UNDER PEER REVIEW

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

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 measurement of mycelia radial growths were recorded at intervals of 24hours 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 ≤ 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 2days 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 2days 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 two days before the arrival of A.niger.*

Keywords: *A. niger*, antagonistic, *in vitro*, *T. 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 and 2]. Yams *(Dioscorea spp)* 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 spp, Fusarium oxysporum, Fusarium solani, Geotrichum candidum, Penicillius chrysogenum, Pennicillium digitatum, Rhizoctonia spp, Penicillium oxalicum, Trichoderma viride and Rhizopus nodosus* [10; 11; 12; 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.

UNDER PEER REVIEW

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, uses 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 *Trichoderma harzianum*, *Trichoderma viride* pers. ex S. Gray, *Penicillium digitatum, Botryodiploidia theobromae* and *Bacillus subtilis* in the control of fungi 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 and 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° $30^{'}$ and 8° $35^{'}$ E, and on latitudes 8° $10^{'}$ 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. 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 dehydrated 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 0.16g/l streptomycin sulphate powder was added to suppress bacterial contaminations [30]. 15ml of the molten

UNDER PEER REVIEW

PDA was poured into sterile 9cm glass Petri dishes and were allowed to cool at room temperature before inoculation.

2.5 Isolation of fungi organism

Small sizes of approximately 2x2mm 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 2minutes; 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 2minutes.

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^{0}C) for 8days. 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 8days. 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 3rd day up to the 8th day of incubation at ambient temperature (30 ± 5^{0}C). 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].

UNDER PEER REVIEW

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)

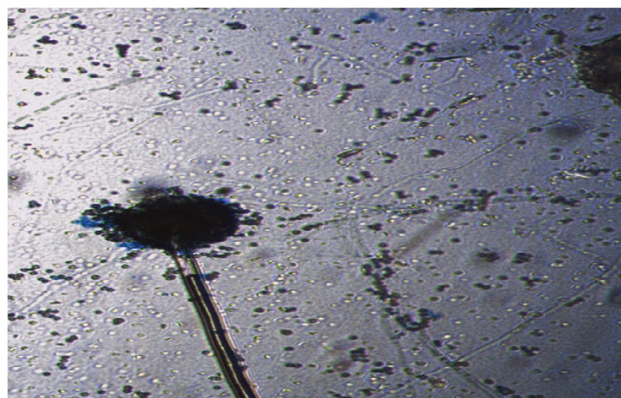
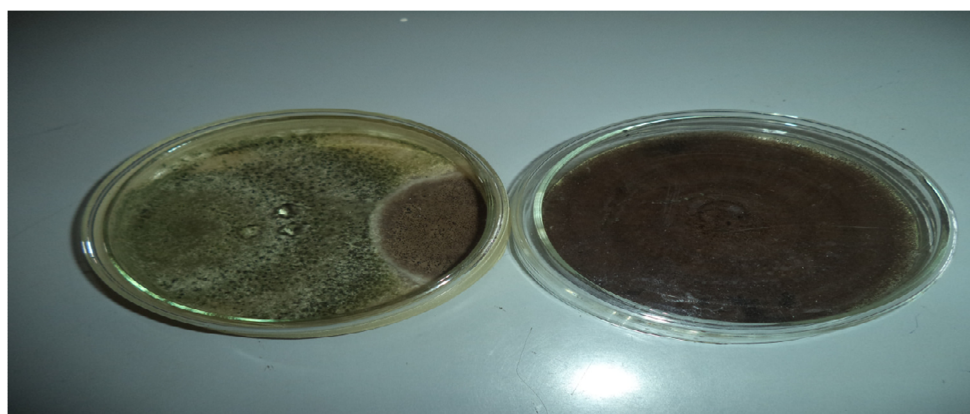


Figure 1: Pure culture of *A. niger* growing on Potato dextrose agar (left); Microscopic structure of *A.niger* (× 10) with conidia borne by phialides on conidiophores (right)

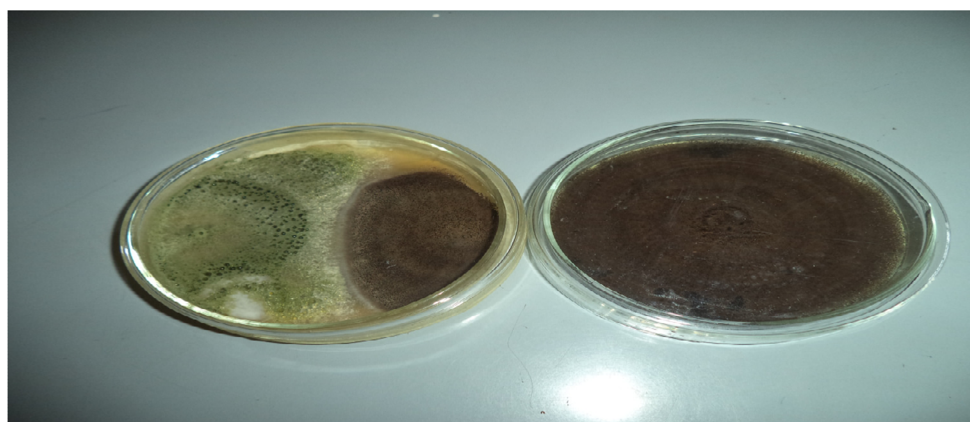
3.2 Evaluation of Dual culture method on agar plates

UNDER PEER REVIEW

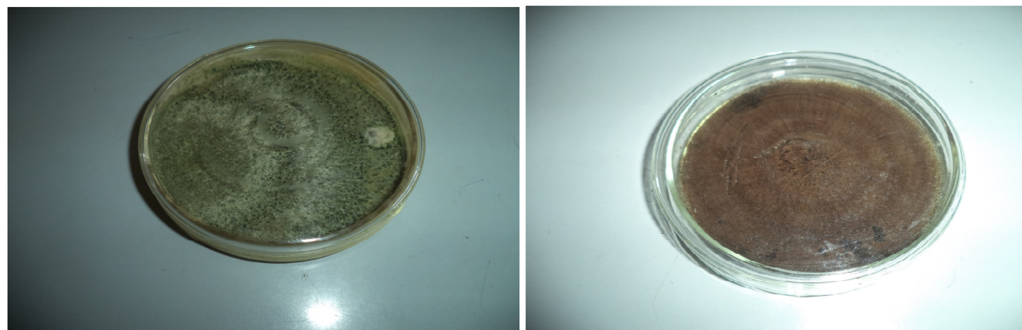
168 The results of dual culture of *T. harzianum* and *A. niger in vitro* on PDA medium, shows that
169 when the mycelium of both cultures came in contact with each other the hyphal growth of the
170 pathogenic fungus were found to be inhibited by the hyphae of *T. harzianum* (Fig. 2and 3).
171 The result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

189 

190 **Figure 2**: Dual culture of *T. harzianum* and *A. niger* on potato dextrose agar inoculated same time
191 (Th×path) (left) and pure culture of *A. niger* on potato dextrose agar as control (right)
192

193 

194 **Figure 3**: Dual culture of *T. harzianum* and *A. niger* on potato dextrose agar (left); *T. harzianum*
195 was introduced 2days after inoculation of *A. niger* (2dai) and pure culture of *A. niger* on potato
196 dextrose agar as control (right)
197



198
199 **Figure 4:** Dual culture of *T. harzianum* and *A. niger* on potato dextrose agar (left); *T. harzianum* was
200 introduced 2days before inoculation of *A. niger* (2dbi) and pure culture of *A. niger* on potato dextrose
201 agar as control (right)
202

203 **Table 1: *In vitro* Percentage Growth Inhibitions (PGI) of *A.niger* by time of**
204 **Introduction of *T. harzianum***

Duration of Incubation	Time of Introduction of *T.harzianum*		
	ThXPath	**Th2dbiPath**	**Th2daiPath**
72 Hrs	11.87±5.68^{d}	11.10±11.0^{b}	19.39±2.63^{d}
96 Hrs	35.82±4.25^{c}	84.40±2.26^{a}	2.09±1.04^{e}
120 Hrs	44.25±2.56^{bc}	89.65±0.21^{a}	19.23±2.63^{d}
144 Hrs	49.95±1.88^{b}	91.81±0.15^{a}	33.13±0.81^{c}
168 Hrs	62.27±1.30^{a}	94.25±0.22^{a}	43.59±0.6^{b}
192 Hrs	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}**

205 Means on the same column with the same superscript are not statistically significant (*P≤ 0.05*)
206 Means on the same row (for Mean) with the same superscript are not statistically significant *(P≤ 0.05)* by time
207 of introduction of *T. harzianum.* Th×path = *T.harzianum* introduced same time with pathogen; Th2dbipath =
208 *T.harzianum* introduced 2days before inoculation of pathogen; Th2daipath = *T. harzianum* introduced 2days
209 after inoculation of pathogen.

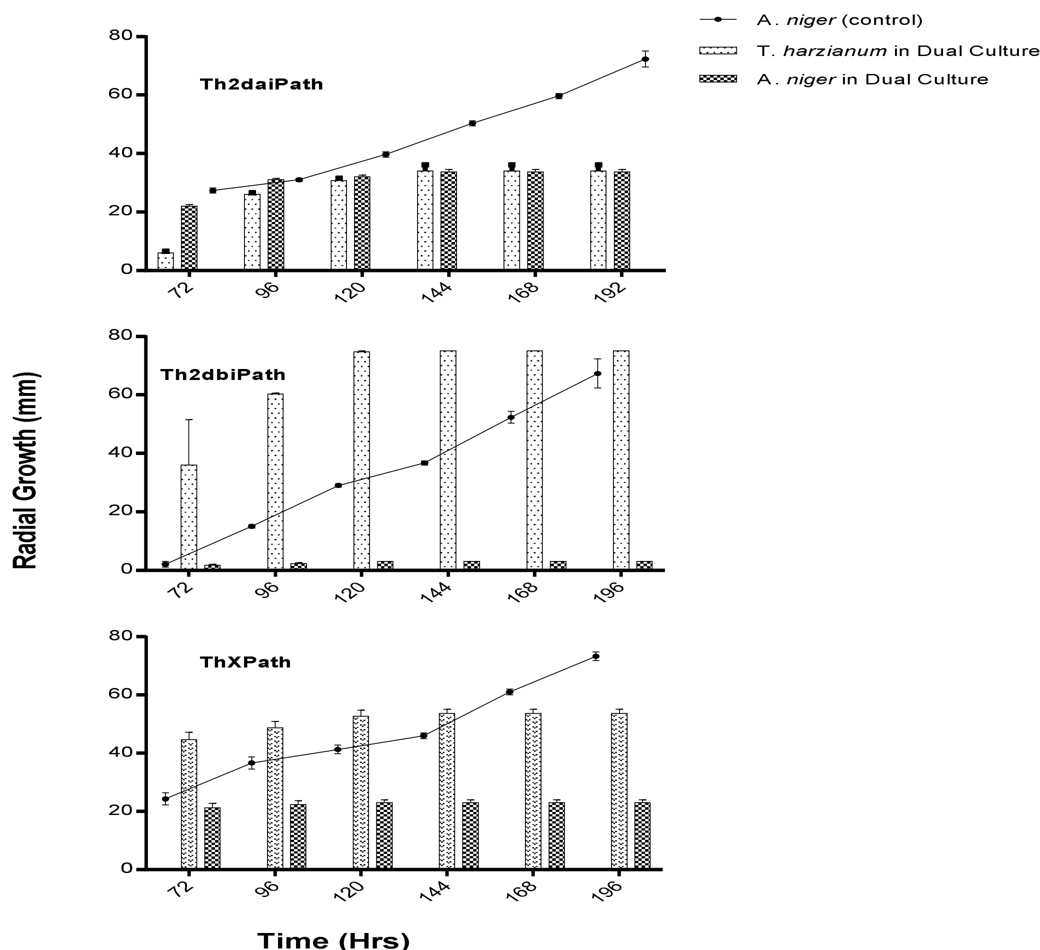
210 **3.3 Radial mycelia growth and determination of inhibition**

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

216 Three levels of treatments of *T. harzianum* were tested on *A. niger* for effectiveness levels as
217 highly effective, effective, moderately effective, slightly effective and not effective across the
218 treatments. *T. harzianum* introduced 2days before inoculation of *A. niger* significantly *(P≤*
219 *0.05)* reduced growth (77.79%) more than that introduced same time with *A. niger* (45.96%)
220 and that introduced 2days after inoculation of *A. niger* (28.47%). Effectiveness levels of *T.*

221 *harzianum* were moderately effective to effective and significant *(P≤ 0.05)* across treatments
222 (Table 2).

223



224

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

229 **Table 2: Mean Percentage growth inhibition of *A. niger* treated with *T. harzianum* at**
230 **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		

UNDER PEER REVIEW

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

236 **4. DISCUSSION**

237 The results of this study revealed that *Trichoderma harzianum* has a high inhibitory effect
238 against *A. niger* with several biological mechanisms like mycoparasitism and food
239 competition [40; 41; 24]. The results of dual culture indicated that *T.harzianum* inhibited the
240 growth of *A.niger* at varying degrees. The bioagent inhibited the growth of the target
241 organism through its ability to grow much faster than the pathogenic fungi thus competing
242 efficiently for space and nutrient even as it develops the system of mycotoxins [42]. *T.*
243 *harzianum* inhibited the growth of the target organisms through its ability to grow much
244 faster than the pathogenic fungus thus competing efficiently for space and nutrients (Fig. 3, 4
245 and 5). Mycoparasitism was the most common cause of death for *A. niger* so that
246 competition for limiting nutrients resulted in biological control of the fungal phytopathogen
247 [41].

248 The antagonistic effect of *T. harzianum* against *A. niger in vitro* on PDA medium showed
249 that when the mycelium of both cultures came in contact with each other the hyphae growth
250 of *A. niger* were found to be inhibited by the hyphae of *T. harzianum*. A clear zone of
251 interaction was formed in all *Trichoderma*-*A. niger* interactions except in the interaction
252 between *T. harzianum* and *A. niger;* where *T. harzianum* was introduced 2days before
253 inoculation of *A. niger* (figure 5). It was observed that *T. harzianum* overgrew *A. niger* and
254 completely stopped its growth. This is similar to the findings of [43] who showed that *T.*
255 *harzianum* suppressed the growth of *Pythium aphanidermatum* and *P. myriotylum* killing
256 their mycelia within three days of inoculation as the test organism were not recovered in the
257 area grown over by the antagonist. In another experiment, [44] found out that *T. harzianum*
258 isolates suppressed the growth of *Colletotrichum capsici* eventually overgrowing it within
259 seven days. The bioagent is known to control plant pathogens by antagonizing them through
260 mycoparasitism, by producing metabolites such as Beta 1-3 and 1-4 glucanases, directly
261 competing with the pathogen and inducing host resistance [45; 46]. [47] discovered that the
262 *Trichoderma sp* isolates have a strong antagonism against wilt diseases caused by *Fusarium*
263 *sp*, *in vitro*, on potato dextrose agar medium. It decrease the growth of *Fusarium* sp by
264 (88%), (86%) and (80%) for *Trichoderma harzianum*, *T.hamatum* and *T. viride* respectively.

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

UNDER PEER REVIEW

279 the test antagonist as reported by [52] and [53]. Minimum inhibition concentration (MIC)
280 showed that *T. harzianum* introduced 2days before the arrival of *A. niger* inhibited the growth
281 of the pathogen at the highest level more than that introduced 2days after the inoculation of
282 the pathogen as well as that introduced same time. *T. harzianum* introduced 2days before
283 inoculation of *A. niger* and was therefore considered more effective in controlling the
284 pathogen.

285 **5. CONCLUSION**

286 It is therefore, concluded that *T. harzianum* has the capability of affecting the survival and
287 control of *A. niger in vitro*, one of the pathogens that caused dry rot of yam tubers in storage;
288 especially when the bioagent is introduced before the arrival of the pathogen on the host.

289 **REFERENCES**

290 [1]. F. I. Nweke, B. O. Ugwu, C. L. Asadu and P. Ay, Production cost in the yam based cropping system South- western Nigeria
291 Research monograph No. 6 IITA Ibadan, Nigeria; 1992, pp 4-12.
292 [2]. A. E. Agwu and J. I. Alu, Farmers perceived constraints to yam production in Benue State, Nigeria. Proceedings of the 39th
293 Annual Conference of the Agricultural society of Nigeria; 2005, pp 347-50.
294 [3]. Food and Agricultural Organization, Food and Agricultural Organisation of the United Nations, 2013,
295 www.fao.org/statistics/en/, FAO Rome
296 [4]. Food and Agriculture Organisation, Production Year Book, 2008, FAO Rome
297 [5]. U. Kleih, D. Phillips, M. Ogbonna and B. Siwoku, Nigeria-Scoping Yam Value Chain Analysis.Yam Improvement for
298 Income and Food Security in West Africa, 2012, pp 1-53
299 [6]. R. N. Okigbo and U. O. Ogbonnaya, Antifungal effects of two tropical plant leaves extract (*Ocimum gratissimum* and
300 *Aframomum melegueta*) on post harvest yam (*Dioscorea* spp.) rot. *Afr. J. Biotech*., 5(9), 2006, 727- 731
301 [7]. R. N. Okigbo and A. N. Emeka, Biological control of rot-inducing fungi of water Yam (Dioscorea alata) with
302 *trichoderma harzianum, pseudomonas syringe* and *pseudomonas chlororaphis J. stored product res.* 1(2), 2010, 18-23
303 [8]. N. A. Amusa, A. A. Adegbite, S. Muhammed and R. A. Baiyewu, Yam diseases and their management in Nigeria. African
304 *Journal of Biotech. Vol. 2(12)***.** 2003, 497- 502 pp
305 [9]. R. N. Okigbo, R. Putheti and C. T. Achusi, Post-harvest deterioration of cassava and its control using extracts of
306 *Azadirachta indica* and *Afromonium meleguata.* E-J Chem, 6(4), 2009a, 1274-1280
307 [10]. J. K. Okoro, and A. O. Nwankiti, Post-harvest Microbial Rot of Yam in Nigeria. *Pathologia:* 2004, 35-40
308 [11]. A. O. Ogunleye, O. T. Ayansola, Studies of Some Isolated Rot-Causing Mycoflora of Yams (*Dioscorea* Spp.). *Amer. J. Microb.*
309 *and Biot.* Vol. 1(1), 2014, pp. 9-20.
310 [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
311 Government Area of Benue State, Nigeria. *Journal of Phytopathology and Plant Health 3*, 2015, 38-43
312 [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.
313 Ezenwa, and A. C. Iloanusi, Control of white yam (*Dioscorea rotundata)* rot pathogen using peel extract of water yam
314 (*Dioscorea alata*) *Advances in Applied Science Research, 6(10), 2015,*7-13
315 [14]. T. Ikotun, Diseases of yam tubers**.** *International Journal of Tropical Plant Diseases (India).* 1989, Pp 21.
316 [15]. A. Taiga, Comparative studies of the efficacy of some selected fungicidal aqueous plant extracts on yam tuber dry rot
317 disease. *Ann Biol Res, 2(2)*, 2011, 332-336
318 [16]. J. Onyeka, P. M. Chuwang, O. Fagbola, Baseline studies on the status of yam research for development: status of yam
319 research in Nigeria, 2011, Pp 1-52. www.iita.org/c/document_library/et_file/p_l_id.
320 [17]. A. E. Arinze, Plant Pathology and Post –harvest Food Loss. An Inaugural Lecture Series, 43, 2005, 29-72
321 [18]. K. A. Aidoo, Identification of yam tuber rots fungi from storage systems at the Kumasi Central market. A dissertation
322 submitted to Faculty of Agriculture, K.N.U.S.T, 2007.
323 [19]. O. A. Nwankiti, Studies on the Aetiology and Control of Acthracnose/Brown blotch disease Complex of Dioscoreaalata
324 in Nigeria.University of Nigeria, Nsukka, 1982.
325 [20]. R. H. Booth, Post harvest deterioration of Tropical root crops: Losses and their control. *Trop. Sci., 16(2),* 1974, 49-63
326 [21]. R.A. Noon, Storage and market diseases of yams. Trop. Sci., 20, 1978
327 [22]. R. N. Okigbo, Biological Control of Postharvest Fungal Rot of Yam (*Dioscorea* spp) with *Bacillus subtilis*. *Mycopathologia*,
328 *159,* 2005, 307-314.
329 [23]. M. O. Adebola and J. E. Amadi, Screening three *Aspergillus spp* for antagonistic activities against the cocoa black pod
330 organism (*Phytophthora palmivora*) *Agric. Bio. J.N. America, 13*, 2010a, 362-365
331 [24]. H. Mokhtar and D. Aid, Contribution in isolation and identification of some pathogenic fungi from wheat seeds, and
332 evaluation of antagonistic capability of *Trichderma harzianum* against those isolated fungi in vitro. *Agric. Bio. J.*
333 *N.Am. 4(2)*, 2013, 145-154
334 [25]. Y. Thakore, The biopesticide market for global agricultural use. *Industrial Biotechnology,* Vol. 2, 2006, pp. 194-208.
335 [26]. D. T. L. Sugar, E. E. Righettic, Sanchez and E. Khemira, Management of nitrogen and calcium in pear trees for
336 enhancement of resistance of post harvest decay. *Hortic. Technol.,* 2, 1997, 382-387.
337 [27]. C. S. Eze, *Studies on storage rot of cocoyam (Colocasia esculenta (L.) Schott) at Nsukka.* MSc Dissertation, Dept of
338 Botany, Univ of Nigeria, Nsukka. 1984,73pp
339 [28]. M. Cheesbrough, District Laboratory Practice in Tropical Counties. Low Price Edition, Cambridge University Press,
340 Cambridge, UK, 2000, 62-70.
341 [29]. M. Jawetz, G. F. Adelberg, Brooks, J.S. Butel, and S. A. Morse, *Medical Microbiology*. 23rd edition. McGraw Hill
342 Companies, Inc. Singapore, 2004, p.818
343 [30]. W. B. Lester, E. K. Timothy, T. Len and T. P. Hien, *Diagnostic manual for plant diseases in Vietnam,* 2008, p213

UNDER PEER REVIEW

344 [31]. B. Ritichie, *Practical techniques in plant pathology*. CAB. Walling ford. UK., 1991.
345 [32]. G. Agrios, *Plant pathology 5 ed.* 2004, Elsevier, London
346 [33]. H. C. Evans, K. A. Hoimes and A. P. Reid, Phylogeny of the Frosty Pod rot pathogen of cocoa. Plant Pathology, 52, 2003,
347 476-485.
348 [34] K. A. Gomez, and A. A. Gomez, *Statistical procedures for Agricultural Research 2nd Edition* John Wiley and sons. 1984 Pp 680
349 [35]. L. Korsten, and E. S. De Jager, Mode of action of *Bacillus subtilis* for control of avocado post harvest pathogens. *S. Afr.*
350 *Avocado Growers Assoc. Yearb, 18,* 1995, 124-130
351 [36]. A. Singh and R. Sharma, Biocontrol and Environmental Studies on Paper Degrading Mycoflora Isolated from
352 Sanganer Area, Jaipur, India *Int.J.Curr.Microbiol.App.Sci* 3(8), 2014, 948-956
353 [37]. T .E. Sangoyomi, Post-*harvest fungal deterioration of yam (Dioscorea rotundata. Poir) and its control.* Ph.D. Thesis.
354 University of Ibadan, Nigeria, 2004, p. 179.
355 [38]. B. Hajiehgrari, T. Mousa, Mohammed Mahdi, Davari Reza Mohammadi, Biological potential of some Iranian
356 Trichoderma isolates in the control of soil borne plant pathogenic fungi. African *Journal Bioteol. Control. 50,* 2008, 143-149
357 [39] G. W. Cochran, and G. M. Cox, Experimental *Designs. 2nd Edn* John willey and Sons Inc., 1992, pp: 611
358 [40]. B. Umamaheswari, B. Thakore and T. More, Post-harvest management of ber (*Ziziphus mauritiana* Lamk) fruit rot
359 (*Alternaria alternata* (Fr.) Keissler) using Trichoderma species, fungicides and their combinations, *Crop Protection, Vol.*
360 *28(6),* 2009, pp.525-32
361 [41]. E. N. Siameto, S. Okoth, N. O. Amugune, and N. C. Chege, Molecular Characterization and idedentification of
362 biocontrol isolates of Trichoderma harzianum from Embu District, Kenya. *Tropical and Subtropical Agroecosystem 13,* 2011,
363 81-90
364 [42]. M. Barbosa, K. Rehm, M. Menezes, and R. L. Mariano, Antagonism of *Trichoderma* species on *Cladosporium herbarum* and
365 their enzymatic characterization, *Brazilian Journal of Microbiology, Vol.32*, 2001, pp.98-104
366 [43]. N. S. Devaki, S. Shankara Bhat, S. G. Bhat and K. R. Manjunatha, Antagonistic activities of *Trichoderma harzianum*
367 against *Pythium* aphanidermatum and Pythium myriotylum on Tobacco. *Journal of Pytopathology 136*, 1992, 82-87.
368 [44]. E. J. Ekefan, A. Jama and S. R. Gowen, Potential of Trichoderma harzianum isolates in biocontrol of *Colletotrichum*
369 *capsici* causing anthracnose of pepper (Capsicum spp.) in Nigeria *Journal of Applied Biosciences 20*, 2009, 1138 - 1145
370 [45]. M. Lorito, S. L. Woo, M. D. D'ambrosio, G. E. Harman, C. K. Hayes, C. P. Kubicek and F. Scala, Synergistic interaction
371 between cell wall degrading enzymes and membrane affecting compounds. *Molecular Plant-Microbe Interaction 9,* 1996,
372 206-213.
373 [46]. B. K. Duiff, D. Pouhair, C. Olivian, C. Alabouvette and P. Lemanceau, Implication of systemic induced resistance in the
374 suppression of *Fusarium* wilt of tomato by *Pseudomonas flourescens* WC417r and by non pathogenic *Fusarium*
375 *oxysporum* Fo47. *European Journal of Plant Pathology 104*, 1998, 903-910.
376 [47]. A. T. Azza and D. A. Allam, Improving production under soikl infestation with Fusarium Pathogen 1 screening of
377 biocontrol agents. *Ass. Univ. Bull.Environ.Res.2*, 2004, 35-45.
378 [48]. J. W. J. Janisienwicz, Biocontrol of post-harvest disease of Apples with antagonistic mixtures. J. Phytopathol., 78, 1988, 194-
379 198.
380 [49]. M. O. Adebola and J .E. Amadi, Screening three *Aspergillus spp* for antagonistic activities against the cocoa black pod
381 organism (*Phytophthora palmivora*) *Agric. Bio. J.N. America, 13*, 2010a, 362-365.
382 [50]. F. Sempere, M. Santamarina, *In vitro* biocontrol analysis of Alternaria alternate (Fr.) Keissler under different environmental
383 conditions, *Mycopathologia*, 163(3), 2007, pp.183-90
384 [51]. D. J. Royse and S. M. Ries, The influence of fungi isolated from peach twigs on the pathogenicity of *Cytospora cinata.*
385 *Phytopathol. 63,* 1977, 603-607.
386 [52]. M. Shankar, D. I. Kurtboke and K. Sivasithamparam, Nutritional and environmental factors affecting growth and
387 antIfungal activity of a sterile red fungus against *Gaeumanomyces graminis var. Tritici. Can. J. Microbiol., 33*, 1994, 515-519.
388 [53]. T. O. Adejumo, T. Ikotun and D. A. Florin, Biological control of *Protomycopsis phaseoli,* the causal agent of leaf smut of
389 Cowpea. J. Phytopathology 147, 1999, 371-375.