Original research paper

In vitro study of antagonistic capability of *Trichoderma harzianum* against *Aspergillus niger* isolated from rotten white yam (*Dioscorea rotundata)* tubers

Gwa, V. I.[1, 2*] Nwankiti, A. O.[1] and Ekefan, E. J.[1]

[1]*Department of Crop and Environmental Protection, Federal University of Agriculture, PMB 2373 Makurdi, Nigeria*
[2]*Department of Crop Production and Protection, Faculty of Agriculture and Agricultural Technology, Federal University, Dutsin-Ma, PMB 5001, Katsina State, Nigeria*
**Corresponding Author's Email & phone: igwa@fudutsinma.edu.ng*

Abstract: *In vitro antagonistic study using dual culture technique was carried out at Advanced Plant Pathology Laboratory, Federal University of Agriculture, Makurdi, Nigeria to assess the potential capability of Trichoderma harzianum as a biocontrol agent against Aspergillus niger isolated from rotten yam tubers. The test antagonist (T. harzianum) was introduced at three different times (same time with pathogen, two days before the inoculation of the pathogen and two days after the inoculation of the pathogen). 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 in vitro interactions between T. harzianum and A. niger revealed that T. harzianum was able to significantly ($P \leq 0.05$) inhibit the growth of A. niger at the three different times of introduction of T. harzianum and this increased with the time of incubation. T. harzianum grew faster than A. niger and produced inhibition zones which completely stopped the growth of A. niger. Mean percentage growth inhibition was found to be highest (77.79%) when T. harzianum was introduced 2 days before inoculation of A. niger followed by introduction of T. harzianum same with A. niger (45.96%). The least percentage growth inhibition (28.47%) was recorded when T. harzianum was introduced 2 days after inoculation of A. niger. In all cases, T. harzianum was observed to be effective at checking the growth of A. niger in vitro and therefore showed the capability for the biological control of the pathogen. It is therefore recommended that for effective in-vitro control of A. niger, T. harzianum should be introduced 2 days before the arrival of A.niger.*

Keywords: *Aspergillus niger*, antagonistic, *in vitro*, *Trichoderma harzianum*, yam.

1. INTRODUCTION

Yams (*Dioscorea spp*) are among the oldest recorded food crops and rank second after cassava in the study of carbohydrates in West Africa [1;2]. Yams *(Dioscorea sp.)* are reported to be a major staple food crop and source of livelihood for most parts of West Africa, East Africa, the Caribbean, South America, India and South East Asia [3]. Nigeria is the largest producer of the crop, producing about 38.92 million metric tonnes annually [4;5]. Yam has very high food value and is a major source of carbohydrate, minerals such as calcium, phosphorus, iron and vitamins including riboflavin, thiamine and vitamins B and C [6;7]. Rot of yam tubers and setts may be caused by a wide variety of micro-organisms including fungi, bacteria, and viruses at all stages of growth and also during storage of tubers [8;9]. These pathogenic fungi includes *Aspergillus flavus*, *Aspergillus niger*, *Botryodiplodia theobromae, Collectotrichum sp, Fusarium oxysporum, Fusarium solani, Geotrichum candidum, Penicillius chrysogenum, Pennicillium digitatum, Rhizoctonia sp, Penicillium oxalicum,*

Trichoderma viride and Rhizopus nodosus [10-13]. A total of 30 different fungi have been reported to be associated with the storage rots of yams [14]. Rot is a major factor limiting the Post-harvest life of yams besides lack of research for development and capacity building in yam-based researches [15;16] and losses can be very high resulting to about 50% reduction of the total stored tubers reported within the first 6 months of storage [17]. Losses due to post-harvest rot significantly affect farmers' and traders' income, food security and seed yams stored for planting. The incidence of rotting varies with the species and with varieties within each species of yam [18]. [19] reported that Rot vary due to variations in the distributions of the microorganisms and does related to the soil mineral status because the differences in the mineral status are not known to be correlated with the type of organism isolated nor total percentage of rot.

Several methods have been adopted for controlling losses due to post harvest disease of yam; these include the use of chemicals, use of antagonistic microorganisms, use of natural plant extracts, as reported by [8]. Because of the low capital income of farmers in Nigeria and lack of expertise in the safe handling of chemical, farmers resorted to the method of crop rotation, fallowing, planting of healthy material and destruction of infected crop cultivars in controlling the diseases of yam tubers [19]. Synthetic chemicals such as borax, captan, thiobendazole, benomyl, bleach (Sodium hypochloride) has been found to significantly reduce storage rot in yams [20; 21; 22] but chemicals have been found to be expensive, can cause environmental pollution and may also induce pathogen resistance. The use of micro-organisms such as *T. harzianum*, *T. viride* pers. ex S. Gray, *Penicillium digitatum, Botryodiploidia theobromae* and *Bacillus subtilis* in the control of fungal pathogens have also been reported [22; 23] but have not been adopted by resource poor farmers in Nigeria. Antagonistic micro-organisms can compete with the pathogen for nutrients, inhibit pathogen multiplication by secreting antibiotics or toxins, or reduce pathogen population through mycoparasitism [24]. It therefore, shows several advantages when compared to chemical products. They decompose more quickly in the environment and are generally less toxic towards non-target species [25]. Fungicides may have a role in the management of yam tuber rots but their cost of application, tolerance of target pathogens, environmental and health concerns may limit application. Thus alternative methods to control post harvest diseases, particularly those that are environmentally safe are urgently needed [26].

In view of this, the application of biological control agent (BCA) using *T. harzianum* on the *in vitro* control of *A. niger* causing dry rot of yam tubers in storage therefore, needs to be explored as an alternative to fungicide use.

2. MATERIALS AND METHODS

2.1 Experimental site

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

2.2 Source of *T. harzianum* isolate

T. harzianum used in this study was obtained 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.

2.3 Collection of diseased yam tubers

Rotten yam tubers of white yam varieties (*Dioscorea rotundata*) showing various diseased symptoms of dry rots were obtained from yam farmers in Kadarko, Keana local government area of Nasarawa State, Nigeria which lies between longitude 8^{o} $30^{'}$ and 8^{o} $35^{'}$ E, and on latitudes 8^{o} $10^{'}$ and 8^{0} $14'$ N. The rotten yam tubers were packaged in sterile polyethylene bags and taken to the laboratory for isolation and identification of pathogens two days after collection. The tubers were protected using wire mesh to prevent rodent attack [27]. *A. niger* which was the most frequently isolated organism was selected as the test fungus.

2.4 Preparation of potato dextrose agar (PDA)

Potato dextrose Agar (PDA) was prepared according to manufacturer's recommendations by dissolving 39g of powdered PDA in 1 litre of distilled water and autoclaved at 121^{0}C for 15min [28; 29] the medium was allowed to cool to 45-50^{0}C. About 0.16 g/l streptomycin sulphate powder was added to suppress bacterial contaminations [30]. 15 ml of the molten PDA was poured into sterile 9 cm glass Petri dishes and were allowed to cool at room temperature before inoculation.

2.5 Isolation of fungal organism

Small sizes of approximately 2 x 2mm were cut out with sterile scalpel from yam tubers infected with rot at inter-phase between the healthy and rotten portions of the tubers. They were first surface sterilized by dipping completely in a concentration of 5% sodium hypochlorite solution for 2 minutes; the sterilized sections to be inoculated were then removed and rinsed in four successive changes of sterile distilled water (SDW) as reported by [31]. The yam pieces were placed on sterile filter papers in the laminar Air flow cabinet to dry for 2 minutes.

2.6 Inoculation

The bits of the rotten yam were aseptically transferred onto solidified sterile Potato Dextrose Agar (PDA) medium in Petri dishes. Four pieces of the yam sections were plated per plate and each plate was replicated three times and incubated at room temperature (30 ± 5^{o}C) for 8 days. The plates were examined daily for the development of fungal growth.

2.7 Characterization and identification

Fungi Isolates were identified after pure cultures were obtained following successive sub-culturing. The culture plates obtained were examined for distinct growth. Microscopic examination and morphological characteristics were noted and compared with existing authorities [32; 30].

2.8 Evaluation of Dual culture method on agar plates

The assay for antagonism was performed on PDA on Petri dishes by the dual culture method [33]. The mycelial plugs (5 mm diameter) of 5 day old fungal antagonist and pathogen were placed on the same dish 6 cm from each other. Isolate of test fungal antagonist was plated same time with pathogen, two days before the pathogen and two days after the pathogen. Paired cultures were incubated at room temperature (30± 5^{0}C) for 8 days. Dishes inoculated only with the test pathogen served as controls. The experiment was replicated three times in completely randomized [34]

2.9 Radial mycelia growth and determination of inhibition

The radial growths of the pathogen in dual culture and control plates were measured after two days of inoculation at 24 hour interval beginning from the 3^{rd} day up to the 8^{th} day of

incubation at ambient temperature (30 ± 5^0C). Percent Growth Inhibition (PGI) of pathogen was calculated as described by [35]

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

Where,
PGI = Percent Growth Inhibition
R = the distance (measured in mm) from the point of inoculation to the colony margin in control plate,
R_1 = the distance of fungal growth from the point of inoculation to the colony margin in treated plate in the direction of the antagonist.
And the width of zone of inhibition (ZI) measured as the smallest distance between the colonies in the dual culture plate if any was determined [36].
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 fungi for the three treatments. Antagonist was also rated for inhibitory effects using a scale by [37] as:
≤ 0% inhibition (not effective),
>0-20% inhibition (slightly effective)
>20-50% inhibition (moderately effective),
>50-<100% inhibition (effective)
100% inhibition (highly effective)
T. harzianum was tested for both antibiosis and mycoparasitic activities against the test fungus [24]. The edges of the parasitized pathogen hyphae by microbial antagonist were transferred from the dual culture dish onto clean slides after 8 days of incubation. Cover slips were mounted on the mycelia with a drop of lactophenol cotton blue (LCB) [38]. Hyphal interaction and morphology were examined under a light microscope.

2.10 Data Analysis

Data collected were subjected to Analysis of variance (ANOVA) using GenStat Discovery Edition 12 for ANOVA and means separation, Minitab Release 14 for descriptive statistics and Graph Pad Prism 6 for trend graphs. Statistical F-tests were evaluated at $P \leq 0.05$. Differences among treatment means for each measured parameter were separated using fishers least significance difference (F-LSD) [39].

3. RESULTS

3.1 Sample collection, isolation and pathogen identification

Test fungus *A. niger* was isolated and identified as one of the fungi causing dry rot of white yam (*D. rotundata*) tubers in the study area. Macroscopic examination of pure cultures of this fungus on PDA showed dark brown colour. Microscopic examination and morphological characteristics and identification showed non-septate conidiophores. Each conidiophores ends in a terminal enlarged spherical swellings. Conidia are borne by phialides arising from a terminal swelling on the conidiophores. It has 'mop-like' head of conidia (Fig. 1)

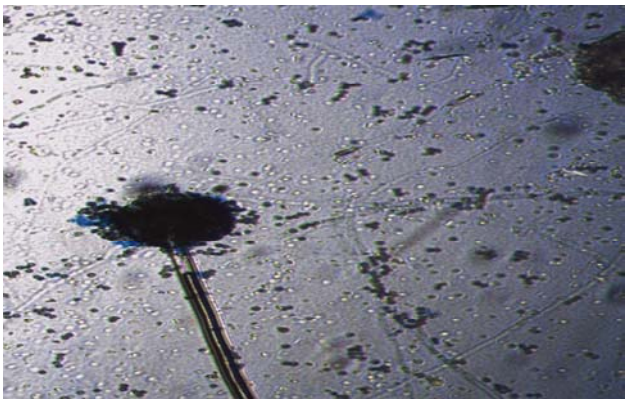

Figure 1: Microscopic structure of *A.niger* (× 10) with conidia borne by phialides on conidiophores (right)

3.2 Evaluation of dual culture method on agar plates

The *in vitro* results of dual culture of *T. harzianum* and *A. niger* shows that when the mycelium of both cultures came in contact with each other the hyphal growth of the pathogenic fungus were found to be inhibited by the hyphae of *T. harzianum* (Fig. 2and 3). The result of dual culture also shows that the antagonist grew much faster than the pathogen, parasitized on the pathogen and deprived it from absorbing the nutrients from the substrate. The pathogen eventually died (Fig. 4). The results of dual culture indicated that *T. harzianum* significantly (P≤ 0.05) inhibited the growth of *A. niger* at varying degrees across duration of incubation (Table 1).When *T. harzianum* was introduced two days before inoculation of *A. niger*, it was observed that the percentage growth inhibition of *A.niger* in dual culture with *T. harzianum* rose steadily from 11.10% at 72 hours to 95.49% at 192 hours after incubation. A similar trend was recorded when both the antagonist and pathogen were introduced same time, with percentage growth inhibition of 11.87% at 72 hours to 68.61% at 192 hours respectively. Inhibitions of 19.39% at 72 hours to 53.40% at 192 hours were computed when *T. harzianum* was introduced two days after inoculation of *A. niger* (Table 1). It was found that when *T.harzianum* was introduced two days before inoculation of *A. niger*, the mean variation in percentage growth inhibition after 192 hours was higher (77.79%) than when *T. harzianum* was introduced same time with *A. niger* (45.96%) and the least percentage growth inhibition (28.47%) was recorded when *T. harzianum* was introduced two days after the inoculation of *A. niger* (Table 2). Mean variation of percentage growth inhibition of *A. niger* tested at three different times of introduction of *T. harzianum* significantly *(P≤ 0.05)* inhibited the growth of *A. niger* (Table 1).

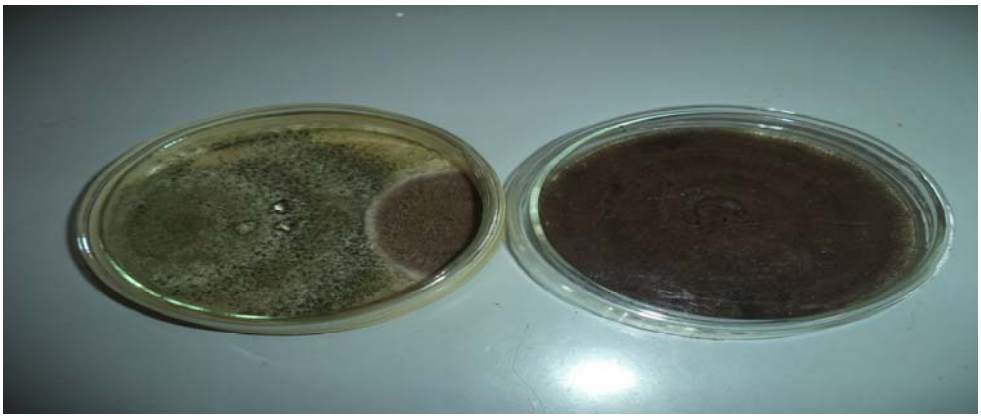

Figure 2: Dual culture of *T. harzianum* and *A. niger* on potato dextrose agar inoculated same time (Th × path) (left) and pure culture of *A. niger* on Potato Dextrose Agar as control (right)

Figure 3: Dual culture of *T. harzianum* and *A. niger* on potato dextrose agar (left); *T. harzianum* was introduced 2 days after inoculation of *A. niger* (2 dai) and pure culture of *A. niger* on Potato Dextrose Agar as control (right)

Figure 4: Dual culture of *T. harzianum* and *A. niger* on potato dextrose agar (left); *T. harzianum* was introduced 2 days before inoculation of *A. niger* (2 dbi) and pure culture of *A. niger* on Potato Dextrose Agar as control (right)

Table 1: *In vitro* Percentage Growth Inhibitions (PGI) of *A. niger* at different times of introduction of *T. harzianum*

Duration of Incubation (Hours)	Time of Introduction of *T. harzianum*		
	ThXPath	**Th2dbiPath**	**Th2daiPath**
72	11.87±5.68^{d}	11.10±11.0^{b}	19.39±2.63^{d}
96	35.82±4.25^{c}	84.40±2.26^{a}	2.09±1.04^{e}
120	44.25±2.56^{bc}	89.65±0.21^{a}	19.23±2.63^{d}
144	49.95±1.88^{b}	91.81±0.15^{a}	33.13±0.81^{c}
168	62.27±1.30^{a}	94.25±0.22^{a}	43.59±0.6^{b}
192	68.61±1.16^{a}	95.49±0.35^{a}	53.40±0.94^{a}
LSD	*10.03*	*14.28*	*5.22*
Mean (LSD= 15.88)	**45.96±4.58^{b}**	**77.79±7.46^{a}**	**28.47±4.17^{c}**

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; Th2daipath = *T. harzianum* introduced 2days after inoculation of pathogen.

3.3 Radial mycelia growth and determination of inhibition

Radial mycelia growth of *T. harzianum* and *A. niger* in dual culture and *A. niger* in control plates for each of the treatments were measured. There was a more rapid growth in the control plates than in the dual culture plates in all the treatments as observed in Figure 5. It was also found that *T. harzianum* grew much faster than *A. niger* in all the treatments when grown in dual culture thereby inhibiting the growth of *A. niger*.

The three levels of treatments of *T. harzianum* tested on *A. niger* 2 days before inoculation of *A. niger* significantly *(P≤ 0.05)* reduced growth (77.79%) more than that introduced same time with *A. niger* (45.96%) and that introduced 2 days after inoculation of *A. niger* (28.47%). Effectiveness levels of *T. harzianum* were moderately effective to effective and significant *(P≤ 0.05)* across treatments (Table 2).

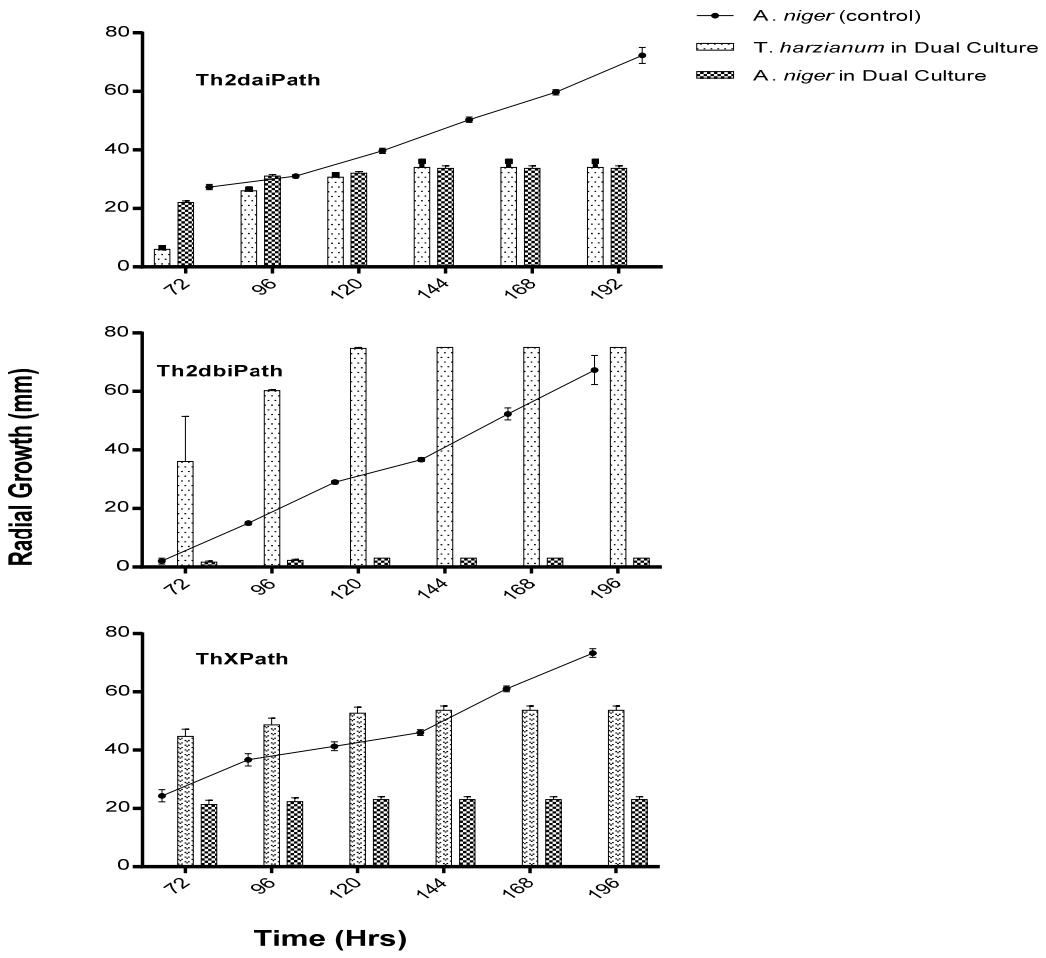

Figure 5: Radial growth of *Aspergillus niger* in dual culture with *T. harzianum* at different times of introduction of *T. harzianum* after 196 hours of incubation. Th2daipath = *T. harzianum* 2 days after inoculation of pathogen; Th2dbipath = *T. harzianum* 2 days before inoculation of pathogen; Th×path = *T.harzianum* introduced same time with pathogen.

Table 2: Mean Percentage growth inhibition of *A. niger* treated with *T. harzianum* at different times showing minimum inhibition concentration

Time of Introduction of *T. harzianum*	Percentage Growth Inhibition (PGI)	MIC (%)	Level of Effectiveness
ThXPath	45.96±4.58[b]	>20-50	Moderately Effective
Th2dbiPath	77.79±7.46[a]	>50<100	Effective
Th2daiPath	28.47±4.17[c]	>20-50	Moderately effective
LSD	15.88		

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)

4. DISCUSSION

The results of this study revealed that *T. harzianum* has a high inhibitory effect against *A. niger* with several biological mechanisms like mycoparasitism and food competition [40; 41; 24]. The results of dual culture indicated that *T. harzianum* inhibited the growth of *A. niger* at varying degrees. The bioagent inhibited the growth of the target organism through its ability to grow much faster than the pathogenic fungi thus competing efficiently for space and nutrient even as it develops the system of mycotoxins [42]. *T. harzianum* inhibited the growth of the target organisms through its ability to grow much faster than the pathogenic fungus thus competing efficiently for space and nutrients (Fig. 3-5). Mycoparasitism was the most common cause of death for *A. niger* so that competition for limiting nutrients resulted in biological control of the fungal phytopathogen [41].

The antagonistic effect of *T. harzianum* against *A. niger in vitro* on PDA medium showed that when the mycelium of both cultures came in contact with each other the hyphae growth of *A. niger* were found to be inhibited by the hyphae of *T. harzianum*. A clear zone of interaction was formed in all *T. harzianum*-*A. niger* interactions except in the interaction between *T. harzianum* and *A. niger;* where *T. harzianum* was introduced 2days before inoculation of *A. niger* (figure 5). It was observed that *T. harzianum* overgrew *A. niger* and completely stopped its growth. This is similar to the findings of [43] who showed that *T. harzianum* suppressed the growth of *Pythium aphanidermatum* and *P. myriotylum* killing their mycelia within three days of inoculation as the test organism were not recovered in the area grown over by the antagonist. In another experiment, [44] found out that *T. harzianum* isolates suppressed the growth of *Colletotrichum capsici* eventually overgrowing it within seven days. The bioagent is known to control plant pathogens by antagonizing them through mycoparasitism, by producing metabolites such as Beta 1-3 and 1-4 glucanases, directly competing with the pathogen and inducing host resistance [45; 46]. [47] discovered that the *Trichoderma sp* isolates have a strong antagonism against wilt diseases caused by *Fusarium sp*, *in vitro*, on potato dextrose agar medium. It decrease the growth of *Fusarium* sp by (88%), (86%) and (80%) for *T. harzianum*, *T. hamatum* and *T. viride* respectively.

The inoculation of *T. harzianum* 2 days before the arrival of *A. niger* was done because there are no biocontrol agents that have enough competitive ability to displace an already established pathogen. The time lapse allows adequate increase in cell concentration and subsequent colonization by antagonist before the arrival of the pathogen [48; 49]. The ability of antagonists to proliferate within a short period of favourable environmental conditions before they encounter plant pathogen is an important factor as more rapid growth and sporulation of fungi from biocontrol formulations may superficially enhance efficacy in the field. In another case, when *T. harzianum* was introduced 2 days after inoculation of *A. niger,* even when *A. niger* had a significant space and time advantage, *T. harzianum* has shown to have an antagonistic influence. This effect is produced because of competition for food and space, mycoparasitism and possible antibiosis [50]. Production of zones of inhibition at the boundary with the pathogen agrees with the report of [51] that *in vitro* fungal interactions resulted in production of a zone of inhibition (ZI), contact inhibition or no inhibition at all. The zones of inhibition produced might be due to the production of antifungal metabolites by the test antagonist as reported by [52] and [53]. Minimum inhibition concentration (MIC) showed that *T. harzianum* introduced 2 days before the arrival of *A. niger* inhibited the growth of the pathogen at the highest level more than that introduced 2 days after the inoculation of the pathogen as well as that introduced same time. *T. harzianum* introduced 2

days before inoculation of *A. niger* and was therefore considered more effective in controlling the pathogen.

5. CONCLUSION

It is therefore, concluded that *T. harzianum* has the capability of affecting the survival and growth of *A. niger in vitro*, one of the pathogens that caused dry rot of yam tubers in storage; especially when the bioagent was introduced 2 days before the arrival of the pathogen on the host and should be used to control yam fungal pathogens as it is eco-friendly and does not induce resistance in host.

REFERENCES

[1]. F. I. Nweke, B. O. Ugwu, C. L. Asadu and P. Ay, Production cost in the yam based cropping system South- western Nigeria Research monograph No. 6 IITA Ibadan, Nigeria; 1992, 4-12.

[2]. A. E. Agwu and J. I. Alu, Farmers perceived constraints to yam production in Benue State, Nigeria. Proceedings of the 39th Annual Conference of the Agricultural society of Nigeria; 2005, pp 347-50.

[3]. Food and Agricultural Organization, Food and Agricultural Organisation of the United Nations, 2013, www.fao.org/statistics/en/, FAO Rome

[4]. Food and Agriculture Organisation, Production Year Book, 2008, FAO Rome

[5]. U. Kleih, D. Phillips, M. Ogbonna and B. Siwoku, Nigeria-Scoping Yam Value Chain Analysis.Yam Improvement for Income and Food Security in West Africa, 2012, 1-53

[6]. R. N. Okigbo and U. O. Ogbonnaya, Antifungal effects of two tropical plant leaves extract (*Ocimum gratissimum* and *Aframomum melegueta*) on post harvest yam (*Dioscorea* spp.) rot. *Afr. J. Biotech*., 5(9), 2006, 727- 731

[7]. R. N. Okigbo and A. N. Emeka, Biological control of rot-inducing fungi of water Yam (Dioscorea alata) with *trichoderma harzianum, pseudomonas syringe* and *pseudomonas chlororaphis J. stored product res.* 1(2), 2010, 18-23

[8]. N. A. Amusa, A. A. Adegbite, S. Muhammed and R. A. Baiyewu, Yam diseases and their management in Nigeria. African *Journal of Biotech. Vol. 2(12)***.** 2003, 497- 502

[9]. R. N. Okigbo, R. Putheti and C. T. Achusi, Post-harvest deterioration of cassava and its control using extracts of *Azadirachta indica* and *Afromonium meleguata.* E-J Chem, 6(4), 2009a, 1274-1280

[10]. J. K. Okoro, and A. O. Nwankiti, Post-harvest Microbial Rot of Yam in Nigeria. *Pathologia:* 2004, 35-40

[11]. A. O. Ogunleye, O. T. Ayansola, Studies of Some Isolated Rot-Causing Mycoflora of Yams (*Dioscorea* Spp.). *Amer. J. Microb. and Biot.* Vol. 1(1), 2014, pp. 9-20.

[12]. I. V. Gwa, A. A. Bem, and J. K. Okoro, Yams (*Dioscorea rotundata* Poir *and D. alata* Lam*.)* fungi etiology in Katsina-Ala Local Government Area of Benue State, Nigeria. *Journal of Phytopathology and Plant Health 3*, 2015, 38-43

[13]. R. N. Okigbo, C. E. Enweremadu, C. K. Agu, R. C. Irondi, B. C. Okeke,S. N. Awah, C. G. Anaukwu, I. O. Okafor, C. U. Ezenwa, and A. C. Iloanusi, Control of white yam (*Dioscorea rotundata)* rot pathogen using peel extract of water yam (*Dioscorea alata*) *Advances in Applied Science Research, 6(10), 2015,*7-13

[14]. T. Ikotun, Diseases of yam tubers**.** *International Journal of Tropical Plant Diseases (India)*. 1989, Pp 21.

[15]. A. Taiga, Comparative studies of the efficacy of some selected fungicidal aqueous plant extracts on yam tuber dry rot disease. *Ann Biol Res, 2(2)*, 2011, 332-336

[16]. J. Onyeka, P. M. Chuwang, O. Fagbola, Baseline studies on the status of yam research for development: status of yam research in Nigeria, 2011, Pp 1-52. www.iita.org/c/document_library/et_file/p_l_id.

[17]. A. E. Arinze, Plant Pathology and Post –harvest Food Loss. An Inaugural Lecture Series, 43, 2005, 29-72

[18]. K. A. Aidoo, Identification of yam tuber rots fungi from storage systems at the Kumasi Central market. A dissertation submitted to Faculty of Agriculture, K.N.U.S.T, 2007.

[19]. O. A. Nwankiti, Studies on the Aetiology and Control of Acthracnose/Brown blotch disease Complex of Dioscoreaalata in Nigeria.University of Nigeria, Nsukka, 1982.

[20]. R. H. Booth, Post harvest deterioration of Tropical root crops: Losses and their control. *Trop. Sci., 16(2),* 1974, 49-63

[21]. R.A. Noon, Storage and market diseases of yams. Trop. Sci., 20, 1978

[22]. R. N. Okigbo, Biological Control of Postharvest Fungal Rot of Yam (*Dioscorea* spp) with *Bacillus subtilis*. *Mycopathologia*, *159,* 2005, 307-314.

[23]. M. O. Adebola and J. E. Amadi, Screening three *Aspergillus spp* for antagonistic activities against the cocoa black pod organism (*Phytophthora palmivora*) *Agric. Bio. J.N. America, 13*, 2010a, 362-365

[24]. H. Mokhtar and D. Aid, Contribution in isolation and identification of some pathogenic fungi from wheat seeds, and evaluation of antagonistic capability of *Trichderma harzianum* against those isolated fungi in vitro. *Agric. Bio. J. N.Am. 4(2)*, 2013, 145-154

[25]. Y. Thakore, The biopesticide market for global agricultural use. *Industrial Biotechnology,* Vol. 2, 2006, pp. 194-208.

[26]. D. T. L. Sugar, E. E. Righettic, Sanchez and E. Khemira, Management of nitrogen and calcium in pear trees for enhancement of resistance of post harvest decay. *Hortic. Technol.,* 2, 1997, 382-387.

[27]. C. S. Eze, *Studies on storage rot of cocoyam (Colocasia esculenta (L.) Schott) at Nsukka.* MSc Dissertation, Dept of Botany, Univ of Nigeria, Nsukka. 1984,73pp

[28]. M. Cheesbrough, District Laboratory Practice in Tropical Counties. Low Price Edition, Cambridge University Press, Cambridge, UK, 2000, 62-70.

[29]. M. Jawetz, G. F. Adelberg, Brooks, J.S. Butel, and S. A. Morse, *Medical Microbiology*. 23rd edition. McGraw Hill Companies, Inc. Singapore, 2004, p.818

[30]. W. B. Lester, E. K. Timothy, T. Len and T. P. Hien, *Diagnostic manual for plant diseases in Vietnam,* 2008, p213

[31]. B. Ritichie, *Practical techniques in plant pathology*. CAB. Walling ford. UK., 1991.

[32]. G. Agrios, *Plant pathology 5 ed.* 2004, Elsevier, London

[33]. H. C. Evans, K. A. Hoimes and A. P. Reid, Phylogeny of the Frosty Pod rot pathogen of cocoa. Plant Pathology, 52, 2003, 476-485.
[34] K. A. Gomez, and A. A. Gomez, *Statistical procedures for Agricultural Research 2nd Edition* John Wiley and sons. 1984 Pp 680
[35]. L. Korsten, and E. S. De Jager, Mode of action of *Bacillus subtilis* for control of avocado post harvest pathogens. *S. Afr. Avocado Growers Assoc. Yearb, 18,* 1995, 124-130
[36]. A. Singh and R. Sharma, Biocontrol and Environmental Studies on Paper Degrading Mycoflora Isolated from Sanganer Area, Jaipur, India *Int.J.Curr.Microbiol.App.Sci* 3(8), 2014, 948-956
[37]. T .E. Sangoyomi, Post-*harvest fungal deterioration of yam (Dioscorea rotundata. Poir) and its control.* Ph.D. Thesis. University of Ibadan, Nigeria, 2004, p. 179.
[38]. B. Hajiehgrari, T. Mousa, Mohammed Mahdi, Davari Reza Mohammadi, Biological potential of some Iranian Trichoderma isolates in the control of soil borne plant pathogenic fungi. African *Journal Bioteol. Control. 50,* 2008, 143-149
[39] G. W. Cochran, and G. M. Cox, Experimental *Designs. 2nd Edn* John willey and Sons Inc., 1992, pp: 611
[40]. B. Umamaheswari, B. Thakore and T. More, Post-harvest management of ber (*Ziziphus mauritiana* Lamk) fruit rot (*Alternaria alternata* (Fr.) Keissler) using Trichoderma species, fungicides and their combinations, *Crop Protection, Vol. 28(6),* 2009, pp.525-32
[41]. E. N. Siameto, S. Okoth, N. O. Amugune, and N. C. Chege, Molecular Characterization and idedentification of biocontrol isolates of Trichoderma harzianum from Embu District, Kenya. *Tropical and Subtropical Agroecosystem 13,* 2011, 81-90
[42]. M. Barbosa, K. Rehm, M. Menezes, and R. L. Mariano, Antagonism of *Trichoderma* species on *Cladosporium herbarum* and their enzymatic characterization, *Brazilian Journal of Microbiology, Vol.32*, 2001, 98-104
[43]. N. S. Devaki, S. Shankara Bhat, S. G. Bhat and K. R. Manjunatha, Antagonistic activities of *Trichoderma harzianum* against *Pythium* aphanidermatum and Pythium myriotylum on Tobacco. *Journal of Pytopathology 136*, 1992, 82-87.
[44]. E. J. Ekefan, A. Jama and S. R. Gowen, Potential of Trichoderma harzianum isolates in biocontrol of *Colletotrichum capsici* causing anthracnose of pepper (Capsicum spp.) in Nigeria *Journal of Applied Biosciences 20*, 2009, 1138 - 1145
[45]. M. Lorito, S. L. Woo, M. D. D'ambrosio, G. E. Harman, C. K. Hayes, C. P. Kubicek and F. Scala, Synergistic interaction between cell wall degrading enzymes and membrane affecting compounds. *Molecular Plant-Microbe Interaction 9,* 1996, 206-213.
[46]. B. K. Duiff, D. Pouhair, C. Olivian, C. Alabouvette and P. Lemanceau, Implication of systemic induced resistance in the suppression of *Fusarium* wilt of tomato by *Pseudomonas flourescens* WC417r and by non pathogenic *Fusarium oxysporum* Fo47. *European Journal of Plant Pathology 104*, 1998, 903-910.
[47]. A. T. Azza and D. A. Allam, Improving production under soikl infestation with Fusarium Pathogen 1 screening of biocontrol agents. *Ass. Univ. Bull.Environ.Res.2*, 2004, 35-45.
[48]. J. W. J. Janisienwicz, Biocontrol of post-harvest disease of Apples with antagonistic mixtures. J. Phytopathol., 78, 1988, 194-198.
[49]. M. O. Adebola and J .E. Amadi, Screening three *Aspergillus spp* for antagonistic activities against the cocoa black pod organism (*Phytophthora palmivora*) *Agric. Bio. J.N. America, 13*, 2010a, 362-365.
[50]. F. Sempere, M. Santamarina, *In vitro* biocontrol analysis of Alternaria alternate (Fr.) Keissler under different environmental conditions, *Mycopathologia*, 163(3), 2007, pp.183-90
[51]. D. J. Royse and S. M. Ries, The influence of fungi isolated from peach twigs on the pathogenicity of *Cytospora cinata. Phytopathol. 63,* 1977, 603-607.
[52]. M. Shankar, D. I. Kurtboke and K. Sivasithamparam, Nutritional and environmental factors affecting growth and antIfungal activity of a sterile red fungus against *Gaeumanomyces graminis var. Tritici. Can. J. Microbiol., 33*, 1994, 515-519.
[53]. T. O. Adejumo, T. Ikotun and D. A. Florin, Biological control of *Protomycopsis phaseoli,* the causal agent of leaf smut of Cowpea. J. Phytopathology 147, 1999, 371-375.