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Fungal Organisms Isolated from Rotted White Yam (*Dioscorea rotundata*) Tubers and Antagonistic Potential of *Trichoderma harzianum* against *Colletotrichum Species*

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Summary

Nine fungal organisms were isolated from rotted white yam (*Dioscorea rotundata*) tubers from Kadarko, Nasarawa, Nigeria between February and May, 2015. *Trichoderma harzianum* was used as a biological agent to evaluate its potential against *Colletotrichum* species isolated from rotted white yam tubers in dual culture method at Advanced Plant Pathology Laboratory, Federal University of Agriculture, Makurdi, Nigeria. Percentage frequency of occurrence of the isolated fungal organisms was determined at monthly interval. The test antagonist (*T. harzianum*) was introduced at three different times (same time with *Colletotrichum spp*, two days before the inoculation of *Colletotrichum spp* and two days after the inoculation of *Colletotrichum spp*). The plates were incubated for 192 hours and measurements of mycelial radial growths were recorded at intervals of 24 hours beginning from the third day. The results of the in vitro interactions between *T. harzianum* and *Colletotrichum spp* revealed that *T. harzianum* was able to significantly ($P \leq 0.05$) inhibit the growth of *Colletotrichum spp*. Mean percentage growth inhibition was found to be highest (50.61%) when *T. harzianum* was introduced 2 days before inoculation of Colletotrichum spp followed by introduction of *T. harzianum* same with *Colletotrichum spp* (37.12%) with the least percentage growth inhibition (12.48%) recorded when the antagonist was introduced 2 days after inoculation of the pathogen. Minimum inhibition concentration (MIC) showed slightly effective to effective control indicating that the antagonist has the potential for biological control of *Colletotrichum spp* at all levels. It is therefore, recommended that the biological agent should be introduced prior to infection in order to be more effective in controlling the pathogenic fungus.

Keywords: Inoculation; Incubation; Inhibition; Effective; Biological agent

Introduction

Yam production is constrained by several factors including those caused by the genus *Colletotrichum* [1,2], causing about 90% loss in yield. The genus Colletotrichum was recently voted the eighth most important group of plant pathogenic fungi in the world, based on perceived scientific and economic importance [3].

The genus *Colletotrichum* includes a number of plant pathogens of major importance, causing diseases of a wide variety of woody and herbaceous plants. It has a primarily tropical and subtropical distribution, although there are some high-profile species affecting temperate crops [4].

The genus *Colletotrichum* affects the leaves, petioles, stems and veins of the plant and even tubers, causing leaf spots, leaf blotches, petiole blights, premature abscission, dieback and eventual death of the entire plant [1,2,5]. The disease usually has a dramatic effect on infected plants, converting a field of initially healthy yam plants from 'green' to 'black' within a few weeks [6]. Other fungal organisms commonly associated with yam rots include *Aspergillus flavus, Aspergillus niger, Botryodiplodia theobromae, Colletotrichum spp, Fusarium oxysporum, Penicillium chrysogenum, Pennicillium digitatum [2,5,7,8]. Colletotrichum* species are occasionally identified in yam tubers

[9]. Several methods of control of fungal organisms have been used. Chemical control of soil borne pathogens provides certain degree of control but at the same time have adverse effects on environment affecting the beneficial soil microorganisms [10]. Therefore, biological control of plant pathogens has been considered as a potential control strategy in recent years and search for these biological agents is increasing. *Trichoderma* is the most commonly used fungal biological control agent and have long been known as effective antagonists against plant pathogenic fungi [11,12]. The possibility of applying *Trichoderma* harzianum as a biocontrol agent of *Colletotrichum spp.* causing yam dry rot in Nigeria has not been reported. This research describes experiments conducted to determine the effect of dual culture of introduced at different intervals on mycelia inhibition of *Colletotrichum spp.* isolated from rotted yam tubers in Nigeria.

Materials and Methods

Experimental site

The experiment was conducted at the Advanced Plant Pathology Laboratory, Federal University of Agriculture, Makurdi, Nigeria.

Source of *T. harzianum* isolate

T. harzianum was collected from yam Pathology Unit of University of Ibadan, Oyo State, Nigeria. Stock cultures of the isolate were maintained on slants of acidified potato dextrose agar (PDA) in McCartney bottles for subsequent studies.

Collection of rotted and healthy yam tubers

Deteriorated white yam tuber (*Dioscorea rotundata*) cultivars of *Ghini* and *Hembankwase* showing varying degrees of rots were collected from yam barns in Kadarko, Keana local government area of Nasarawa State, Nigeria, which lies between longitude 8º 30' and 8º 35' E, and on latitudes 8º 10' and 8º 14' N. The rotten yam tubers were packaged in sterile polyethylene bags and taken to the laboratory for isolation and identification of fungal organisms two days after collection. The healthy yam tubers were used for pathogenicity test. Test isolate in this study was *Colletotrichum spp.*

Preparation of Potato dextrose agar (PDA)

Potato dextrose Agar (PDA) was prepared according to manufacturer's recommendations by dissolving 39g of dehydrated PDA in 1 litre of distilled water and autoclaved at 121 °C for 15min [13] and medium was allowed to cool to 45-50 °C. About 0.16g/l streptomycin sulphate powder was added to suppress bacterial contaminations [14]. 15ml of the molten PDA was poured into sterile 9cm glass Petri dishes and were allowed to cool at room temperature before inoculation of the test organisms.

Isolation and identification of fungi organisms

Deteriorated yam tubers were cut approximately 2 x 2mm with sterile scalpel from at the inter-phase between the healthy and rotted portions of the tubers. The cut pieces were first surface sterilized by dipping completely in a concentration of 5% sodium hypochlorite solution for 2min; and rinsed in four successive changes of sterile distilled water (SDW) [15]. Infected yam tissues were later picked onto sterile filter paper using a sterile forceps and then blotted with filter paper for 2-3 minutes in the laminar Air flow cabinet. The dried infected tissues were aseptically plated on Petri dishes containing acidified sterile potato dextrose agar (PDA) and the plates were incu¬bated at ambient room temperature (30±5 °C) for 7 days. Fungi organisms were identified following sub-culture of growing fungus after 7 days of incubation [16]. The culture plates obtained were examined and spores were collected from distinct growths. This was inoculated onto sterile PDA plates and incubated. When pure cultures were obtained, the growth pattern was examined for uniformity. Microscopic examination and morphological characteristics were noted and compared with existing authorities [14,17].

Determination of frequency of occurrence of isolates

To determine the frequency of occurrence of the isolates, records of organisms isolated were kept on periodic basis. Since isolation and characterization were carried out at monthly interval, the number of times each fungi pathogen was isolated in a month was expressed as a percentage of the total of all the different organisms over the period [18], which was calculated as follows:

$$\%\ \text{frequency of occurrence} = \frac{x}{n} \times \frac{100}{1}$$

Where,

x = number of times of occurrence of the individual isolates over the period

n = total number of micro organisms isolated in the study over the period

Stock cultures of the isolates were maintained on slant of acidified potato dextrose agar (PDA) in McCartney bottles for subsequent studies.

Pathogenicity test

Some healthy yam tuber (*Discorea rotundata*) cultivars were washed under running tap water to remove soil (dirt). Surface contaminants were removed by dipping each yam tuber in 5% sodium hypochlorite solution for 2min and rinsed in four successive changes of sterile distilled water (SDW) [15]. A disc of five days old culture of the fungal isolates obtained from rotted yam tubers grown on PDA was the source of inoculum for the pathogenicity test. A five-millimeter diameter cork borer was used to remove 4mm tissue from the healthy *D. rotundata* tuber surfaces aseptically [19]. A five-millimeter diameter cork borer (Sterilized by dipping in alcohol followed by flaming) was used to cut plugs from the five day old cultures of *Colletotrichum spp.*

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to be tested. These fungal plugs were put in the holes created in the yam tubers. Petroleum jelly was used to seal the edges of the replaced yam tissues [12]. The same procedure was used for the control except that discs of uninoculated PDA were placed in the holes created in the tubers [20]. The inoculated yam tubers were placed at room temperature in a completely randomized design [21] for 14 days under sterile condition to determine rot. Rot was determined by using a sterilized and flamed knife to cut open the inoculated yam tubers from the point of inoculation.

Evaluation using dual culture on agar plates

The assay for antagonism was performed on Potato Dextrose Agar (PDA) on Petri dishes by the dual culture method [22]. The mycelial plugs (5mm diameter) of *T. harzianum* used as antagonist and *Colletotrichum spp.* isolated from rotted yam tubers were both inoculated on the same dish with *T. harzianum* 6cm from *Colletotrichum spp. T. harzianum* was plated same time with the pathogen, two days before the inoculation of pathogen and two days after inoculation of pathogen on Petri dishes containing 15ml of PDA. Paired cultures were incubated at room temperature (30±5 °C) for 192 hours. In the control plates, a PDA plug was used instead of the antagonist against the pathogen. Treatments comprised times of introduction of antagonist which were replicated three times for each treatment and arranged in completely randomized design [21].

Measurement of radial mycelia growth

Measurement of radial mycelia growths of the *T. harzianum* and *Colletotrichum spp.* in dual culture and *Colletotrichum spp.* in control plates were done after three days of inoculation on a 24 hour interval beginning from the 72nd hour up to the 192nd hour of incubation at ambient room temperature (30± 5 0C). Percentage Growth Inhibition (PGI) of *Colletotrichum spp.* was calculated as described by [23].

$$PGI(\%) = \frac{R - R_1}{R} \times 100$$

Where,

PGI = Percent Growth Inhibition

R = the distance (measured in mm) from the point of inoculation to the colony margin in control plate,

R1 = the distance of fungal growth from the point of inoculation to the colony margin in treated plate in the direction of the antagonist.

The percent growth inhibition was determined as a guide in selecting the minimum inhibition concentration (MIC) that will be effective in controlling the rot-causing fungus for the three treatments. Antagonist was also rated for inhibitory effects using a scale by Sangoyomi [24] as:

- ≤0% inhibition (not effective),
- >0-20% inhibition (slightly effective)
- >20-50% inhibition (moderately effective),
- >50-<100% inhibition (effective)
- 100% inhibition (highly effective)

Data analysis

Data collected were subjected to Analysis of variance (ANOVA) using GenStat Discovery Edition 12 for ANOVA and means separation, Minitab Release 17 for descriptive statistics and Graph Pad Prism 6 for trend graphs. Statistical F-tests were evaluated at P≤0.05. Differences among treatment means for each measured parameter were separated using Fisher's least significant difference (FLSD) [25].

Results

Sample collection, isolation and identification of *Colletotrichum species*

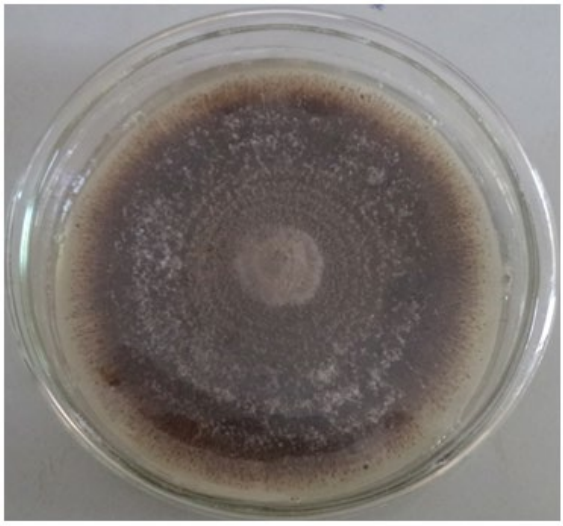

Figure 1: Culture of *Colletotrichum spp* on PDA.

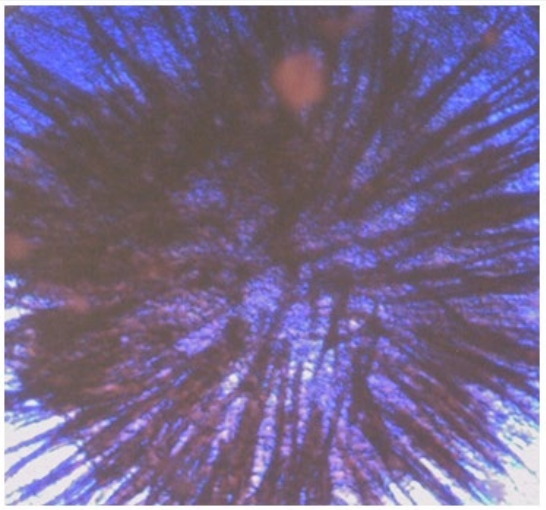

Figure 2: Micrograph showing Acervulus with setae of *Colletotrichum spp* (×10).

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Fungi organisms were isolated and identified from the rotted yam tubers. *Colletotrichuim spp*. was one of the fungi organisms identified as causing dry rot of white yam (*D. rotundata*) tubers in the study area. Macroscopic examination of pure cultures of this fungus on PDA showed slow growth rate taking more than 7 days to fill the plates (Figure 1). The colour of *Colletotrichum spp.* was grey, to dark orange while the reverse side of the growth pattern was either circular with the mycelia showing a uniform growth pattern and radial in a ring. Acervuli were rounded or elongate, separate or confluent, superficial, erumpent, with conspicuous multicellular, darkly pigmented setae (Figure 2). Setae were brown with a dark swollen base and a pale rounded tip dark brown colour.

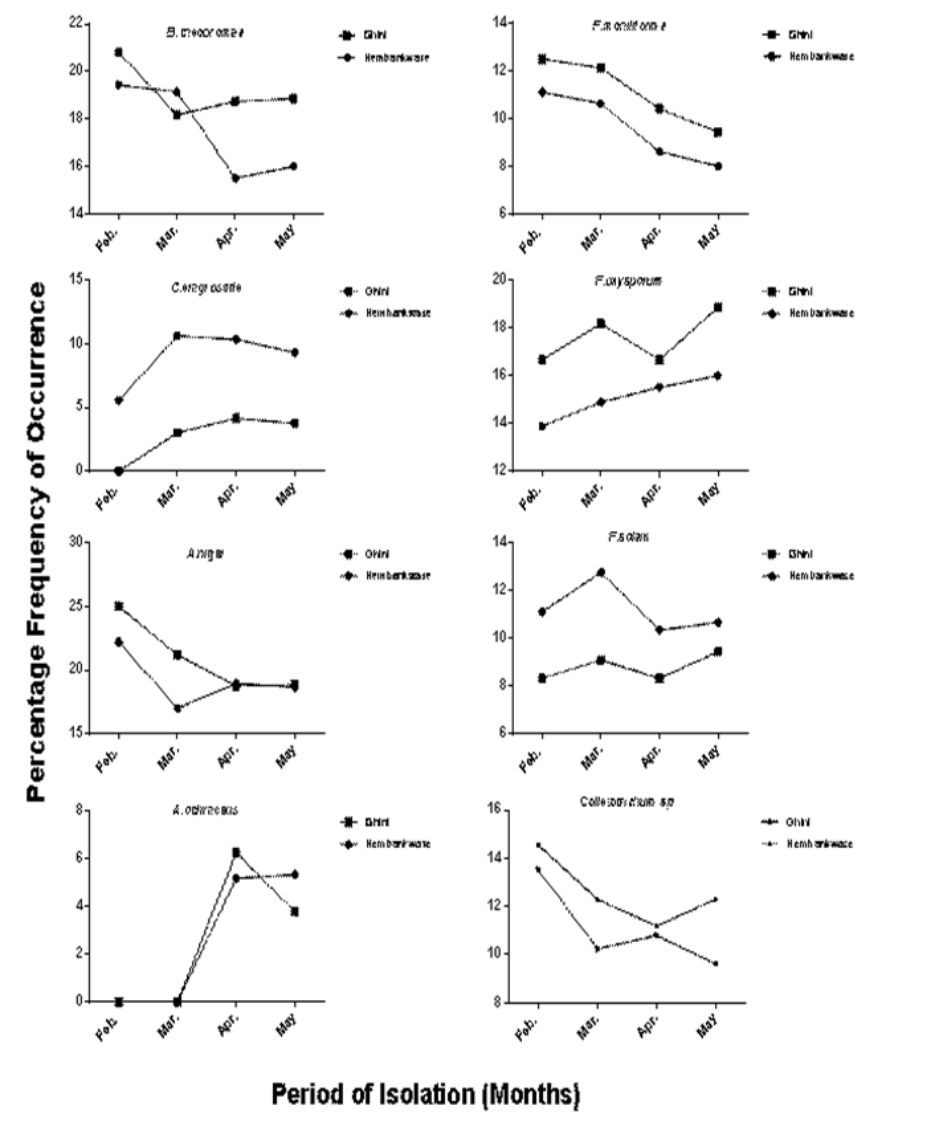

Figure 3: Percentage frequency of occurrence of fungal isolates from Hembankwase and Ghini white yam tuber cultivars from February to May 2015 in Kadarko.

Table 1: Variation of mean percentage frequency of occurrence of fungal isolates from Ghini and Hembankwase cultivars of white yam tuber after four months of isolation in Kadarko.

Pathogens	White Yam Cultivar		T-Value	P-Value
	Ghini	Hembankwase		
B. theobromae	18.66±0.73	16.48±1.01	1.75	0.14
A. flavus	16.66±0.35	16.82±0.38	3.55	0.01*
A. niger	20.44±1.63	18.07±1.07	1.22	0.27
A. ochraceus	2.41±1.47	2.46±1.42	-0.02	0.98
F. moniliforme	10.83±0.78	9.02±0.73	1.69	0.15

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F. oxysporum	17.12±0.48	14.16±0.39	4.71	0.01*
F. solani	8.56±0.24	10.55±0.51	-3.50	0.02*
C. eragrostide	2.64±0.90	8.42±1.09	-4.08	0.01*
Colletotrichum spp.	2.64±0.90	5.99±0.26	-3.54	0.03*

*indicates statistical significance at (P≤0.05)

Percentage frequency of occurrence of fungal isolates in Kadarko

Figure 3 shows fungi organisms isolated and identified on rotted *Hembankwase* and *Ghini* cultivars of white yam tuber samples collected from farmers' barns in Kadarko, which is one of the yam producing settlements in Nasarawa State, Nigeria. The fungi organisms include *Botryodiplodia theobromae, Aspergillus flavus, A. niger, A. ochraceus, Fusarium moniliforme, F. oxysporum, F. solani, Curvularia eragrostide* and *Colletotrichum spp.* Percentage frequence of occurrence of *A. ochraceus* showed that it was same in *Hembankwase* and *Ghini* in February and March. The occurrence of the pathogen increased in *Ghini* compared with *Hembankwase* in April but decreased in *Ghini* compared with *Hembankwase* in May. The occurrence of A. niger in *Hembankwase* was less than in *Ghini* in February and March but recorded an increase in *Hembankwase* slightly above *Ghini* in April. The difference in frequency of occurrence was nearly the same in *Ghini* which rose slightly above *Hembankwase* in May. *C. eragrostide, F. solani* and *Colletotrichum spp.* occurred higher in *Hembankwase* compared with *Ghini* throughout the period of isolation. In *F. oxysporum* and *F. moniliforme,* the frequency was higher in *Ghini* compared with *Hembankwase* within the same period. *B. theobroma*e showed the highest level of occurrence in *Hembankwase* than in *Ghini* in February, April and May while the pathogen displayed higher frequency of occurrence in *Ghini* only in March. Occurrence of *A. flavus* in *Ghini* and *Hembakwase* was the same in February but increased in *Ghini* far above *Hembankwase* in March and decreased in April and May but was still above Hembankwase. Mean percentage frequency of occurrence of *B. theobromae, A. niger, A. ochraceus, F. moniliforme,* between *Hembankwase* and *Ghini* in Kadarko after four months of isolation showed no significant difference (P≤0.05) between the two cultivars. However, there were significant differences (P≤0.05) in mean percentage frequency of occurrence between *Ghini* and *Hembankwase* in *A. flavus*, *F. oxysporum, F. solani, C eragrostide* and *Colletotrichum sp.* after four months of isolation (Table 1).

Pathogenicity test

The results of the pathogenicity test of *Colletotrichum spp* is presented in Figure 4. The pathogenicity test shows that *Colletotrichum spp* induced rot in the healthy looking yam tubers after 14 days of inoculation. Symptoms of infections were seen on the inoculated yam tubers. The yam tubers that were not inoculated with the test fungi used as control experiments however, did not show any sign of rot indicating absence of reproductive propagules in the bored yam tissues (Figure 5)

Figure 4: Rot caused by *Colletotrichum spp.*

Figure 5: Control (no organism inoculated).

Evaluation using dual culture method on agar plates

Results of dual culture method used indicated that *T. harzianum* inhibited the growth of Colletotrichum spp at varying degrees. The dual culture interactions showed significant success in biocontrol of *Colletotrichum spp.*, under in vitro conditions. Result of the study revealed that *T. harzianum* exhibited antagonistic activities on Colletotrichum spp in all the treatments. The potential antagonist was seen to grow faster than the fungus in the dual culture. The control plates also grew much faster when compared with their respective dual cultures. *Colletotrichum spp.* had its growth stopped either when the antagonist was introduced same time with it (Figure 6) or when

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was introduced 2 days before inoculation of *Colletotrichum spp.* (Figure 7), in these interactions, the antagonist continued with its growth uninhibited and over grew the pathogen resulting into complete degradation of the fungus and sporulation of the antagonist over the entire plate. When *T. harzianum* was introduced 2 days after the inoculation of the pathogen, there was a clear zone of inhibition (Figure 8). Table 2 shows the percentage growth inhibition of Colletotrichum spp increases with increase in time of incubation from 72 hours to 192 hours irrespective of the treatment. When *T. harzianum* was introduced same time with Colletotrichum spp, it was observed that the percentage growth inhibition of the fungus in dual culture with *T. harzianum* increased steadily from 24.94% at 72 hours to 54.06% at 192 hours respectively. A similar trend was recorded when *T. harzianum* was introduced two days before inoculation of the pathogenic fungus, with percentage growth inhibition of 27.80% at 72 hours to 80.93% at 192 hours respectively. Percentage growth inhibitions rose from 16.03% at 72 hours to 24.04% at 192 hours when *T. harzianum* was introduced two days after inoculation of *Colletotrichum spp.* Duration of incubation for each of the treatments showed significant differences at 24 hour interval. Mean variation of percentage growth inhibition of *Colletotrichum spp.* tested at different times of introduction of *T. harzianum* significantly (P≤0.05) inhibited the growth of Colletotrichum spp (Table 2).

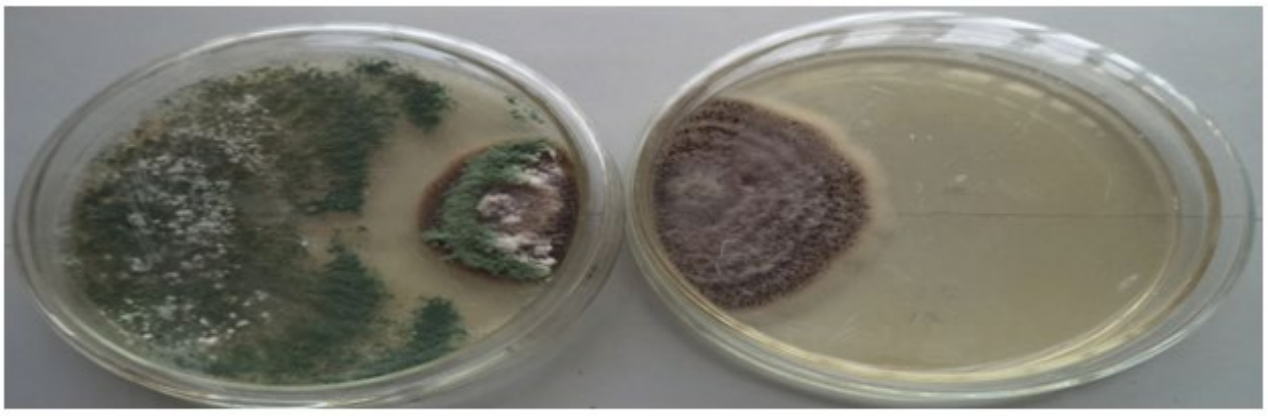

Figure 6: Dual culture of *T. harzianum* and *Colletotrichum spp*. on potato dextrose agar inoculated same time (Th×path) (left) and pure culture of *Colletotrichum spp*. as control (right).

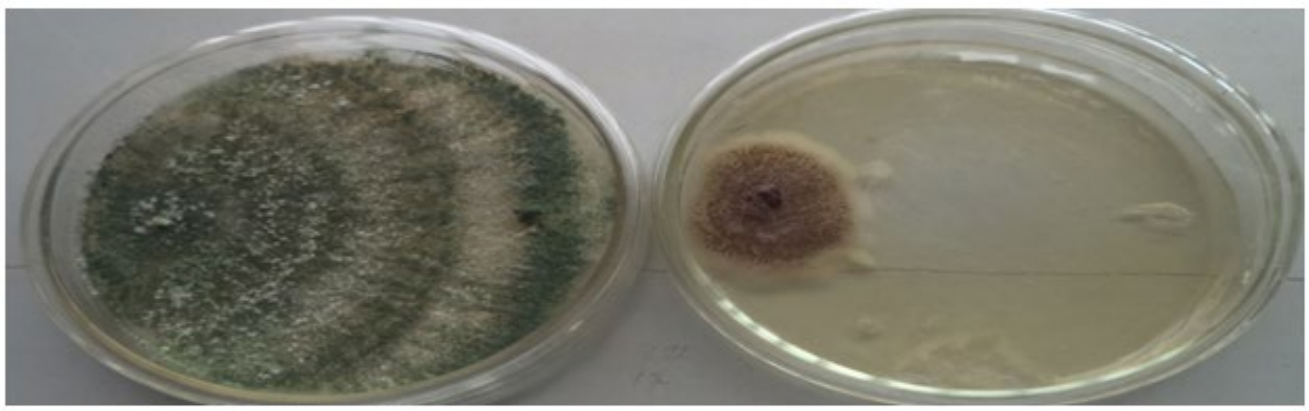

Figure 7: Dual culture of *T. harzianum* and *Colletotrichum spp.* on potato dextrose agar (left); *T. harzianum* was introduced 2days before inoculation of *Colletotrichum spp*. (2dbi) and pure culture of *Colletotrichum spp.* as control (right).

Table 2: *In vitro* Percentage Growth Inhibitions (PGI) of *Colletotrichum spp.* at different times of introduction of *T. Harzianum.*

Duration of Incubation (Hours)	Time of Introduction of *T. harzianum*		
	ThXPath	Th2dbiPath	Th2daiPath
72	24.94±6.76^{cd}	27.80±14.70^{cd}	16.03±5.01^{ab}
96	19.77±2.63^{d}	11.11±5.56^{d}	8.26±1.91^{bc}
120	32.93±2.49^{bc}	45.96±6.45^{bc}	4.94±0.01^{c}
144	41.34±4.37^{ab}	62.70±2.86^{ab}	9.28±3.57b^{c}

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168	50.86±3.56[a]	75.20±0.94[a]	18.98±2.58[a]
192	54.06±3.52[a]	80.93±0.64[a]	24.04±2.50[a]
LSD	*12.77*	*26.71*	*9.16*
Mean(LSD= 12.48)	37.32±3.38[b]	50.61±6.54[a]	12.48±1.97[c]

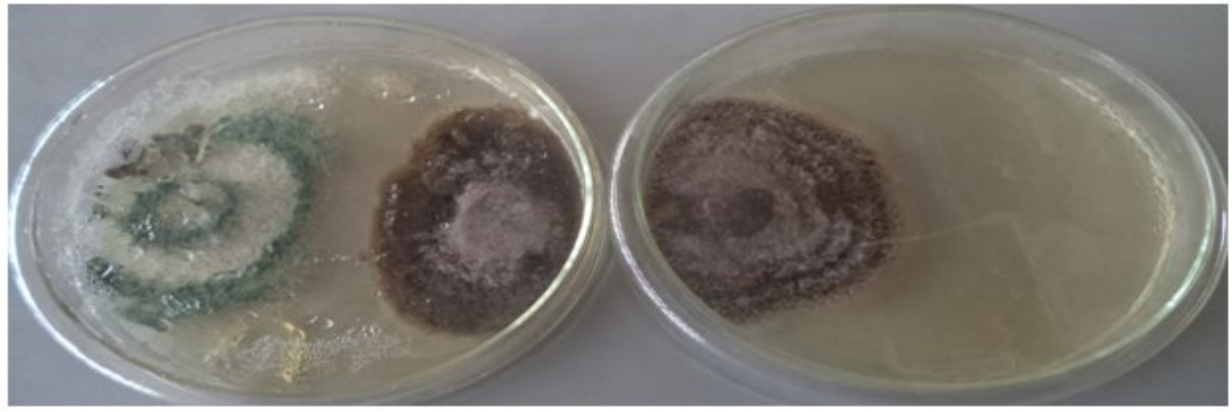

Figure 8: Dual culture of *T. harzianum* and *Colletotrichum spp.* on potato dextrose agar (left); *T. harzianum* was introduced 2days after inoculation of *Colletotrichum spp.* (2dai) and pure culture of *Colletotrichum spp.* as control (right).

Means on the same column with the same superscript are not statistically significant (P≤0.05). Means on the same row (for Mean) with the same superscript are not statistically significant (P≤0.05) by time of introduction of *T. harzianum*. Th×path = T. harzianum introduced same time with pathogen; Th2dbipath = *T. harzianum* introduced 2 days before inoculation of pathogen; Th2daipat = *T. harzianum* introduced 2 days after inoculation of pathogen.

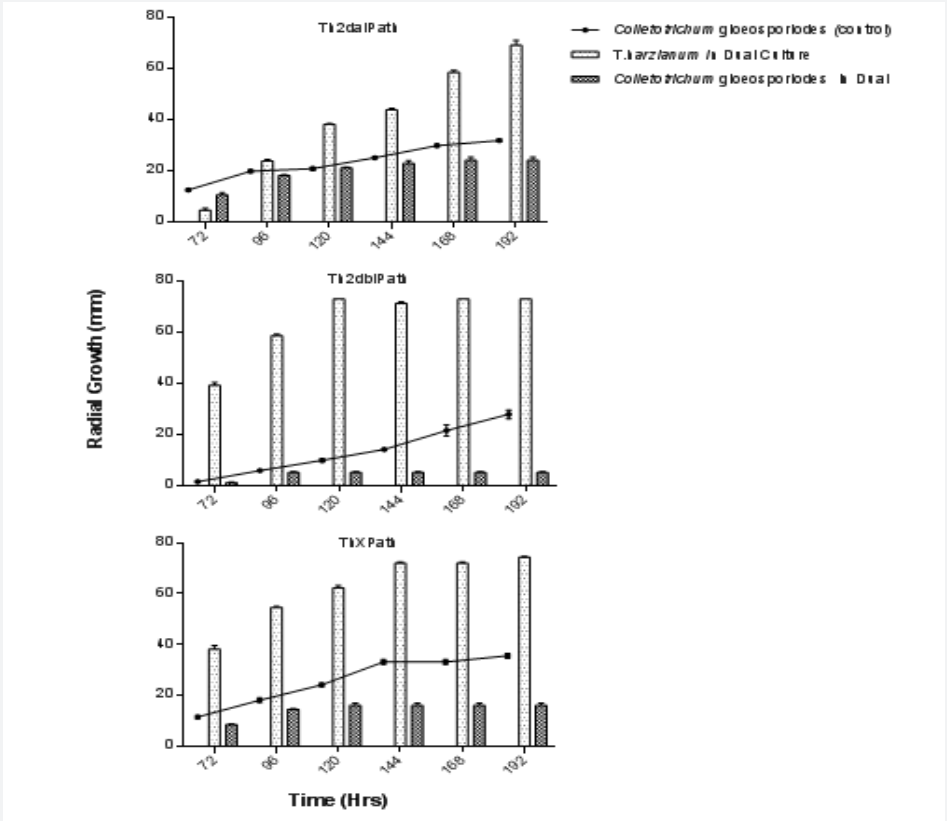

Figure 9: Radial growth of *Colletotrichum spp*. in dual culture with *T. harzianum.* Th×path = *T. harzianum* introduced same time with pathogen; Th2dbipath = *T. harzianum* introduced 2days before inoculation of pathogen; Th2daipath = *T. harzianum* introduced 2days after inoculation of pathogen.

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Radial mycelia growth and determination of inhibition

Radial mycelia growth of *T. harzianum* and Colletotrichum spp in dual culture and *Colletotrichum spp.* in control plates for each of the treatments were measured from 72 hours to 196 hours of incubation. Figure 9 shows that growth was rapid in the control plates than in the dual culture plates in all the treatments. It was also found that *T. harzianum* grew much faster than Colletotichum spp in all the treatments in dual culture thereby inhibiting the growth of the fungus.

Effectiveness levels of *T. harzianum* were found to be slightly effective to effective and significant (P≤0.05) across treatments (Table 3).

Table 3: Mean Percentage growth inhibition of *Colletotrichum spp.* treated with *T. harzianum* at different times showing minimum inhibition concentration (MIC).

Time of Introduction of T. harzianum	Percentage Growth Inhibition (PGI)	MIC (%)	Level of Effectiveness
ThXPath	37.32.±3.38[b]	>20-50	Moderately effective
Th2dbiPath	50.61±6.54[a]	>50-<100	Effective
Th2daiPath LSD	12.48±1.97[c] 12.48	>0-20	Slightly effective

Th×path = *T.harzianum* introduced same time with pathogen; Th2dbipath = *T.harzianum* introduced 2 days before inoculation of pathogen; Th2daipath = *T. harzianum* introduced 2 days after inoculation of pathogen; MIC = minimum inhibition concentration (%); ≤0% inhibition (not effective); >0-20% inhibition (slightly effective); >20-50% inhibition (moderately effective); >50-<100% inhibition (effective); 100% inhibition (highly effective)

Discussion

Fungal pathogenic organisms are the main causative agents of rot in yam tubers in storage in major yam producing areas reducing the yield and productivity of yam per annum. As a result, the demand for yam tubers has always exceeded its supply [26]. Fungal organisms isolated and identified had been previously linked with post harvest yam tuber rot in different locations in Nigeria [5,8,19,27,28]. Results of inoculation of Colletotrichum spp into the healthy yam tubers shows that the fungus was able to cause rot. This was probably due to the ability of the pathogen to utilize the nutrients of the tubers as substrates for growth and development. The control tubers were however not infected suggesting the absence of inoculum in the tissues. The occurrence of *Colletotrichum spp.* in yam tubers may probably be due to the fact that *Colletotrichum spp.* overwinters in leaves, stems and seeds and in infected soil [9]. It has been found that most of these microorganisms infect the tubers in the field reducing their capacity to germinate and affect their survival in the field and then subsequently manifest in storage barns; occurring even when infected tubers do not have any sign of external symptoms [9]. The results of the dual culture revealed the capability of *T. harzianum* to reduce mycelia growth of Colletotrichum spp irrespective of the time of introduction of the antagonist. This was probably due to the ability of the antagonist to grow much faster than the pathogenic fungus and cover the entire plates thus competing efficiently for space and nutrients. The mechanism of action was mainly competition for limited nutrient and space resulting to starvation and subsequently death of the pathogen [29,30]. Microscopic observations showed the effect of *T. harzianum* and its volatile metabolite substances on mycelia and on spore formation of the pathogenic fungus at different times of the introduction of the antagonist. The interaction between *T. harzianum* and *Colletotrichum spp.* showed that spores of *Colletotrichum spp.* were only seen when *T. harzianum* was introduced two days after inoculation of pathogen. The result also revealed that *T. harzianum* mycelia entangled the hyphae fragments of *Colletotrichum spp.* and eventually plasmolysed and lysed them. As a result, no zones of inhibitions were formed in the interactions between the antagonist and these isolates in the dual culture plates. The conidia of Colletotrichum spp in the presence of *T. harzianum* mycelia were torrulose [30-33]. The actions of *T. harzianum* could be due to the possible role of chitinolytic and/or glucanases enzymes in bio-control by *Trichoderma* [30]. They showed that the enzymes function by breaking down the polysaccharides, chitin, and glucans that are responsible for the rigidity of fungal cell walls, thereby destroying the cell wall integrity and limiting the growth of the pathogen. The growth inhibition of *Colletotrichum spp.* by *T. harzianum* in dual culture in this study could be due to its fast growing nature. *Trichoderma* species have been successfully used as biocontrol agents due to their high reproductive capacity, efficient utilization of nutrients, and strong aggressiveness against other pathogens. It is also reported that *T. harzianum* isolates suppressed the growth of Colletotrichum capsici eventually overgrowing it within seven days [34]. It was found that the antagonism in vitro of *T. harzianum* against *Fusarium oxysporium* showed an inhibition on the pathogenic fungus growth with a ratio more than 65%. Moreover, the volatile metabolism substances of the antagonism reduced the pathogenic fungus growth by 63% compared with controls [35]. The dual culture inoculation and the introduction of potential antagonist two days before the pathogen was in agreement with earlier work [36]. The author believed that there are no biocontrol agents that have enough competitive ability to displace an already established pathogen. The time lapse between inoculation of the antagonist and the pathogens contributed to the success recorded with the antagonist against the pathogens. This allows adequate increase in cell concentration and subsequent colonization by antagonist before the arrival of the pathogen [37]. When *T. harzianum* was introduced 2 days after inoculation of the pathogen even when the pathogen had

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a significant space and time advantage, it could not compete effectively with the antagonist in culture. *T. harzianum* has shown to have an antagonistic influence. This effect is produced because of competition for food and space, mycoparasitism and possible antibiosis [38]. Minimum inhibition concentration (MIC) showed that *T. harzianum* introduced 2 days before the arrival of Colletotrichum spp inhibited the growth of the pathogen at the highest level compared with that introduced same time with the pathogen with least percentage growth inhibition recorded when the antagonist was introduced 2 days after inoculation of the fungus. *T. harzianum* introduced 2 days before inoculation of Colletotrichum spp was therefore considered more effective in controlling the pathogenic fungus in vitro.

Conclusion

Fungal pathogens are the main cause of rot in yam tubers in different parts of Nigeria. The use of biological antagonist which is environmentary safe has the capability of inhibiting the growth and survival of *Colletotrichum spp.* and can therefore, provide alternative ways of controlling fungal pathogens of yams. This can be mostly achieved when the bioagent (*T. harzianum*) is introduced on the host (yam tubers) before the arrival of fungal pathogen (*Colletotrichum spp.*) as this has achieved the highest percentage growth inhibition.

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