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BIOCONTROL POTENTIAL OF *TRICHODERMA HARZIANUM* AGAINST ROT CAUSING FUNGI OF WHITE YAM (*DIOSCOREA ROTUNDATA* POIR*)* TUBERS

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ABSTRACT: *Biological control potential with Trichoderma harzianum in the control of postharvest fungal pathogens of Pepa white yam tubers in storage was carried out for two years. Rotted Ogoja and Pepa white yam tubers were collected from farmers' barns in Zaki-Biam, Benue State, Nigeria. Pathogenicity tests conducted on healthy Pepa yam tuber cultivars after fourteen days of inoculation revealed that the tubers were susceptible to A. flavus, F. moniliforme and P. expansum. Treatments comprised either inoculation of yam tubers with A. flavus, F. moniliforme and P. expansum alone or paired with T. harzianum as well as a control where the tubers were neither inoculated with antagonist nor with fungi pathogens and were stored for five months between December, 2015 and April, 2016 and between December, 2016 and April, 2017. Results obtained in the first year of storage showed that tubers treated with fungi pathogens alone caused mean percentage rot of between 8.89 % (P. expansum) and 20.00 % (A. flavus) while those treated with T. harzianum alone produced only 2.22 %. In the paired treatments, mean percentage rots were between 4.44 % (P. expansum × T. harzianum) and 6.67 % (A. flavus × T. harzianum). The Findings in the second year revealed 13.33 % (P. expansum), 22.22 % (A. flavus) and 4.44 % (T. harzianum) in the alone treatments while paired treatments produced mean rot of between 4.44 % (P. expansum × T. harzianum) and 8.89 % (A. flavus × T. harzianum). The Results revealed that P. expansum was the most antagonized while A. flavus was the least inhibited. The findings revealed that T. harzianum (biological control agent) was more effective in inhibiting the growth of A. flavus, F. moniliforme and P. expansum in the first year of storage compared with the second year of storage. The antagonist therefore has biological potentials in controlling fungi pathogens of yam in storage*.

KEYWORDS: Aspergillus flavus; Biocontrol; Pathogenicity Test; Trichoderma harzianum; Zaki-Biam.

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INTRODUCTION

Trichoderma harzianum is an antagonistic fungus that is widely recognized as an effective biocontrol agent for a range of important airborne and soil borne plant pathogens (Papavizas, 1985; Harman *et al.,* 2004; Mokhtar and Aid 2013). The genus *Trichoderma* is known to be promising members against soil-born plant parasitic fungi. Rot of crop produce has been known to reduce quantity and quality of yam both in the field and in storage (Amusa *et al.,* 2003). and fruits (Amusa *et al.,* 2003).

Studies conducted in different parts of the country have shown that fungal rot is the greatest cause of tuber losses in storage (IITA, 1993; Amusa *et al.,* 2003). These pathogenic fungi associated with yam rot in Nigeria are: *Aspergillus flavus, A. niger, Botryodiplodia theobromae, Colletotrichum spp, Fusarium oxysporum, F. solani, F. moniliforme, Penicillium purpurogenum, P. digitatum, P. oxalicum, Rhizoctonia sp, Rhizopus nodosus* (Markson *et al.,* 2012; Ogunleye and Ayansola, 2014; Gwa *et al.,* 2015; Gwa and Akombo, 2016; Shiriki *et al.,* 2015, Gwa and Abdulkadir, 2017; Gwa and Nwankiti, 2017a; Gwa and Ekefan, 2018; Gwa and Okrikata, 2019). Rot of yam tubers in storage is caused by up to 30 different pathogenic fungi (Ikotun, 1989 and Nahunnaro, 2008).

There are different methods of control of these pathogens such as chemical, use of natural plant extracts as well as biological control methods with antagonistic micro organisms. Chemical control is fast but with lots of adverse effects such as killing beneficial micro and macro organisms, risk to humans, pollution of the environment (Yadav, 2010; Lakshmeesha *et al.,* 2013a). The use of plant extracts in controlling these pathogens of crops has proven to be effective (Taiga *et al.,* 2008; Nweke 2015; Gwa and Akombo 2016; Gwa and Nwankiti 2017a; Zubairu and Gwa, 2019). Biological control using fungi belonging to the genus *Trichoderma* such as *T. harzianum, T. viride* and the genus *Pseudomonas* such as *P. syringae, P. chlororaphis* as well as *Bacillus subtilis* and *Gliocladium roseum* has been considered very effective as bio-control agents in controlling postharvest and storage rots of yam tubers (Okigbo, 2004; Okigbo and Emeka, 2010; Gwa *et al.,* 2016; Gwa and Nwankiti, 2017b, Gwa and Ekefan, 2017; Gwa and Abdulkadir, 2017; Gwa *et al.,* 2019). The bio control agents have no phytotoxic effects, target specific, eco-friendly with no pollution problem as well as promote plant growth (Mausam *et al.,* 2007; Harman *et al.,* 2004). The study therefore, focuses on the capabilities of *T. harzianum* as a bio control agent of postharvest rot causing fungal pathogens of yam tubers as alternative to synthetically used fungicides.

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MATERIALS AND METHODS

Source of *T. harzianum*

T. harzianum was collected from yam pathology unit, University of Ibadan, Nigeria. Stock cultures of the isolate were aseptically prepared and maintained on slants of acidified potato dextrose agar (PDA) in McCartney bottles and stored for subsequent studies.

Collection of rotted and healthy yam tubers

Ogoja and *Pepa* tubers of white yam (*D. rotundata*) tubers showing various degrees of rot symptoms were collected from yam farmers' barns in Zaki-Biam, Benue State, Nigeria which lies between longitudes $9^{o}\ 25^{'}$ and $9^{o}\ 28^{'}$E, and latitude $7^{o}\ 32^{'}$ and $7^{0}\ 35'$N respectively. The yam tubers were packaged in sterile polyethylene bags to avoid wounding before taken and to Advanced Plant Pathology Laboratory, Federal University of Agriculture, Makurdi, Nigeria for subsequent isolation and identification of pathogens two days after collection. The medium used for isolation of the pathogens was Potato Dextrose Agar (PDA).

Isolation and identification of fungi associated with rots of yam tubers

Tubers were washed in running tap water and were cut into approximately 2 x 2mm from the advancing edge of lesion with sterile scalpel. The cut tissues were surface sterilized for 2 minutes in 5 % Sodium hypochlorite solution in order to remove surface contaminants. The pieces were then rinsed in four successive changes of sterile distilled water and dried on sterile filter paper (Gwa and Nwankiti, 2017a). Four pieces of the sterilized tissues were plated out on the solidified potato dextrose agar (PDA). The plates were neatly covered with mastic tapes and incubated at ambient room temperature (30±5°C) for 7 days and growths were observed daily for the development of fungi. Sub-cultures of growing fungi mycelial were identified after 7 days of incubation when pure cultures were fully established (Gwa and Nwankiti, 2018). The grown pure cultures were used for identification of the fungi with the aid of a compound microscope and identification guide (Navi, *et al.,* 1999; Burgess *et al*., 2008).

Pathogenicity test of the isolated fungi

B. theobromae, A. flavus, A. niger, F. moniliforme, F. oxysporum, P. purpurogenum P. expansum and *Pestalotia* sp isolated from the rotted yam tubers were inoculated into healthy *Pepa* yam tubers. Healthy yam tubers were washed under running tap water and surfaced sterilized in 5 % Sodium hypochlorite solution for 2 minutes. The tubers were rinsed in four

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successive changes of sterile distilled water. Cylindrical discs of 5 mm were removed from the tubers using a sterile cork borer. Mycelial discs of each fungus measuring 4 mm in diameter were taken from 5 day-old culture of each of the fungi and each fungal disc was put into a hole in each of the tubers. Same procedure was replicated for the control experiment except that sterile agar discs were used instead of the inoculum obtained from the fungi in the holes created in the tubers (Gwa *et al.,* 2017). Petroleum jelly was used to completely seal the remaining holes to prevent contamination by other pathogenic organisms. The inoculated yam tubers were replicated three times for each of the pathogens and also in the control experiments. A total of 27 tubers of Pepa yam varieties were used in this experiment (three tubers for each of the eight pathogens and three tubers for control). The treatments were completely randomized and incubated for 14 days at ambient room temperature (30±5^{o}C) under sterile condition to allow for growth and establishment of the fungi organisms after which the tubers were examined for infection and disease development by cutting transversely at point of inoculation. Disease symptoms produced by artificial inoculation of the yam tubers with the pathogens after the incubation period were compared with those observed on the naturally infected tubers initially collected from farmers' barns. The fungi were re-isolated from the inoculated diseased yam tubers and cultured on PDA plates. The morphology of each pathogenic fungus was compared with that of the original culture obtained from the naturally infected tubers.

Preparation of fungal spore suspension and culture of *T. harzianum*

Spores suspensions of *A. flavus*, *F. moniliforme* and *P. expansum* and the antagonist; *T. harzianum* were prepared from 5 days old cultures grown on Potato dextrose agar (PDA) plates. Conidia from the surface of agar plate from these fungi were scrapped with a sterile glass rod to dislodge the spores (Nduagu *et al.,* 2008) and were each re-suspended in 1L of sterile distilled water containing 5 % Tween 80 (Ismet *et al*., 2012). The spore suspensions obtained were filtered through four folds layer of sterile cheese cloth into a sterile 1000 mL Pyrex glass beaker. The suspension concentrations were determined by using an improved Neubauer haemocytometer (model BS 748) and adjusted to 1×10^6 spores per mL.

Determination of the interaction between rot fungi (*A. flavus, F. moniliforme* and *P. expansum*) and biological antagonist (*T. harzianum*) on healthy *Pepa* white yam tubers

Healthy white yam tubers of *Pepa* cultivar (*D. rotundata*) which were used for storage were when applied with different treatments. Treatments comprising *A. flavus*, *F. moniliforme* and *P. expansum* were each paired with *T. harzianum* separately to determine their effects on rot

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development in *Pepa* white yam tubers during storage. The tubers were also inoculated with each of the fungal isolates separately without *T. harzianum* as a bio control agent. Yam tubers without fungal isolates and *T. harzianum* served as the control. *T. harzianum* was paired with the three pathogenic fungi and yam tubers were inoculated separately according to the following inoculation regime (Okigbo and Emeka (2010) :

(a) Uninoculated yam tubers (control);
(b) Tubers inoculated with *A. flavus* alone;
(c) Tubers inoculated with *P. expansum* alone;
(d) Tubers inoculated with *F. moniliforme* alone;
(e) Tubers inoculated with *T. harzianum* alone;
(f) Tubers inoculated with *T. harzianum* and *A. flavus* simultaneously;
(g) Tubers inoculated with *T. harzianum* and *P. expansum* simultaneously;
(h) Tubers inoculated with *T. harzianum* and *F. moniliforme* simultaneously;

Three tubers formed a treatment; each of the eight treatments was replicated three times giving a total of nine tubers per treatment. 72 tubers of yams were used in this experiment for the eight different treatments. The suspension for each of the treatments was poured in a hand sprayer and the yam tubers were sprayed accordingly (Sarma 1984; Wilson and Pusey, 1985). The yam tubers were arranged in completely randomized design and stored at ambient room temperature ($30 \pm 5\ ^0C$) for five months in the first years and also five months in the second year for two years. Record of rotted tubers were kept on periodic basis and cumulative percentage rot during storage of yam tubers that were inoculated with *T. harzianum* and the post harvest pathogens of yams in different combinations were calculated at monthly interval for five months according to the method described by Dapaah, (2013), thus, calculated as follows;

$$Percentage\ rot\ (\%) = \frac{N}{T} \times \frac{100}{1}$$

Where,
% = Percentage rotten of tubers
N = Number of rotten tubers at the time of evaluation
T = Total number of tubers stored per the treatment

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Data analysis

Data that were collected from the different treatments were subjected to Analysis of variance (ANOVA) using GenStat Discovery Edition 12, Graph Pad Prism 6 for trend graphs and significant means for each measured parameter were separated using Fisher's least significant difference (FLSD) ($P \leq 0.05$) (Cochran and Cox, 1992).

Results and Discussion

Isolation and identification of pathogenic fungi from rotted yam tubers

B. theobromae, A. flavus, A. niger, F. moniliforme, F. oxysporum, P. purpurogenum P. expansum and *Pestalotia* sp. were identified from the rotted Ogoja and *Pepa* white yam tuber cultivar. These fungi are rot-causing pathogens of *D. rotundata* in different parts of Nigeria (Ogunleye and Ayansola, 2014; Okigbo *et al.,* 2015, Gwa and Akombo, 2016; Gwa and Nwankiti, 2017b; Gwa *et al.,* 2018a,b). The occurrence of *B. theobromae, A. flavus, A. niger, F. oxysporum* in high numbers in major yam producing areas in Nigeria have been previously demonstrated by Ogunleye and Ayansola (2014), Okigbo *et al.,* (2015); Shiriki *et al.,* (2015) Gwa and Richard, (2018)

Pathogenicity tests carried out on the healthy *Pepa* white yam tubers produced typical symptoms of the pathogens isolated from the naturally infected tubers and the re-isolation of identical fungi from the artificially inoculated rotted yam tubers fulfilled Koch's postulates and established the pathogenicity of the yam fungi isolates. The tubers that were treated without mycelial from different fungi (control tubers) did not produce rot suggesting the absence of inoculum in the bored yam tissues. Table 1 shows the result of inoculation of healthy white yam tuber of *Pepa* with *T. harzianum* and rot-causing fungi alone and in combination for two years. There was no significant difference ($P \leq 0.05$) in mean percentage rot in the treatments for the five months storage period between for the two years. However, *P. expansum* was most antagonized followed by *F. moniliforme* while the least antagonised pathogen was *A. flavus*. The result revealed that *T. harzianum* was more antagonistic in the first year of storage compared with the second year of storage (Table 2). Similar results were obtained by Markson *et al.,* (2012) and Nikolajeva *et al.,* (2012) who showed that *Trichoderma* species are useful in the control of rot fungi of fruits, vegetables and tuber diseases.

Result obtained by Okigbo and Emeka (2010) showed the biological control of rot-inducing fungi of water yam (*D. alata*) using *T. harzianum*, *Pseudomonas syringae* and *P. chlororaphis*

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which they both revealed that the three antagonists significantly inhibited the growth of *B. theobromae* and *F. solani* on stored yam tubers. Result obtained by Okigbo (2005) showed the inhibitory potentials of *Bacillus subtilis* in controlling post harvest fungal pathogens of yam tubers in storage

The use of *T. harzianum* in controlling postharvest fungal pathogens of yam tubers in storage for five months is similar to the result obtained by Okigbo and Ikediugwu, (2000) that used a single application of *T. harzianum* and protected yam tubers in storage for up to 6 months. Though *T. harzianum* was able to reduce rot pathogen infections, the effect of the antagonist pairing with *P. expansum* was more potent than the inhibition recorded between the interaction of *T. harzianum* and *F. moniliforme* and *P. expansum*. The difference in antagonistic potency could probably be due to production of antifungal phenolic compounds which function by breaking down the polysaccharides, chitin, and glucans that are responsible for the rigidity of fungal cell walls, thereby destroying cell wall integrity and limiting the growth of these pathogens (Anita *et al*., 2012).

The tubers that were neither treated with pathogenic fungi nor antagonist (control tubers) showed between 8.89 % and 15.56 % rot in the first and second year respectively after five months of storage similar to the result obtained by Ekundayo and Naqvi (1972) who reported losses in yam tubers due to rots pathogens to be between 10 % and 15 % in the first three months of storage. The results however, disagreed with studies conducted by Okigbo and Ikediugwu (2000) who estimated an average of between 20 and 39.5 % of stored tubers lost to rot pathogens. This result is also different with the work of Arinze (2005) and Okigbo *et al*., (2009b) that reported that about 50 % reduction of the total stored tubers has been reported lost to diseases within the first 6 months of storage in Nigeria. The findings revealed that *T. harzianum* (biological control agent) was more effective in inhibiting the growth of *A. flavus, F. moniliforme* and *P. expansum* in the first year of storage compared with the second year of storage. The difference in the antagonistic potential of *T. harzianum* could probably be due to favourable environmental condition such as adequate moisture or relative humidity which increased the interaction of the rot-causing fungi with the host yam tissues and decreased the potentials of *T. harzianum* in the second year. It has been reported that fungal species occurred more abundantly in the more humid months where the environmental conditions favoured the production of inoculum more than in the drier less humid period (Ekundayo, 1986; Agrios, 2005).

Table 1: Cumulative percentage rot of *Pepa* white yam tubers inoculated with bio control agent (BCA) and the postharvest fungi pathogens of white yam in different combinations for five months

Treatment	**Period of Storage**					
	Dec., 2015	**Jan., 2016**	**Feb., 2016**	**Mar., 2016**	**Apr., 2016**	**Mean**
1st Storage Period						
Control	0.00±0.00	0.00±0.00[b]	11.10±11.10	11.10±11.10	22.20±22.20	8.89±5.11
A. flavus alone	0.00±0.00	22.20±11.10[a]	22.20±11.10	22.20±11.10	33.33±0.00	20.00±4.36
F. moniliforme alone	0.00±0.00	0.00±0.00[b]	11.10±11.10	11.10±11.10	22.20±11.10	15.56±6.40
P. expansum alone	0.00±0.00	0.00±0.00[b]	11.10±11.10	33.30±19.20	33.30±19.20	8.89±3.94
T. harzianum alone	0.00±0.00	0.00±0.00[b]	0.00±0.00	0.00±0.00	11.10±11.10	2.22±2.22
A. flavus X T. harzianum	0.00±0.00	0.00±0.00[b]	0.00±0.00	11.10±11.10	22.20±11.10	6.67±3.56
F. moniliforme X T. harzianum	0.00±0.00	0.00±0.00[b]	0.00±0.00	11.10±11.10	11.10±11.10	4.44±3.03
P. expansum X T. harzianum.	0.00±0.00	0.00±0.00[b]	0.00±0.00	11.10±11.10	11.10±11.10	4.44±3.03
LSD	-	*11.78*	*23.55*[ns]	*35.33*[ns]	*40.80*[ns]	*18.25*[ns]
2nd Storage Period						
	Dec., 2016	**Jan., 2017**	**Feb., 2017**	**Mar., 2017**	**Apr., 2017**	**Mean**
Control	0.00±0.00	11.10±11.10	22.20±11.10	22.20±22.20	33.30±19.20	15.56±6.40
A. flavus alone	0.00±0.00	11.10±11.10	11.10±11.10	33.33±0.00	44.40±11.10	22.22±5.31
F. moniliforme alone	0.00±0.00	0.00±0.00	11.10±11.10	22.20±22.20	33.30±19.20	17.78±6.40
P. expansum alone	0.00±0.00	11.10±11.10	11.10±11.10	33.30±19.20	33.30±19.20	13.33±6.34
T. harzianum alone	0.00±0.00	0.00±0.00	0.00±0.00	11.10±11.10	11.10±11.10	4.44±3.03
A. flavus X T.harzianum	0.00±0.00	0.00±0.00	11.10±11.10	11.10±11.10	11.10±11.10	8.89±3.94
F. moniliforme X T.harzianum	0.00±0.00	0.00±0.00	0.00±0.00	11.10±11.10	11.10±11.10	8.89±3.94
P. expansum X T.harzianum.	0.00±0.00	0.00±0.00	11.10±11.10	11.10±11.10	11.10±11.10	4.44±3.03
LSD	-	*20.40*[ns]	*28.85*[ns]	*45.61*[ns]	*44.07*[ns]	*19.71*[ns]

Means on the same column with different superscript are statistically significant (P≤0.05). ns = not significant

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Table 2: Mean percentage rot of *Pepa* white yam tubers inoculated with *T. harzianum* (BCA) and the postharvest fungi pathogens of white yam in different combinations for two Years

Treatment	Time of Storage		T-Value	Df	P-Value
	1st Year	2nd Year			
Control	8.89±5.11	15.56±6.40	-0.81	26	0.42
A. flavus alone	20.00±4.36	22.22±5.31	-0.32	26	0.74
F. moniliforme alone	15.56±6.40	17.78±6.40	-0.60	23	0.55
P. expansum alone	8.89±3.94	13.33±6.34	-0.25	28	0.80
T. harzianum alone	2.22±2.22	4.44±3.03	-0.59	25	0.55
A. flavus X T.harzianum	6.67±3.56	8.89±3.94	-0.42	27	0.67
F. moniliforme X T.harzianum	4.44±3.03	8.89±3.94	-0.89	26	0.37
P. expansum X T. harzianum.	4.44±3.03	4.44±3.03	0.00	28	1.00

CONCLUSION

The result has demonstrated that *T. harzianum* has potentials to control *A. flavus*, *F. moniliforme* and *P. expansum* which are postharvest fungi pathogens of in post harvest *Pepa* white yam tubers. It is therefore, concluded that the application of the antagonist will provide better alternative measures in reducing rot of yam tubers in storage compared with the use of synthetically produced fungicides which are in many cases destructive to the ecosystem, expensive, non target specific and toxic to the applicator as well as beneficial organisms.

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