tons per year (Adams *et al.*, 2009; Olsen and Schaal, 1999). Usually, it is mainly used for human consumption, than for animal consumption and for industrial purposes, though this may vary depending on the country, for instance in Arua, NorthwesternUganda and Lugbara cassava is grown as a staple food (Sadik 1988)It has been reported that cassava provides a major source of calories for poor families in the tropics because of its high carbohydrate content and cassava flour is used for making a lot of things, ranging from cookies, loaves, pancakes, doughnuts and dumplings, strong liquor, fufu, gariri (Sadik, 1988). Nigeria however is reported as the world's largest producer of cassava (Olsen and Schaal, 1999; Pope *et al*., 2001). Based on several reports, Nigeria is the largest producer of cassava though Thailand has the largest exportation of dried cassava of about 77% as at 2005. The second largest exporting country is Vietnam, with 13.6%, followed by Indonesia (5.8%) and Costa Rica (2.1%) (Adams *et al*., 2009).

There will always be the occurrence of Postharvest physiological deterioration (PPD) once the roots are separated from the main plant even though there might be healing response mechanism from the interaction between the phytoalexic property of the root and the fungi associated with the damage (Vinale *et al*., 2004). However, the same mechanism involves coumaric acid, which initiates about 15 minutes after damage and fails to switch off in harvested tubers which may continue until the entire tuber is oxidized and blackened within two to three days after harvest, rendering it unpalatable and useless (Morante *et al.*, 2010). (PPD) is one of the main obstacles currently preventing farmers from exporting cassavas abroad and generating income (Van *et al.*, 1998; Naseby *et al.*, 2000). Researchers

have shown over the years that cassava growers have experienced epidemic threats by the activities of pathogens; leading to massive destruction of plants parts (leaves, stem and roots) thereby culminating in a huge financial loss (Coyne and Talwana, 2000; Makumbi-kidza*et al.*, 2000). Hence, there are growing concerns over the use of synthetic pesticides and fumigants to manage and protect the plant and its produce from pathogens (Morante *et al.*, 2010). However,*Trichoderma* species has been severally reported to have antifungal potential against different pathogens of plant (Sobowale *et al.*, 2010). It has been reported by several researchers that *Trichoderma* secrete hydrolytic enzymes at a constitutive level and detects the presence of another fungus by sensing the molecules released from the host by enzymatic degradation (Harman *et al.*, 2004; Lorito *et al.*, 1996). The bio-control activity of *T. harzianum* against some fungal pathogens of certain crops through microbial interactions proved that *T. harzianum* induced defence responses and systemic resistance on those crops (Yedidia *et al.*, 2001 and 2003). It was also reported that *T. harzianum* is one of the best bio-control inoculants and can be used safely as an alternative to chemical substances (Whipps 2001; Grondona *et al.*, 2002). Hence the reason of this study is to manage the devastation of the root crop and possible bio-inhibition of the pathogens associated with postharvest of cassava from further development and spreading.

Materials and Method

Sample collection: Rotting cassava (*M. esculenta*) tubers were collected from three different markets in Ibadan. At each of the markets, three root samples were obtained from three different vendors each. A total of nine different samples were collected from the markets. Cassava (*M. esculenta*) stems, leaves, roots and soil were also randomly collected from two different cassava farms at the University of Ibadan, Oyo Sate, Nigeria. The samples were taken to the Plant Pathology Research Laboratory in the Department of Botany, University of Ibadan, Oyo State, Nigeria, for isolation and further experiments.

Isolation and Identification of Pathogens
Pieces of the diseased cassava samples (3.0mm – 4.0mm) were washed with 15ml distilled water for 10 minutes and inoculated onto the plates containing Potato Dextrose Agar (PDA) using a pair of forceps. The plates were incubated at room temperature for 5 days to allow fungi growth. These were sub-cultured severally to obtain pure cultures and incubated at 28±2^{0}C. The fungi isolated were identified at the Pathology laboratory, Department of Botany, University of Ibadan.

Extraction of Metabolites from *T. harzianum*
Five mm mycelia (30) discs from 7 day old cultures of *T. harzianum* isolate were inoculated into 500 ml flasks containing 250 ml of $^{1}/_{5}$ strength Potato Dextrose Broth (PDB) and then, incubated in horizontal rotary shaker incubator at 28±2^{0}C. Thereafter, the spores and mycelia of *T. harzianum* were removed from broth culture by filtration using. Blotter paper and then subjected to centrifugation at 5000rpm. Further purification and sterilization were done using 0.22millipore. The metabolite was extracted from cell pellets using N-hexane, and ethyl acetate as the extraction solvents.

Introduction of *T. harzianum* metabolite
One (1ml) of 100%, 50%, 25% and 10% concentrations of the metabolite of *T. harzianum* were introduced into some disposable sterile Petri dish after which 10ml of Potato Dextrose Agar (PDA) was poured into the Petri dish, swerved gently for about 10 minutes and allowed to set. The test fungi isolates *A. flavus, B. theobromae* and *P. notatum* were inoculated onto the surface of the medium using a 5mm cork borer. The treatments of the various concentrations were in triplicate, including their control experiments and incubated at 28±2^{0}C. Examination and measurement of the radial mycelia growth were taken daily for 7 days of incubation using a meter rule for each of the concentrations and controls. Wonglom *et al.* (2019) was adopted for estimation of fungal growth inhibition zone as thus; I = (C-T)/C x 100. Where C is the radial mycelium growth in the control plate, T is the radial mycelium growths in the inserted or treated plate while I is the radial mycelia growth inhibition.
Data collection and Analysis: Data collected was subjected to statistical analysis of variance (ANOVA) using Generalized Linear Model (SAS, version 9.3). Least significant difference (LSD) for multiple means comparisons of the growth diameter of mycelia was used to evaluate the significant differences among treated samples.

Results: Table 1 shows the means comparison of the growth inhibition of selected fungi at 10% concentration of *T. harzianum* metabolite with their controls. There was significant difference between the fungal isolates within the treatment. There was significant difference (0.4) in the growth inhibition of *P. notatum* when compared to the control. But there was no significant difference in the growth inhibitions of *A. flavus* and *B. theobromae* when compared to the control (0.02 & 0.17) at ($LSD_{0.05}$). However, *P. notatum* was mostly inhibited at 10% (3.46) *T. harzianum* metabolite.

Table 1: Effect of 10% Concentration of *T. harzianum* Metabolite on Growth Inhibition of Fungal Isolates from Rotting Cassava Root

Fungi isolate	*T. harzianum* metabolite(cm)	Control (cm)
P. notatum	3.46	3.86
A. flavus	3.72	3.70
B. theobromae	3.65	3.82
$LSD_{0.05}$	0.18	

Means lower than $LSD_{0.05}$ across the rows is not significantly different

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Table 2 shows the means comparisons of growth inhibitions of the selected fungi at 25% concentration *T. harzianum* metabolite. The growth inhibition of *P. notatum* was significantly different from that of *B. theobromae* while there was no significant difference between the inhibitory effect of *T. harzianum* metabolite on *P. notatum, A. flavus* and *B. theobromae.* But there was significant difference in the growth inhibitions of *P. notatum, A. flavus* and *B. theobromae* between the treatments when compared to the control. However, *P. notatum* was mostly inhibited at 10% (3.34) *T. harzianum* metabolite.

Table 2: Effect of 25% Concentration of *T. harzianum* Metabolite on Growth Inhibition of Fungal Isolates from Rotting Cassava Root

Fungi isolate	*T. harzianum* metabolite (cm)	Control (cm)
P. notatum	3.34	3.86
A. flavus	3.48	3.70
B. theobromae	3.65	3.82
$LSD_{0.05}$	0.20	

Means lower than $LSD_{0.05}$ across the rows is not significantly different

Table 3 shows the mean comparison of growth inhibition of the selected fungi from rotting cassava at 50% concentration of *T. harzianum* metabolite with the controls. There was significant difference in the growth inhibitions of all selected fungi compared to the controls. But there was no significant difference between the test fungi. The growth of *P. notatum* was mostly inhibited by *T. harzianum* metabolite.

Table 3: Effect of 50% Concentration of *T. harzianum* Metabolite on Growth Inhibition of Fungal Isolates from Rotting Cassava Root

Fungi isolate	*T. harzianum* metabolite(cm)	Control (cm)
P. notatum	3.16	3.86
A. flavus	3.30	3.70
B. theobromae	3.34	3.82
$LSD_{0.05}$	0.22	

Means lower than $LSD_{0.05}$ across the rows is not significantly different

Table 4 shows the mean comparison of growth inhibitions of the selected fungi from rotting cassava at 100%concentration of *T. harzianum* metabolite with their controls. There was significant difference in the growth inhibition of all the selected fungi by *T. harzianum* metabolite when compared with the two controls. *A. flavus* was mostly sensitive to the treatment at 100% concentration.

Table 4: Effect of 100% Concentration of *T. harzianum* Metabolite on Growth Inhibition of Fungal Isolates from Rotting Cassava Root

Fungi isolate	*T. harzianum* metabolite(cm)	Control(cm)
P. notatum	2.88	3.86
A. flavus	1.50	3.70
B. theobromae	3.38	3.82
$LSD_{0.05}$	0.41	

Means lower than $LSD_{0.05}$ across the rows is not significantly different

DISCUSSION

T. harzianum metabolite showed positive inhibitory potential against several fungal pathogen; *A. flavus, P. notatum and B. theobromae*obtained from rotting cassava (*M. esculenta*). The presence of these fungi in the post harvest cassava roots induced post-harvest physiological deterioration despite the phytoalexic effect of the root against fungal pathogens. This observation agrees with the report of Vinale*et al*. (2004) who noted that there will always be the occurrence of Postharvest physiological deterioration (PPD) once the roots are separated from the main plant even though there might be healing response mechanism from the interaction between the phytoalexic property of the root and the fungi associated with the damage.

The significant inhibitory impact of *T. harzianum* metabolite on the selected fungi compared to controls at all concentrations proved that the growth inhibitory capacity of the metabolite even at low concentration was very potent. This implied that the test post harvest fungal pathogens of cassava root are sensitive to the metabolite. It could however be as a result of secretion of hydrolytic enzymes even at the minimal level of antagonistic and mycoparasitic interaction of Trichoderma spp. and fungal pathogens. This corresponds to the works of Lorito *et al.* (1996), Harman *et al.* (2006) and Sobowale *et al.* (2010) who reported that *Trichoderma* species have antifungal potential against different pathogens of plant secrete hydrolytic enzymes at a constitutive level

and detects the presence of another fungus by sensing the molecules released from the host by enzymatic degradation. The bio-control activity of *T. harzianum* against some fungal pathogens of certain crops through microbial interactions proved that *T. harzianum* induced defence responses and systemic resistance on those crops (Yedidia *et al.*, 2001 and 2003). The significant difference recorded in growth the inhibition of selected fungi at 10%, 25%, 50% and 100% concentrations of *T. harzianum* metabolite proved that the metabolite depended significantly on its concentration (Tables 1, 2, 3 & 4). Furthermore, the fungal isolate at different concentrations of the metabolite showed that *P. notatum* was mostly inhibited of the three test fungi and *B. theobromae* was least inhibited (Tables 2, 3 & 4). This means implies that *P. notatum* and *B. theobromae* can be inhibited at relatively low concentration of *T. harzianum* metabolite while growth inhibition of *A. flavus* may be more effective and appreciable at higher concentrations of the metabolite (Table 4).

The non-significant difference that was shown for growth inhibitory of *A. flavus* at 10% concentration when compared to controls may mean that the inhibitory potential of the metabolite on the fungus is only effective and appreciable at higher concentrations. This relative resistance could be as a result of the concentration of *T. harzianum* metabolite used or the ability of the fungus to tolerate or degrade the metabolite. The natural healing mechanism that cassava root respond to when damaged might be as a result of native *T. harzianum* on the root. This agrees with the findings of Vinale *et al*. (2004) and Morante *et al.* (2010) who noted that damages trigger healing response mechanism from the root against the activities of fungi associated with post harvest diseases of cassava. Whipps (2001) and Grondona *et al.* (2002) also reported that *T. harzianum* is one of the best bio-control inoculants and can be used safely as an alternative to chemical substances.

Conclusion:The metabolite of *T. harzianum* exhibited antifungal activity against the selected post harvest fungal pathogens of cassava root. Growth inhibition of *B. theobromae* was significantly higher (P = 0.05) than those of the other two fungi. *B. theobromae* was least sensitive to *T. harzianum* metabolite. The three fungi responded differently to various concentration levels of *T. harzianum* metabolite. Therefore, the effectiveness of the *T. harzianum* metabolite depended significantly on its concentrations but can elicit biocontrol effect on post harvest fungal pathogens of cassava even at a lower concentration and should be incorporated into management of crops especially post harvest diseases caused by *B. theobromae*, *P. notatum* and *A. flavus.*

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