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EFFECT OF *Trichoderma* sp. ON MICROBIAL ROT OF YAM-TUBERS

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ABSTRACT

The effect of Trichoderma sp. on the rot of yam tuber by B theobromae, A niger and P oxalicum was investigated by inoculating Trichoderma sp. with the individual pathogens either simultaneously or with advanced inoculation of a pathogen using white yams (Discorea rotundata) obtained from Ondo State or Edo State. The result shows that inoculation of Trichoderma sp. simultaneously or not more than one day after prior inoculation of a pathogen, decreased rot incited by B theobromae, A niger or P oxalicum. However, whereas the reduction of rot caused by A niger or B theobromae in Ondo yam was statistically insignificant (p 0.05), that caused by P oxalicum in Edo yam (55.7 mm, control; 25.7 mm simultaneously; 26.3 mm, one day later) was statistically significant (p 0.05). Edo State yam were rotted faster (51.5 mm) than the Ondo State yams (25.0 mm) suggesting the cultivars of white yams varying level of susceptibility to rot pathogen. A Trichoderma sp. isolated from yam tuber surface inhibited mycelia extention growth of B theobromae (52.5 %), A niger (17.8 %) and P oxalicum (42.5 %) in vitro indicating its potentials as a biocontrol agent of post harvest rot of yams.

Key words: Trichoderma sp., Yam-tubers, Pathogen, Mycelia, Antagonism.

INTRODUCTION

The major areas of cultivation of yams in Nigeria are Anambra, Imo, Kwara, Ondo, Oyo, Edo states. Benue, Cross River Nasarawa and Plateau states. Thus it can be seen that the crop is produce in various ecological regions; that is forest, derived savanna and southern Guinea Savanna regions.

Yam is the common name for some plant species in the genus *Discorea* (family *Dioscoreaceae*) that form edible tubers. These are perennial herbaceous vines cultivated for the consumption of their starchy tubers in Africa, Asia, Latin America, the Caribbean and Oceania. Nigeria alone accounts for considerable more than half of the world's total production of yam (Kay, 1987) that is for over seventy percent.

Few statistical records available indicate the loss of our precious yam to microbial attack; bacteria as well as fungi (Okigbo and Ogbonnaya, 2006), for a country like ours to produces over 70 % of the world total output measures have to be taken to reduce loss (Okigbo and Ikediugwu, 2002, Okigbo, 2004).

There are more than thirty different genera of fungi that are associated with yam rot (Ikotun, 1989). They include *Trichoderma* spp., *Aspergillus* spp., *Penicillum* spp., *Geotrichum* spp., *Roellnia bunodes. Cladosponium herbarium, Collectotrichum* spp., *Mucor* spp., *Botrydiplodia theobromea*

Synthetic chemicals such as benzyl, carbonyl-2 benzlnidazal, and carbonate are used in controlling fungi responsible for yam rot and other root crops while carbonate is found to be most effective against fungi(Ekundayo, 1972). The use of synthetic chemicals have some disadvantages; they are expensive, cause environmental pollution (since some could contain substances that are of environmental and health concern such as the aromatics) and may induce pathogen resistance (Okigbo and Nmeka, 2005). The effect of biological control for the treatment of plant diseases have been described by various Scientists (Templetion and Smith, 1977; Cook and Baker, 1983, Okigbo and Ikediugwu, 2000). This method has the advantage of been less expensive when compared to synthetic chemicals, environmental friendly also there is the absence of toxicity due to bioaccumulation of chemicals.

There are apparently, three distinct forms of tuber rots including (a) wet rot; in which the interior of the tuber disintegrate into a watery mash (Amusa and Baiyewu, 1999) (b) soft rot; where the tissues are slighly softened and become discolored pink(Amusa and Baiyewu, 1999) and (c) dry rot; in which the tissue become discolored depending on the causative organism either brown (*Penicillum oxalicam* and *penicillum cyclopium)*, brown with yellowish margins (*Aspergillus niger* and *Aspergillus tamari*) grey and black (*Roellnia bunodes* and *Botrydiplodia theobromea)* crubles away to a dry powder (Nwauzer and Fawole, 1981; IITA, 1993).

The study aim at; (i) demonstrate the capacity of *Trichoderma* sp. to control on-going rot by major post harvest pathogens of yam tubers (ii) investigate the pathogenicity of rot causing pathogen on different cultivars of white yam.

MATERIALS AND METHODS

Isolation of Rot Pathogen from Affected Yam Tuber

Rot affected yam tuber was washed under tap water to dislodge adhering soil particles. Pieces of yam tissue of about 3mm were cut from across the interface between healthy and rot affected tissue. They were then surface sterilized in 70 % ethanol for 10 seconds, rinsed in sterile water. They were then dried in sterile tissue paper and these were plated out on potato dextrose agar. In which 0.6ml of antibiotic mixture (5g of streptomycin was dissolved in 100ml of sterile water plus five tablets, each containing 500 unit of penicillin dissolved in 10ml of sterile water, 20 ml of the streptomycin solution was mixed with10ml of the penicillin solution to provide the antibiotic mixture) were already been added, four pieces per Petri dish and then incubated under light 10 hrs period for up to 5 days at room temperature(28 ± 2^0C) , during which growths were isolated and identified using colony morphology, cell micro morphology and identification guides (Sutton, 1980)

Isolation of *Trichoderma* sp. from yam tuber surface

Isolation of species of *Trichoderma* was made from the tuber surface of white yams (*Discorea rotundata*) which were bought

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from Edaiken market, Benin City. Isolation was made using the method of Ikediugwu and Ejale (1980). The yam tuber was washed under tap water to dislodge adhering soil particles, the peels was obtained by scraping the outer brown skin which made up of periderm of the yam tuber. These were obtained from the head, middle and tail regions. It was then cut to sizes of about 3mm x 3mm and washed in 25 changes of sterile water. Both water and test tube were discarded for the first five washes and only water in the subsequent washes. The washed peels were then dried on sterile filter paper and plated out on PDA into which two drops of antibiotic mixture have been added. About five peels of the yam tuber were inoculated on each PDA plate. All culture plates were inoculated at room temperature (28 ± 2^0C) under light 10 hours photo-period for five days, after which isolation and identification were made using colony morphology, cell micro morphology and identification guides (Sutton, 1980) .

Screening for Sensitivity of Rot Pathogen to Antagonism by *Trichoderma* sp.

Agar disc inocula, 5mm in diameter, of *Trichoderma* sp. *Botryodioplodia theobromae, Penicillium oxalicum* and *Aspergillus niger* obtained from the edge of their respective actively growing three days old culture were incubated simultaneously on potato dextrose agar, *B theobromae* and *Trichoderma* sp. 2cm apart while *A niger, P oxalicum* and *Trichoderma* 3cm apart. Three replicate plate of each organism were incubated at room temperature under 10 hours photo period for up to five days. The growth of the sensitive organism toward (b) and away from *Trichoderma* sp. was measures each day until the zone of inhibition was established. Magnitude of inhibition was expressed as percentage difference between the growth of the sensitive organism away (a) and toward (b) *Trichoderma* sp. as described by Ferreina *et al.,* (1991).

Effect of *Trichoderma* sp. on Rot of Yam Tuber by Selected Rot Pathogen

Freshly harvested yam tuber were washed under running tap water and surface sterilized in 70% ethanol. Wells (5mm deep) were created using cork borer in the tuber, three replicates wells were made on tuber of about 25cm in length. Inoculations of the wells were separately made with two drops spore suspension of *A. niger* and *P. oxalicum*, either in sterile water or in potato dextrose broth. Mycelial fragments of *B. theobromae* were inoculated into the wells and 2 drops of sterile water or potato dextrose broth was added. Inoculated wells were then sealed with candle wax.

The following treatments were carried out:

i. *Trichoderma* alone
ii. Sterile water/broth suspension alone
iii Pathogen alone
iv. Pathogen + *Trichoderma* simultaneously
v. Pathogen + *Trichoderma* 1 day later
vi. Pathogen + *Trichoderma* 2 days later
vii. Pathogen + *Trichoderma* 3 days later

The treatments were carried out separately for each of the three pathogens, *A niger, P. oxalicum* and *B. theobromae*. Each of the treated tubers was incubated at room temperature for 21 days during which they were examined for rotting. The degree of rotting was assayed by measuring the extent of necrotic tissue. Rotted tissues aseptically removed from the cut-open surface of the

affected tuber were plated out on PDA for the re-isolation of the causal organism.

Pathogenicity of *B.theobromae* on Different Cultivars of White Yam

This experiment was prompted by the observation that some white yam cultivars rt faster than others when inoculated with the same pathogen. The virulence of *B. theobromae* was tested against two different tubers from Ondo and Edo states. Disks of each cultivar measuring 5.5cm in diameter and 2cm thickness were surface sterilized with 70% ethanol and washed in several changes of sterile water to reduce the microbial load. They were then placed in sterile Petri dish containing sterile tissue paper and sterile glass rod for support. 2mm x 2mm agar disc inoculum agar of three days actively growing *B. theobromae* was inoculated on the tissue disks. Three replicate of each cultivar of yam as well as control disks without inoculation were prepared. Each of the plates were incubated under light, (ten hours photo period) at room temperature for (28 ± 2^0C) three days. Measurement of surface mycelia growth of *B. theobromae* on the disks was taken each day and recorded.

RESULTS

The Fungi associated with rot of tubers were identified as *Aspergillus niger, Penicillium oxalicum* and *Botryodiplodia theobromae. Trichoderma sp* was isolated from the peel as shown in plate 1 and Table 1.

In the sensitivity test in agar-plate. Trichoderma sp. inhibited strongly the mycelial extension growths of all the three post harvest pathogen of yam tested. The percentage inhibition of the pathogen ranged from 52.2 % against *B. theobromae* to 17.7 % against *Apergillus niger* (Table 2). It causes 42.5 % inhibition on the growth of *P. oxalicum* (Table 2). Yellowish pigmentation was observe in agar plates on *A niger* and *P oxalicum* were paired respectively with *Trichoderma sp*. while brownish pigmentation was observed in agar plate in *B theobromae* and *Trichoderma sp*. were paired.

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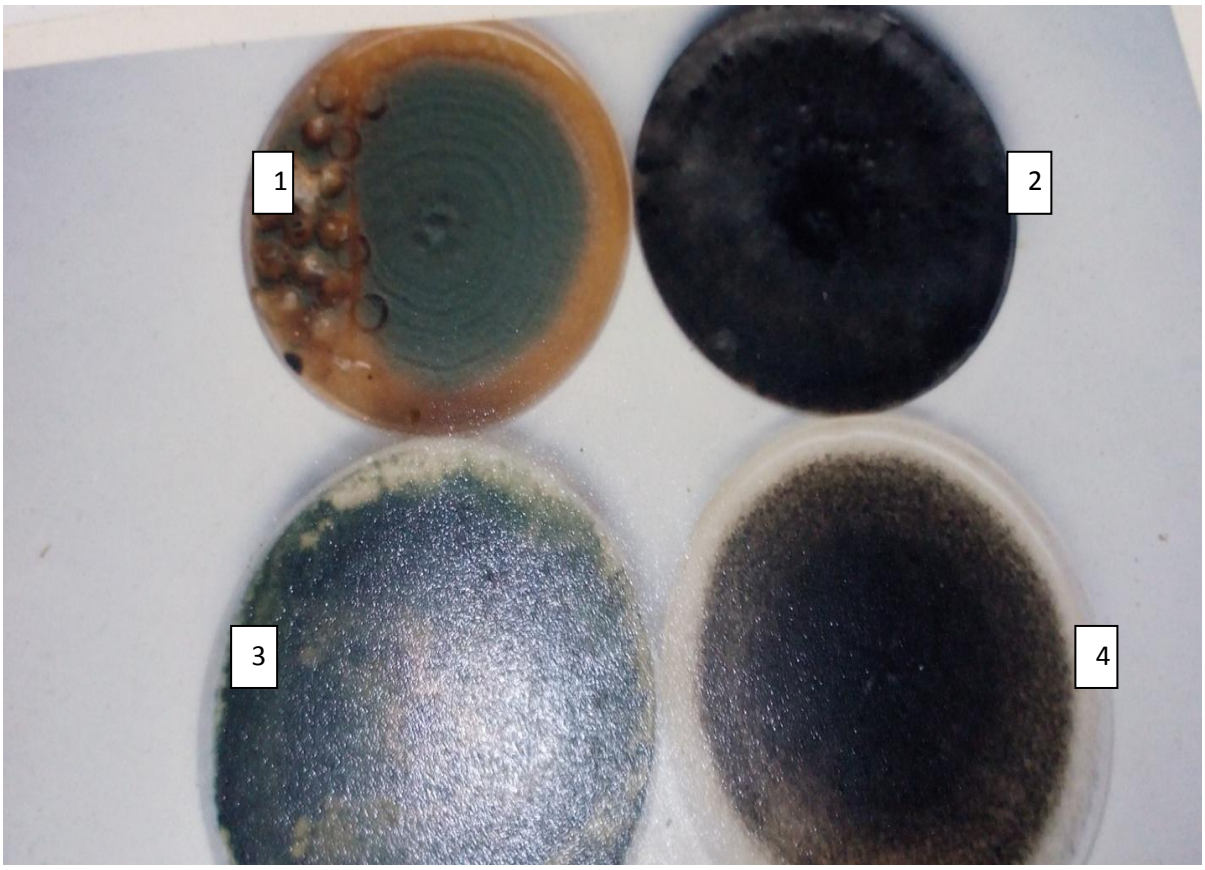

Plate 1: Colony morphology of microorganism isolated
1: *Trichoderma sp*. 2 : *B theobromae*, 3 : *P oxalicum*, 4: *A niger*

Table 1: Cultural characteristics of Fungal Isolates

Cultural characteristic	Probable genus
Dark brown surface having yellow cracked reverse	*Aspergillus* niger
Blue-green velvet surface and light reverse	Penicillium *oxalicum*
Dark green mold surface	*Trichoderma* spp
Dark grey fluffy surface having black smooth reverse	*B. theobromeo*

Table 2: Sensitivity of Rot Pathogen to *Trichoderma sp*. Antagonism

Botrydioplodia sp. vs. *Trichoderma* sp.

Time (Days)	Replicate	a(mm)	b(mm)	a- b = x	$^{x}/_{a}$* 100%	X±δx (%)
1	1	9	9	0	0	
	2	15	10	5	33.0	
	3	13	9	4	30.8	21.4± 15.14
2	1	20	12	8	40.0	
	2	30	13	17	56.7	
	3	25	10	15	60.0	52.2 ±8.75
Aspergillus sp. vs. *Trichoderma* sp.						
1	1	5	4	1	20	
	2	5	4	1	20	
	3	4	4	0	0	13.33 ± 9.43
2	1	8	6	2	25	
	2	9	7	2	25	
	3	8	7	1	12.5	20.83 ± 5.89
3	1	9	7	2	22.2	
	2	10	8	2	20.0	
	3	9	8	1	11.1	17.77 ± 4.79
Penicillium sp. vs *Trichoderma* sp.						
1	1	6	4	2	33.3	
	2	8	7	1	12.5	
	3	4	4	0	0	13.3± 13.7
2	1	11	7	4	36.4	
	2	13	8	5	38.5	
	3	6	5	1	6.7	30.5 ± 9.8
3	1	15	6	9	60.0	
	2	15	7	8	53.3	
	3	7	6	1	14.3	42.5± 20.2

key: a = growth of pathogen away from *Trichoderma*
b = growth of pathogen towards *Trichoderma*
Note: ≤ 0% inhibition (not effective), > 0 - 20% inhibition (slightly effective), > 20 - 50 inhibition (moderately effective), > 50 - 100% inhibition (effective), and 100% inhibition (highly effective).

The effect of *Trichoderma* sp. on rot of yam tuber by selected rot pathogen after 3 – 6 weeks incubated at room temperature was observed as discoloration and softening of tissues. Yam tuber inoculated with specific rot pathogen for 3 – 6 weeks show different

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degree of rot when the tubers were cut open. The rot which generally were mild ranging in width from 16.2 mm (control) to 8.8 mm for inoculation of *A niger* and *Trichoderma* sp. simultaneously (Table 3). Inoculation with *B theobromae* alone gave rot of width 9.3 mm in compares to 8.3 mm when the pathogen was inoculated simultaneously with *Trichoderma* (Table 4). The result shows that the presence of Trichoderma sp. decreased the rot induced by *A niger* and *B theobromae* but these decreased was found statistically insignificant p (0.05).

Inoculation involving *P oxalicum* and *Trichoderma* using cultivar of yam from Edo State instead of Ondo State as with *A niger* and *B theobromae* gives the greatest rot of 55.7 mm P oxalicum alone in contrast to 25.7 mm and 26.3 mm respectively for *P oxalicum* and *Trichoderma* sp. incubated one day later (Table 5). This suggests that cultivars of white yam vary in their susceptibility to rot pathogens. However, when the inoculation of *Trichoderma* was delayed for two days following the inoculation of *P oxalicum* the degree of rot (40.0 mm) was comparable to that of *P oxalicum* alone. The degree of rot were comparable in respective of whether the inoculum was suspended in water or PDA broth suggesting that the pathogen derive its' nutrient from wounded yam tuber tissue.

Table 3: Mean Length of Rotted Yam Tuber by *A niger* in the presence of *Trichoderma* sp.

Test Pathogen	Regions	length(broth) (mm)	Mean (mm)	Length (water)(mm)	Mean(mm)
Aspergilus	Tail	10		15	
	Middle	11		17	
	Head	11		15	
	Tail	11		30	
	Middle	12		10	
	Head	11	11	10	16.2
Aspergilus	Tail	12		6	
Trichoderma	Middle	17		10	
Simultaneously	Head	14		6	
	Tail	13		8	
	Middle	10		13	
	Head	10	12.7	10	8.8
Aspergilus	Tail	12		12	
Trichoderma	Middle	11		14	
1 day later	Head	11		11	
	Tail	10		12	
	Middle	10		8	
	Head	7	10.2	12	11.5
Aspergilus	Tail	13		10	
Trichoderma	Middle	11		14	
2 day later	Head	16		12	
	Tail	15		12	
	Middle	16		25	
	Head	23	15.7	22	15.8
Aspergilus	Tail	17		12	
Trichoderma	Middle	14		18	
3 day later	Head	11		14	
	Tail	17		10	
	Middle	17		17	
	Head	16	15.2	14	14.2

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Table 4:Mean length of Rotted yam tuber by *B theobromae* in the presence of *Trichoderma* sp.

Test Pathogen	Regions	length(broth) (mm)	Mean (mm)	Length (water) (mm)	Mean (mm)
Botrydioplodea	Tail	10		9	
	Middle	10		19	
	Head	13		9	
	Tail	10		10	
	Middle	10		8	
	Head	14	11.2	10	9.3
Botrydioplodea	Tail	11		8	
Trichoderma	Middle	15		9	
Simultaneously	Head	9		7	
	Tail	12		6	
	Middle	7		9	
	Head	12	10.7	10	8.2
Botrydioplodea	Tail	10		10	
Trichoderma	Middle	12		9	
1 day later	Head	11		15	
	Tail	8		7	
	Middle	10		9	
	Head	8	9.8	11	10.2
Botrydioplodea	Tail	12		25	
Trichoderma	Middle	15		8	
2 day later	Head	11		9	
	Tail	8		10	
	Middle	22		11	
	Head	6	12.3	11	12.3
Botrydioplodea	Tail	8		9	
Trichoderma	Middle	9		9	
3 day later	Head	10		9	
	Tail	12		7	
	Middle	11		8	
	Head	11	10.2	8	8.3

Table 5: Mean length of rotted of yam tuber by *P oxalicum* in the presence of *Trichoderma sp.*

Test Pathogen	Regions	length(broth) (mm)	Mean (mm)
Penicillium	Tail	67	
	Middle	56	
	Head	44	55.7
Pencillium	Tail	17	
Trichoderma	Middle	23	
Simultaneously	Head	37	25.7
Pencillium	Tail	26	
Trichoderma	Middle	26	
1 day later	Head	27	
Pencillium	Tail	56	
Trichoderma	Middle	33	
2 day later	Head	31	40

The pathogenicity of *B theobromae* on two different cultivars of white yam appears to differ in their susceptibility to rot pathogens. The mycelia extension growth of *B theobromae* were compared in tuber tissues of two cultivars of white yam , one from Edo State and the other from Ondo State. The growth of the Edo State was found to be approximately twice as fast (51.5mm) as on the Ondo cultivar (25.0mm) (Figure 1) suggesting that the former cultivar was more susceptible to the pathogen than the later.

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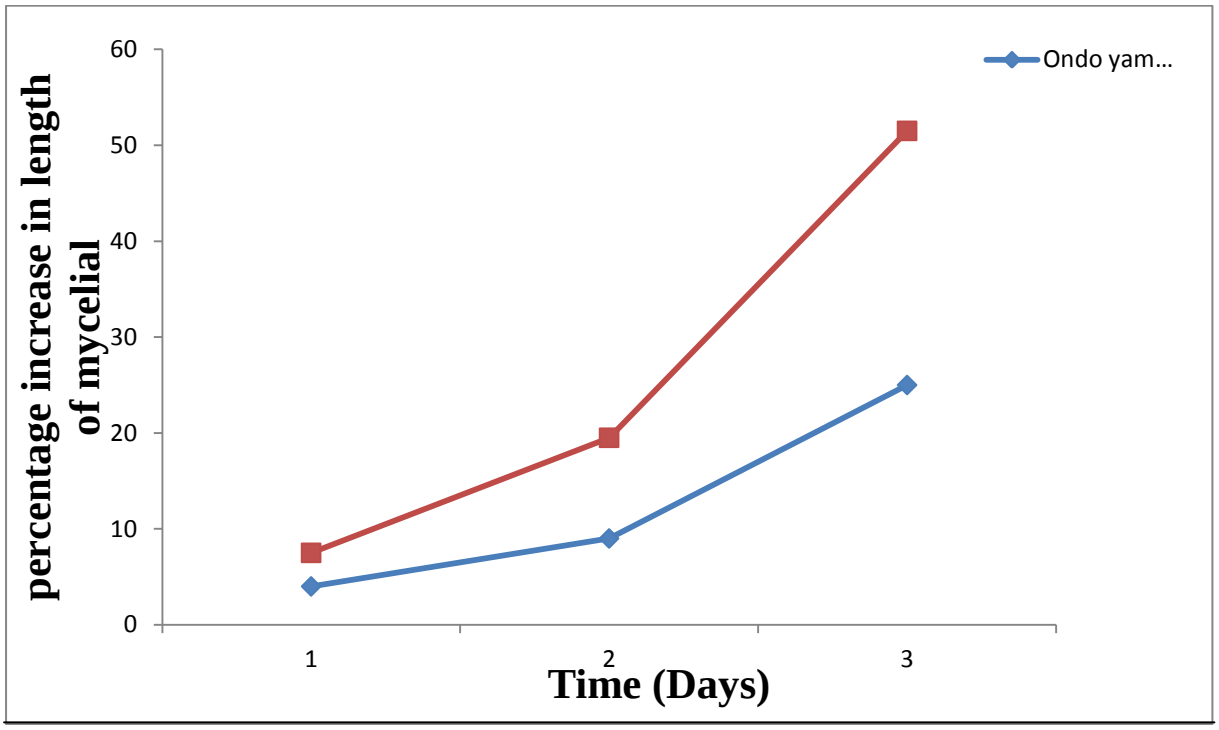

Fig :1 Mycelial extension growth of *B theobromae* on tissue discs of two Cultivars of white yam (*Dioscore rotundata*)

DISCUSSION

Results from this study demonstrated that when the rot pathogens, *B theobromae*, *A niger* and *P oxalicum* were inoculated either simultaneously or not later than one day following the inoculation of the pathogen, rot incited on the yam tubers was ameliorated. Rot inhibition in Ondo yam involving *B theobromae* and *A niger* with *Trichoderma* sp. were not statistically significant. Inhibition in Edo yam involving P. oxalicum with Trichoderma sp. was significant. The results indicate that the use of *Trichoderma* sp. in the control of Post harvest yam tuber is a viable project. Okigbo and Ikediugwu (2000) demonstrated an effective biological control of post harvest rot of yam tuber using *Trichoderma viride* in storage.

The result of the present investigation suggests that an on-going post harvest rot infection can be controlled using the strains of *Trichoderma* sp. Species of *Trichoderma* have been reported to be strong antagonist against a number of fungi (Okigbo 1994). They are also known to produce volatile and non volatile antimicrobial substance in-vitro and the present bio-control effect might have occurred through these antimicrobial agents. The result also shows that different cultivars of yam vary in their susceptibility to the same pathogen.

Trichoderma sp. have a suppressive effect on the rot incited by *B theobromae*, *A niger* and *P oxalicum* when inoculated into yam tuber either simultaneously or with advance inoculation of the pathogen, although different cultivars of white yam have varying level of susceptibility to the rot pathogens.

Trichoderma sp. has the capacity to control on-going rot of tubers therefore it can be used as a bio-control agent of post harvest

rot of yam tubers. It is recommended that individuals should spray their yam tubers with spore suspension of *Trichoderma* sp. for protection against rot of their yams.

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