Mycoparasitic capabilities of *Trichoderma harzianum* and two botanicals against fungi associated with postharvest rots of *Ipomoea batatas* (L.) Lam

ABSTRACT

The mycoparasitic potentials of *Trichoderma harzianum*, and growth inhibitory effects of *Vernonia amygdalina* (bitterleaf) and *Zingiber officinale* (ginger) on rot fungi in *Ipomoea batata* were examined. Rotting tubers were collected from Agbowo, Ojoo and Bodija markets in Ibadan. They were taken to the laboratory under sterile conditions. Different concentrations of the plants' extracts and spore suspensions of *T. harzianum* were prepared. The fungi isolated from the rotting tubers were later cultured on plates impregnated with different concentrations of the extracts and *T. harzianum*. Incubation was done at 28^0C for 14 days. Data collection was done at 24 hours interval. The fungi isolated were *Aspergillus niger* and *Rhizopus stolonifer*. *T. harzianum* at 1 x 10^{-3} had a significantly ($p \leq 0.05$) better pathogens' inhibition than 1 x 10^{-5}. Inoculation of *T. harzianum* before the pathogens gave total inhibition. Inhibition of *A. niger* was significantly ($p \leq 0.05$) higher than *R. stolonifer*. Plant extracts from ethanol gave significantly ($p \leq 0.05$) better pathogens' inhibitions than that from distilled water. Extracts from both plants gave significantly ($p \leq 0.05$) better growth inhibition than control. Growth inhibition was significantly ($p \leq 0.05$) higher at absolute concentration of both extracts than other concentrations. Extract from ginger gave significantly ($p \leq 0.05$) better inhibition than that from bitter leaf. F-values for model ($P > 0.0001$) and concentrations ($P > 0.0024$) for the *T. harzianum* were highly significant. F-values for model ($P > 0.0001$), concentration ($P > 0.0001$) and treatment ($P > 0.0001$) for the plants extracts were also highly significant. The results further underscore the mycoparasitic potentials of *T. harzianum* as well as growth inhibitory effects of *Z. officinale* and *V. amygdalina* on fungi rot pathogens of *Ipomoea batata*.

Keywords: *Trichoderma harzianum*, *Vernonia amygdalina, Zingiber officinale,* mycoparasitic, pathogens, Inhibition

INTRODUCTION

Sweet potato is the most important food crop produced globally after wheat, rice, maize, potato, barley and cassava (FAO, 2008) and plays an important role in household food security in many countries (Mutuura *et al*., 1992; Ray *et al*., 2010; Tomlins *et al*., 2010). Sweet potato has a high economic value and ranks seventh among the world's major crops with an annual production of over 100 million tonnes (Nwokocha, 1992).

Sweet potato is eaten fresh, steamed, or boiled. The leaves are eaten as vegetables or may be processed into flour or starch while the vines are fed to livestock (Hu *et al.,* 2004).
Sweet potato is prone to attack by microorganisms especially fungi. This occurs at different stages including; field, harvest and storage stages. Infection is mainly facilitated by mechanical injuries of the root and environmental conditions, but the physiological condition of the root may influence infection (Wills *et al*., 1998). In addition, environmental and cultural stresses during growth also directly or indirectly predispose the roots to post harvest microbial infection (Ray and Ravi, 2005; Ray *et al*., 2010).

The most pathogenic fungi associated with sweet potato globally include several species of *Rhizopus* including; *Rhizopus nigricans, Rhizopus stolonifer* (commonly called bread mold) and *Rhizopus oryzae* (Scot, 2009; Ray *et al*., 2010). However, rot causing fungi associated with sweet potatoes include; dry rot (*Aspergillus niger, Aspergillus fumigates*, soft rot (*Rhizopus stolonifer*) among others (Agu *et al.,* 2015). Other fungi reported to be associated with rottening of sweet potato include *Fusarium oxysporum*, *Ceratocysts fimbriata*, *Macrophomina phaseolina*, *Fusarium solani, Aspergillus ochraceus, Fusarium moniliforme, Fusarium oxysporum* among others (Clark and Hoy, 1994). Onuegbu (2002) implicated *Penicillium* sp., *Certocystis fimbriata*, *Diaporthe batatalis*, *Aspergillus niger and Aspergillus flavus*, as fungi responsible for decay of sweet potato tubers.

These fungi create local discoloration and disruption of surrounding tissues of infected tubers (Snowdon, 1991), resulting in changes in appearance, deterioration of texture and possibly flavor or taste. These pathogens have lead to enormous loss of sweet potato tubers despite its economic and nutritive value. The presence of the rot-causing fungi on these tubers most especially *Aspergillus niger* poses a serious threat to health of consumers as the organism could produce mycotoxins, which are lethal when consumed (Agu *et al*., 2015).

Fungicides such as Dichloronitroanline are used to protect tubers against *Rhizopus* soft rot (Clark and Moyer, 1988). However, the use of synthetic fungicides apart from their potential danger to both the farmer and environment are unaffordable by most farmers (Obagwu *et al*., 1997).

Biological Antagonists of phytopathogenic fungi have been used to control plant diseases and 90 per cent of such applications have been carried out with different strains of *Trichoderma* (Monte, 2001). *Trichoderma* is free living, asexually reproducing and filamentous fungi. It is an exceptionally good model of biocontrol agent as it is widely spread, easy to isolate and culture, multiply rapidly on many substrates, act as mycoparasite, strong opportunistic invaders, avirulent plant symbionts, competes for food and site, prolific producers of spores and powerful antibiotics, antifungal compounds, secondary metabolites and enzymes. These properties make these fungi ecologically very successful and are the reasons for their ubiquitousness (Kubicek *et al*., 2002).

Plant extracts are products that are made out of plants in form of decoctions, infusions and powders (Adodo, 2004). Plant extracts have been known for their medicinal and antimicrobial properties since ancient times (Jabeen, 2006; Lalitha *et al*., 2010). They offer a greater scope than synthetic chemicals as they are relatively safe, easily biodegradable and ecofriendly (Enikuomehin, 2005; Khan and Nasreen, 2010; Sukanya *et al*., 2011; Gurjar *et al*., 2012). Plants have ability to synthesize aromatic secondary metabolites, like phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins. Phenolic structures such as eugenol and thynol with these components show high antimicrobial effect and aid defense mechanism in plant against pathogens (Cowan, 1999; Das *et al*., 2010). Recent studies on the use of plant extracts have opened a new avenue for the control of plant diseases. These plants extracts have been reported to be safe, non-phototoxic to man, but effective against plant pathogens (Shivpuri *et al*., 1997).

Vernonia amygdalina also known as Bitter leaf is a member of the squash family of plants. The leaf extracts of bitter leaf are used to combat fungal infections as the plants are widely grown and used in different parts of Nigeria in traditional health care services (Oliver–Bever, 1986; Gill, 1992). The antifungal property of bitter leaf was also reported by Iwu (1993).

Leaf extracts of *Vernonia amygdalina* (bitter leaf) have inhibitory effects on fungal pathogens. *Zingiber officinale* (ginger) belongs to the Zingiberaceous plants which are characterized by their

tuberous or non-tuberous rhizomes, and have strong aromatic and medicinal properties (Chen *et al*., 2008). studies show that the ginger's constituents acted as strong antioxidants and effective antimicrobial agents (Mahady *et al*., 2005). The extract of *Zingiber officinale* (ginger) at various concentrations has been reported by Wokocha and Okereke (2005) to possess fungicidal effect against the growth of some soil borne fungi.

MATERIALS AND METHODS

Collection of samples

Diseased Sweet potato tubers were obtained from various markets within Ojoo, Bodija and Agbowo, Ibadan. These diseased samples were kept in polyethene bags and were appropriately labeled.

Media preparation

The culture media (PDA) used was prepared by mixing 19.5g potato dextrose agar powder and 500ml of distilled water in a 500ml conical flask. The conical flask was corked with cotton wool wrapped in aluminum foil. The mixture was gently shaken and autoclaved at 121^0C for 15 minutes after which it was allowed to cool. To prevent bacterial contamination, the resultant mixture (PDA) was acidified using lactic acid (fifty drops) after which they were poured into Petri-dishes and allowed to gel.

Isolation of fungal species from rotting sweet potato tubers

Diseased sweet potato tubers were cut into pieces using a blade and sterilized with 70% ethanol. The pieces plated unto PDA plates by means of a sterilized inoculating needle and were placed in the Petri dishes containing the solidified medium. The Petri dishes were then marked and labeled after which they were incubated at room temperature. Further sub-culturing was carried out until pure cultures of single species isolates were obtained.

Characterization and identification of isolated fungi

This was done based on the description of the gross morphological appearance of fungal colonies on the potato dextrose agar culture medium and the slide culture technique for microscopic evaluation according to method by Watanabe (2002).

Pathogenicity Test

Fresh, healthy and matured sweet potato tubers were obtained from the market and were surface sterilized with 70% ethanol. Each potato was wounded by removing a cylindrical cone from a portion of the tuber with the aid of a sterilized cork borer. Sterile inoculating needle was used to pick from the pure cultures of the pathogen and placed gently in the wounded part. The cylindrical cones were replaced back. The wounded parts were sealed with Vaseline. The sweet potatoes were then incubated at room temperature. These tubers were then examined daily to access and record the extent of fungal spoilage on each potato after which the pathogen is re-isolated from the inoculated sweet potato tubers. This test which conforms to Koch's postulate confirms the pathogenicity of the isolated fungi.

Collection of Biocontrol Fungus (*Trichoderma harzianum*)

A mixed culture of fungi containing *Trichoderma* sp. was obtained from the department of botany in the University of Ibadan, Nigeria. The *Trichoderma* sp. was re-isolated onto a freshly prepared potato dextrose agar medium until pure culture of *Trichoderma* sp. was obtained.

Plant extracts preparation

Two plant species were used in this study to develop extract formulation, namely *Vernonia amygdalina* and *Zingiber officinale*. These plants have been proven to possess inhibitory activity against fungi (Sharma *et al*., 2011; Suleiman and Emua, 2009). The leaves of *Vernonia amygdalina* and Rhizome of *Zingiber officinale* were collected, washed under a running tap water, air dried for weeks at room temperature and grounded into powdered form. The powdered samples were added to a different conical flask containing the extraction solvent (sterile distilled water and Ethanol). These mixtures were allowed to stand for 48 hours with periodic shaking in order to homogenize. Filtration was done through a double layered muslin cloth and No.1 Whatman filter paper prior to evaporation (Sawsan *et al*., 2011).

Evaluation of the effects of the plant extracts and *Trichoderma harzianum* on the growth of fungal isolates

These extracts were tested *in vitro* at various concentrations (25%, 50%, 75% and 100%) on the rot causing fungi associated with sweet potato spoilage by growing each fungus on a solidified PDA medium impregnated with 1ml of each plant extract concentration. Controls were set up in which the test fungi were inoculated on PDA with no plant extract. Three replicates were set up for each treatment. Aqueous suspension of the biocontrol fungus (*Trichoderma harzianum*) at different concentrations 10^{-5} (1.35×10^{6}) spores/ml and 10^{-3} (7.2×10^{9}) spores/ml was prepared in a test tube and 1ml of the *Trichoderma* suspension was introduced into the growth medium (PDA) in a Petri dish before and after inoculation with the rot pathogens. Controls were set up in which the test fungi were inoculated on the PDA with no *Trichoderma harzianum*. Three replicates were set up for each treatment and Fungi toxicity was recorded in terms of Mean mycelia inhibition.

Statistical Data Analysis

The experiment was laid out in a Completely Randomized Design (CRD). The data collected was subjected to analysis of variance (ANOVA) using the Generalized Linear Model (GLM) procedure of SAS (version 9.1). The differences between means were separated using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$.

RESULTS

The isolated fungi associated with post harvest rot of sweet potato were *Aspergillus niger* and *Rhizopus stolonifer.* Table 1 shows the antifungal activity of *T. harzianum* on *A. niger* and *R. stolonifer* from sweet potato tubers. The F-values for model, concentrations, days, pathogens and treatments were all highly significant ($P \leq 0.05$, $R^2 = 0.99$). The F-values for the interactions

between variables were also highly significant except for the interactions between concentrations and days; pathogens and concentrations.

The introduction of *T. harzianum* before the pathogens was observed to be the best treatment in the growth inhibition of *Aspergillus niger* and *Rhizopus stolonifer* compared to other treatments as shown in Table 2 with total growth inhibition of both fungi ($P \leq 0.05$, $R^2 = 0.99$). *Aspergillus niger* was observed to be better inhibited by *T. harzianum* with mycelia growth of 15.87mm than *Rhizopus stolonifer* with mycelia growth of 21.15mm (Table 3). At concentration 1×10^{-3} both *A. niger* and *R. stolonifer* were better inhibited by *T. harzianum* having radial growth of 15.41mm and 20.52mm respectively when compared to concentration 1×10^{-5} with radial growth of both pathogens at 16.34mm and 21.77mm respectively (Table 4). Table 5 shows General performance of *A. niger* and *R. stolonifer* isolated from the rotting sweet potato among incubation days after treating with two concentrations of *T. harzianum***.** The growth inhibition of the isolated fungi differed significantly with respect to incubation periods i.e. *Trichoderma harzianum* had significantly different inhibitory effect on the growth of the test fungi amongst days of incubation ($P \leq 0.05$, $R^2 = 0.99$).

Table 6 shows the for antifungal activity of *Zingiber officinale* and *Vernonia amygdalina* extract on *A. niger* and *R. stolonifer* from rotting sweet potato tubers. The F-values for model, concentrations, days, pathogens and treatments were all highly significant ($p \leq 0.05$, $R^2 = 0.99$). The F-value for the interactions between variables were also highly significant except for the interactions between concentrations and days; treatment, concentrations and days; pathogen, concentration and days. The growths of *A. niger* and *R. stolonifer* were better inhibited by ethanol extract of ginger with radial growth of 7.42mm and 8.01mm respectively when compared with other treatments including controls as shown in Table 7. The absolute ethanol extracts of *Zingiber officinale* had the highest growth inhibitory effect on both *A. niger* (0.52mm) and *R. stolonifer* (0.00mm) compared to all other concentrations of the plant extract while 25% concentration had the least growth inhibitory effect on both fungi compared to 75% and 50% concentrations ($P \leq 0.05$, $R^2 = 0.99$) (Table 8). The absolute ethanol extracts of *Vernonia amygdalina* had the highest growth inhibitory effect on both *A. niger* (15.98mm) and *R. stolonifer* (11.92mm) compared to all other concentrations of the plant extract as shown in Table 9 ($P \leq 0.05$, $R^2 = 0.99$). Table 10 shows general performance of *A. niger* and *R. stolonifer* among incubation days after treating with various concentrations of extracts. The growth inhibition of

the isolated fungi differ significantly with respect to incubation periods i.e. The plants extracts had significantly different inhibitory effect on the growth of the isolated fungi among days of incubation ($P \leq 0.05$, $R^2 = 0.99$).

Table 1: ANOVA table for antifungal activity of *T. harzianum* on *A. niger* and *R. stolonifer* from sweet potato tubers

Source	DF	SS	Mean Square	F value	Pr > F
Model	157	3386.91	21.57	114.46	0.0001**
Concentration	1	1.75	1.75	9.30	0.0024**
Days	9	84.77	9.42	49.97	0.0001*
Pathogen	1	39.31	39.31	208.5	0.0001**
Treatments	4	2715.47	678.87	3601.82	0.0001**
Concentration*Days	9	1.42	0.16	0.84	0.5827
Pathogen*Concentration	1	0.06	0.06	0.32	0.5724
Treatment*Concentration	4	8.65	2.16	11.47	0.0001**
Pathogen*Days	8	15.64	1.95	10.37	0.0001**
Treatments*Days	36	345.80	9.61	50.96	0.0001**
Treatments*Pathogen	4	54.13	13.53	71.79	0.0001**
Pathogen*Concentration*Days	8	3.08	0.39	2.05	0.0401*
Treatment*Concentration*Days	36	12.32	0.34	1.82	0.0034**
Treatment*Pathogen*Concentration	4	47.04	11.76	62.40	0.0001**
Treatment*Pathogen*Days	32	32.28	1.01	5.35	0.0001**
Error	412	77.65	0.19		
Corrected Total	569	3464.56			

**=highly significant

Table 2: Mean growths (mm) of *A. niger* and *R. stolonifer* in the presence of *T. harzianum* at different treatments

Treatment	*Aspergillus niger*	*Rhizopus stolonifer*
Pathogens alone (control)	52.50[a]	62.34[a]
Trichoderma sp after pathogens	20.59[b]	35.91[b]
Trichoderma sp and pathogens	6.28[c]	7.49[c]
Trichoderma sp before pathogens	0.00[d]	0.00[d]
Trichoderma sp alone	0.00[d]	0.00[d]
R^2	0.99	0.99

Means with different letter are significantly different ($p \leq 0.05$)

Table 3: Comparisons of the pathogens' growth inhibitions by *T. harzianum*

Pathogen	Mean growth (mm)
Rhizopus stolonifer	21.15[a]
Aspergillus niger	15.87[b]
R^2	0.99

Means with different letters are significantly different ($p \leq 0.05$)

Table 4: Effectiveness of *T. harzianum* at different concentrations on mean growths (mm) of the pathogens

Concentration (spores/ml)	*Aspergillus niger*	*Rhizopus stolonifer*
1×10^{-5}	16.34[a]	21.77[a]
1×10^{-3}	15.41[b]	20.52[b]
R^2	0.99	0.99

Means with different letters are significantly different ($p \leq 0.05$)

Table 5: General performance of *A. niger* and *R. stolonifer* among incubation days after treating with two concentrations of *T. harzianum*

Days	*Aspergillus niger*	*Rhizopus stolonifer*
10	19.77^{a}	19.38^{d}
9	19.45^{ab}	20.33^{d}
8	19.20^{ab}	22.28^{c}
7	18.40^{bc}	23.82^{b}
6	17.77^{cd}	24.35^{b}
5	16.98^{d}	16.98^{f}
4	16.00^{e}	25.88^{a}
3	14.10^{f}	23.72^{b}
2	11.67^{g}	19.45^{d}
1	05.43^{h}	11.13^{e}
R^2	0.99	0.99

Means with different letter are significantly different ($p \leq 0.05$)

Table 6: ANOVA table for antifungal activity of *Zingiber officinale* and *Vernonia amygdalina* extract on *A. niger* and *R. stolonifer* from sweet potato tubers

Source of variation	DF	SS	Mean Square	F value	Pr > F
Model	344	12378.18	35.98	72.10	0.0001**
Concentration	3	67.05	22.35	44.78	0.0001**
Days	9	1191.66	132.41	265.31	0.0001**
Pathogen	1	668.58	668.58	1339.65	0.0001**
Treatment	5	8655.10	1731.02	3468.50	0.0001**
Concentration*Days	27	7.55	0.28	0.56	0.9665
Pathogen*Concentration	3	6.62	2.21	4.42	0.0042**
Treatment*Concentration	15	221.69	14.78	29.61	0.0001**
Pathogen*Days	9	113.31	12.59	25.23	0.0001**
Treatment*Days	45	562.05	12.49	25.03	0.0001**
Treatment*Pathogen	5	565.97	113.19	226.81	0.0001**
Pathogen*Concentration*Days	27	3.34	0.12	0.25	1.0000
Treatment*Concentration*Days	135	44.54	0.33	0.66	0.9987
Treatment*Pathogen*Concentration	15	128.36	8.56	17.15	0.0001**
Treatment*Pathogen*Days	45	42.37	3.16	6.34	0.0001**
Error	1095	546.48	0.50		
Corrected Total	1439	12924.66			

**=Highly significant

Table 7: Mean growths (mm) of the pathogens on plates impregnated with extracts of *Zingibe officinale* and *Vernonia amygdalina*

Treatment	*Aspergillus niger*	*Rhizopus stolonifer*
Pathogen alone (control)	77.37[a]	55.49[a]
Aqueous extract of bitter leaf and Pathogen	76.66[a]	46.06[b]
Aqueous extract of ginger and pathogen	71.03[b]	46.34[b]
Pathogen alone (control)	12.42[d]	2.90[d]
Ethanol extract of bitter leaf and Pathogen	24.54[c]	18.88[c]
Ethanol extract of ginger and pathogen	7.42[e]	8.01[e]
R^2	0.99	0.99

Means with different letter are significantly different ($p \leq 0.05$)

Table 8: Impact of different concentrations of *Zingiber officinale* extracts on the growth (mm) of *A. niger* and *R. stolonifer* from sweet potatoes

Concentrations (%)	*Aspergillus niger*	*Rhizopus stolonifer*
25	18.38[a]	26.42[a]
50	16.62[a]	13.23[b]
75	16.10[a]	10.03[b]
100	0.52[b]	0.00[c]
Control	77.37[c]	55.49[d]

Means with different letter are significantly different ($p \leq 0.05$)

Table 9: Impact of different concentrations of *Vernonia amygdalina* extracts on the growth (mm) of *A. niger* and *R. stolonifer* from sweet potatoes

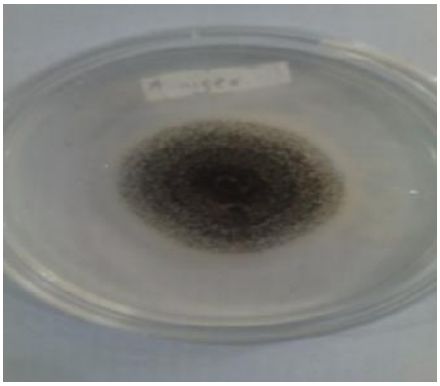
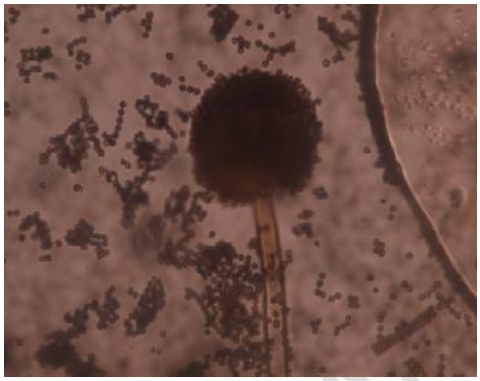
Concentrations (%)	*Aspergillus niger*	*Rhizopus stolonifer*
25	22.78[a]	31.65[b]
50	20.07[b]	41.28[a]
75	16.72[c]	13.33[c]
100	15.98[c]	11.92[c]
Control	77.37[d]	55.49[d]

Means with different letter are significantly different ($p \leq 0.05$)

Table 10: General performance of *A. niger* and *R. stolonifer* among incubation days after treating with various concentrations of extracts.

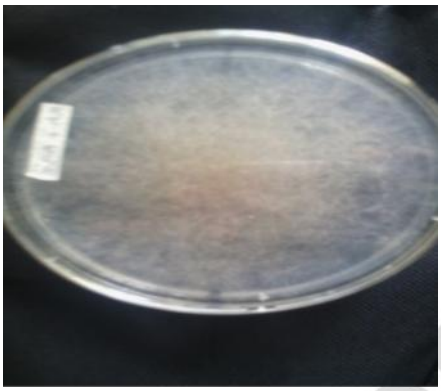
Days	*Aspergillus niger*	*Rhizopus stolonifer*
10	50.77[a]	45.01[a]
9	49.85[ab]	43.56[b]
8	49.47[b]	41.47[c]
7	48.44[c]	37.74[d]
6	48.26[c]	35.10[e]
5	46.79[d]	32.99[f]
4	46.18[d]	28.91[g]
3	43.78[e]	23.74[h]
2	38.91[f]	16.53[i]
1	26.67[g]	07.78[j]
R^2	0.99	0.99

Means with different letter are significantly different ($p \leq 0.05$)

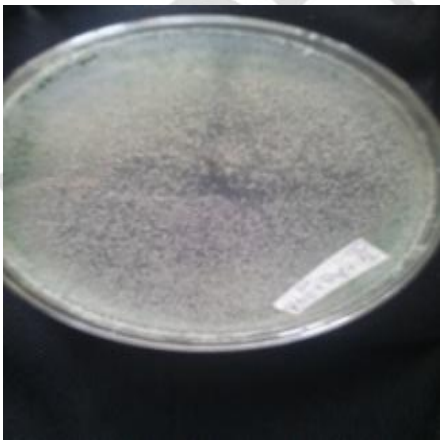
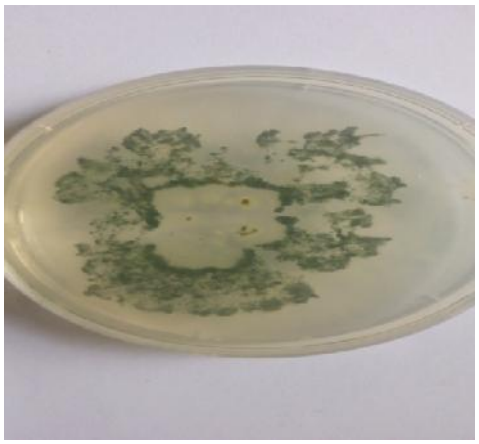
a **b**

Plate 1: Pure culture (a) and Photomicrograph (b) of *Aspergillus niger*




a **b**

Plate 2: Pure culture (a) and Photomicrograph (b) of and *Rhizopus stolonifer*

a **b**

Plate 3: Young (a) and old cultures (b) of *Trichoderma harzianum*

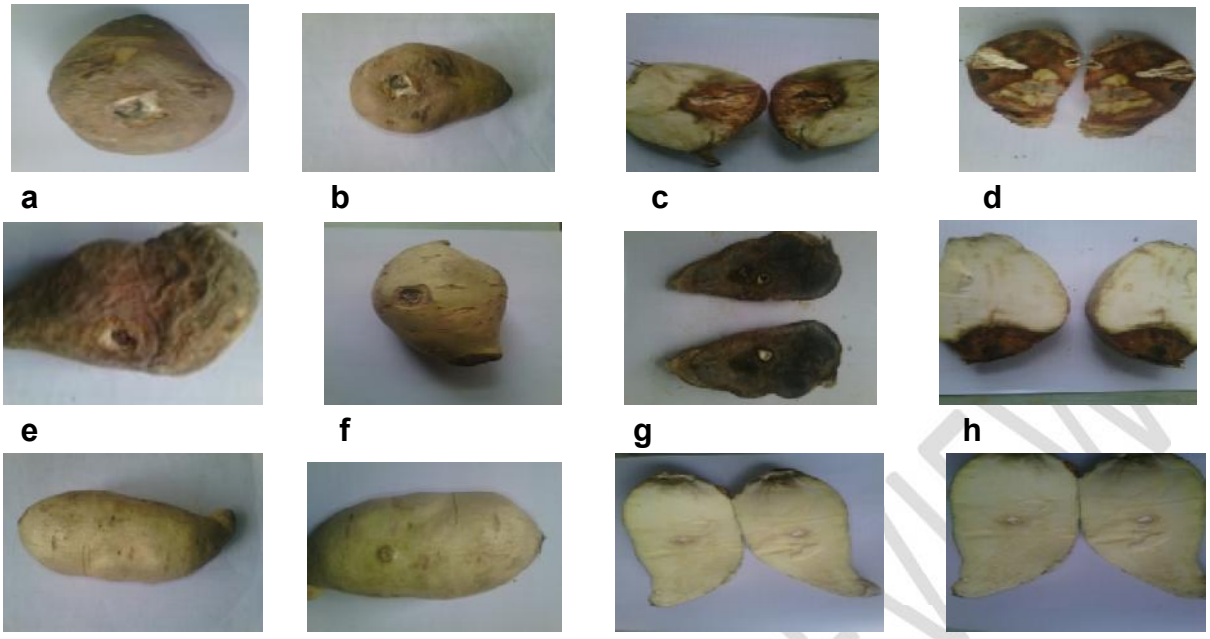


Plate 4: Pathogenicity of *A. niger (a-d); R. stolonifer* (e-h) and control (i-l).

Plate 7: Growth inhibition of *A. niger* using *Zingiber officinale* at 75% (a) and 50% (b, c)

Plate 8: Growth inhibition of *R. stolonifer* using *Vernonia amygdalina* at 25% (b), 75% (a) and 100% (c) concentrations

DISCUSSION

This study revealed that *Rhizopus stolonifer* and *Aspergillus niger* are among the common fungi associated with rotting of sweet potato tubers around Agbowo, Ojoo and Bodija, Ibadan. This finding agreed with the work of Salami and Popoola (2007) that isolated and identified different fungi including *R. stolonifer* and *A. niger* from diseased sweet potato tubers. The highly significant F- value (P>0.0001) for Model of growth inhibition of fungi isolated from a rotting sweet potato tubers by *Trichoderma harzianum* shows the correctness of the fitted model.

The highly significant F-value (P>0.0001) for treatment shows that the various *T. harzianum* treatment had highly significant impact in growth inhibition of the pathogens. The effectiveness of *Trichoderma harzianum* in growth inhibition of *R. stolonifer* and *A. niger* corroborates the work of Durrel (1968) that reported the ability of *Trichoderma* sp. to directly attack different fungi. The highly significant F-value (P>0.0001) for pathogen means that the isolated fungi were sensitive to the mycoparasitic effect of *T. harzianum*. The introduction of *T. harzianum* before the pathogen which shows a total inhibition of mycelia growth of pathogens corroborates the findings of Benitez *et al*. (2004); Monte and Llobell (2003) who reported *Trichoderma* sp. to impede spore germination, kill cells, occupy a physical space and avoid multiplication of pathogen.

The highly significant F-value (P>0.0024) for concentration means that the impact of the different *T. harzianum* concentration on the growth of *R. stolonifer* and *A. niger* were highly significant. The better effectiveness of *T. harzianum* at $1x10^{-3}$ cells/ml supported the work of Campbell (1988) that reported the relatedness of the aggressive ability of *T. harzianum* to its sporulation capacity.

The highly significant F-value (P>0.0001) for days shows that the impact of *T. harzianum* in growth inhibitions of *A. niger* and *R. stolonifer* among incubation days is highly significant at $p \leq 0.05$. Effectiveness of *T. harzianum* in plant disease control points to the high potential and reproducibility of the biological control agent (Elad *et al*., 1979).

The non-significant F-value (P>0.5827) for interactive effect of concentrations and days shows that growth inhibitions of *A. niger* and *R. stolonifer* by different concentrations of *T. harzianum* were not significant among the days of incubation. This means that the days of incubation does not interact with the *Trichoderma harzianum* concentrations to significantly impact the growth of *A. niger* and *R. stolonifer*.

The non-significant F-value (P>0.5724) for the interactive effect of pathogen and concentration means that both variable did not interact to produce a significant impact on the growth of the *A. niger* and *R. stolonifer.*

The highly significant F-value (P>0.0001) for the interactive effects of concentration and treatment reveals that any particular concentration of the treatment have significant impact on the growth of *A. niger* and *R. stolonifer.* The highly significant F-value (P>0.0001) for the interactions between pathogen and days; treatment and days; treatments and pathogen mean that the two variables in each case interacted to produce a significant impact on the growth of *A. niger* and *R. stolonifer.* The highly significant F-value (P>0.0034) for the interactive effect of treatment, concentration and days means that the effect of any particular treatment with any particular concentration among the days of incubation have significant impact on the growth of *A. niger* and *R. stolonifer.* The significant F-value (P>0.00401) for the interactions of pathogen, concentration and days means the three variables interacted to produce significant impact on the growth of the isolated fungi. The highly significant F-value (P>0.0001) for the interactive effects of treatment, pathogen and concentration; treatment, pathogens and days means that the three variables in each case interacted to have significant impact on the growth of *A. niger* and *R. stolonifer.*

The study also revealed that fungitoxic compounds were present in *Zingiber officinale* and *Vernonia amygdalina* since they were able to inhibit the growth of the test fungi. This is in accordance with the findings of Okigbo and Nmeka (2005) on the use of ginger extract among other plant extract in controlling yam tuber rot caused by *Aspergillus flavus*, *A. niger* and *Fusarium oxysporum*. The two plant extracts screened *in vitro* showed varying levels of toxicity to the fungi which is expressed as mean inhibition of mycelia growth. The inhibitory effect of the plant extracts on growth of the pathogens agrees with the work of Suleiman and Emua (2009) that reported the inhibition of a rot fungus using ginger extract.

The highly significant F- value (P>0.0001) for Model with regards to growth inhibition of fungi isolated from rotting sweet potato tubers by the plant extracts shows the appropriateness of the fitted model.

The highly significant F-value (P>0.0001) for treatment shows that the various treatments of *Zingiber officinale* and *Vernonia amygdalina* were effective in inhibiting growth of the pathogens. The extract of *Zingiber officinale* had significant inhibitory impact on the growth of

the pathogens which may be due to presence of antifungal compounds in the plant as reported by Akinpelu (1999). The highly significant F-value (P>0.0001) for pathogen means that the isolated fungi were sensitive to the inhibitory effect of *Zingiber officinale* and *Vernonia amygdalina*. There was a significant difference in statistical test at $p \leq 0.05$ among mycelia radial growth values observed on the different plant extracts used and on the various concentrations employed when compared with the control. This is in agreement with the works of Suleiman and Emua (2009); Suleiman and Falaiye (2013) that both employed different plant extracts in controlling different pathogens. The highly significant F-value (P>0.0001) for concentration means that the impact of the different concentration of *Zingiber officinale* and *Vernonia amygdalina* on the growth of the fungi were highly significant. There was a positive correlation between the growth inhibition of pathogens and the various concentrations of the two plant extracts employed. This is in agreement with the work of Onuh *et al*. (2005) that reported higher fungitoxicity of plant extracts at increased concentrations. The highly significant F-value (P>0.0001) for days shows that the growth inhibitory effects of *Zingiber officinale* and *Vernonia amygdalina* on *A. niger* and *R. stolonifer* among incubation days were highly significant.

The non-significant F-value (P>0.9665) for interactive effect for concentrations and days means that the impact of any plant extract concentration on the growth of *A. niger* and *R. stolonifer* were not significantly different among the days of incubation.

The highly significant F-values for interactions between pathogens and concentrations (P>0.0042); treatments and concentrations (P>0.0001); pathogens and days (P>0.0001); treatments and days (P>0.0001); treatments and pathogens (P>0.0001) means that both variables in each case interacted to produce highly significant impact on the growth of *A. niger* and *R. stolonifer*. The non-significant F-value (P>0.9987) for the interactive effect of treatment, concentration and days means shows that the effect of any particular plant treatment with any particular plant extract concentration among the days of incubation does not have significant impact on the growth of *A. niger* and *R. stolonifer*. The highly significant F-values for the interactions of pathogens, concentrations and days (P>0.0001); treatment, pathogen and concentrations (P>0.0001); treatment, pathogens and days (P>0.0001) means that all three variable in each case interacted to have significant impact on the growth of the isolated pathogens.

CONCLUSION

The results obtained in this work showcase the promising mycoparasitic potential of *Trichoderma harzianum* and growth inhibitory effect of extracts of *Zingiber officinale* and *Vernonia amygdalina* against plant pathogens. The use of these biocontrol measures in fungal disease management could go a long way in reducing over dependence on chemicals by the farmers, cost of production and economic loss of crops thus should be suggested as a component of integrated disease management procedures for the control of rot pathogens of sweet potato in the field. Further research geared towards understanding how disease control as well as the mode of action by *Trichoderma* species and these plant extrcats is achieved will however be needed for credible assertions.

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