Antimicrobial Abilities of Xylopia Aethiopica (Dunal.) Linn. and Syzygium Aromaticum (Linn.) Merr. Extracts Against Postharvest Rot of Discorea Alata Poir and Discorea Rotundata L.

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ABSTRACT

A greater percentage of yam tubers cultivated in Nigeria are lost to infection by several pathogens. Management of plant pathogens using integrated disease management (IDM) such as plant extracts continues to gain more attention. The antimicrobial activities of *X. aethiopica* and *S. aromaticum* extracts on fungi associated with rotting white and water yam was investigated. Diseased and healthy yam species of *Dioscorea* spp were obtained from Bodija and Gbaghi markets in Ibadan, Nigeria. The fungi were isolated from the samples using standard procedures and were later identified after obtaining pure culture. Leaves and fruits of *X. aethiopica* and *S. aromaticum* were obtained from the botanical garden, University of Ibadan, Ibadan. Crude extracts of the plants were obtained by soaking them separately into ethanol and aqueous solvents for 72hours, sieved and allowed to evaporate on a water bath. Preliminary screening of phytochemical constituents of the crude extracts was later done. After pathogenicity tests, the isolated fungi were cultured on acidified Potato Dextrose agar (APDA) that were impregnated separately with the leaves and fruits of *X. aethiopica* and fruits of *S. aromaticum* extracts at concentrations of 35%, 50% and 75% for 10 days. Two controls were set up: Control 1 (0% with agar) and Control 2 (0% with ethanol). The experiment was laid out in a completely randomized design (CRD) with three replicates. The mycelial extension of the fungi was measured every 24 hours using a meter rule. Data were subjected to statistical analysis using SAS software. Means separation was done using LSD (DMRT) at $p \leq 0.05$. The isolated fungi were identified as *Aspergillus niger, A. fumigatus,* and *Penicillium chrysogenum*. The pathogenicity test showed that the three fungi caused rotting in the yams. Growth inhibition of the fungi was significantly ($p \leq 0.05$) higher with ethanol extracts than aqueous extract. Highest mycelial growth inhibitory effect was recorded in the *S. aromaticum* fruit ethanol extracts on all the organisms. Likewise, *X. aethiopica* leaf aqueous extract showed high mycelial growth inhibition on *A. fumigatus* at 50% and 75% concentrations while *X. aethiopica* fruit ethanol and aqueous extracts was noted to have inhibitory effects on the growth of *A. niger* and *P. chrysogenum* at 50% and 75% concentrations respectively. The *in vitro* result underscores the antifungal abilities of these plant extracts and is also suggestive of their promising potential in vivo. Further works are underway to examine their antimicrobial potentials in the field.

Keywords: Dioscorea alata, Dioscorea rotundata, Postharvest rot, Syzygium aromaticum, Xylopia Aethiopica

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1. BACKGROUND TO THE STUDY

Yams are tuber crops widely cultivated in West Africa, East Africa, the Caribbean, South America, India and South East Asia (Etim *et al*., 2013, FAO, 2013, Iduma *et. al*., 2014). Nigeria is known to be the largest producer of yam (Ezeike, 1995). It is rated as one of the important staple food (Nweke *et al*., 1991, Okigbo and Ogbonnaya 2006). White yam (*Discorea rotundata* Poir.) and water yam (*Discorea alata* L.) are reported to be the most preferable varieties and they constitute about 80% of the total yam produced in Nigeria.

However, yam tubers are prone to several diseases. (Onuh *et al.,* 2015, Emmanuel, 2017, Nweke, 2017). Management of plant pathogens using biological control measures has continued to gain more attention.

2. STATEMENT OF PROBLEM

Yam is a tuber crop cultivated worldwide due to its high nutritional value and economic importance. White and water yam are known to form part of the major diet in many countries including Nigeria and over 60% of post-harvest losses of yam tubers recorded are due to infection which has greatly reduced its production and quality in storage. There have been increased attention on management of plant diseases using biological control measures. The extracts of *Xylopia aethiopica* and *Syzygium aromaticum* have been reported to have high antimicrobial activity against several plant pathogens.

3. OBJECTIVE

To isolate and identify fungi associated with post- harvest rot of *Dioscorea rotundata* (white yam) and *Dioscorea alata* (water yam).
To obtain crude leaf and fruits extracts of *Xylopia aethiopica* and *Syzygium aromaticum.*
To evaluate the effectiveness of the extracts on growth of the isolated rot pathogens *in-vitro.*
To examine impact of concentration on the effectiveness of the extracts.
To evaluate the effectiveness of *Xylopia aethiopica* and *Syzygium aromaticum* extracts (in-vitro) on the mycelia growth of the rot pathogens.
To compare the effectiveness of *Xylopia aethiopica* (Linn) and *Syzygium aromaticum* plant parts extracts on the isolated fungi

4. METHODOLOGY

4.1 Research Design

The experiment was conducted in a completely randomized design (CRD). Diseased yam tubers (*D. alata and D. rotundata*) were obtained from Bodija market in Ibadan, Oyo state. Leaf and fruits of *X. aethiopica* and *S. aromaticum* were collected from the Botanical garden, University of Ibadan, Oyo state.

4.2 Data Presentation

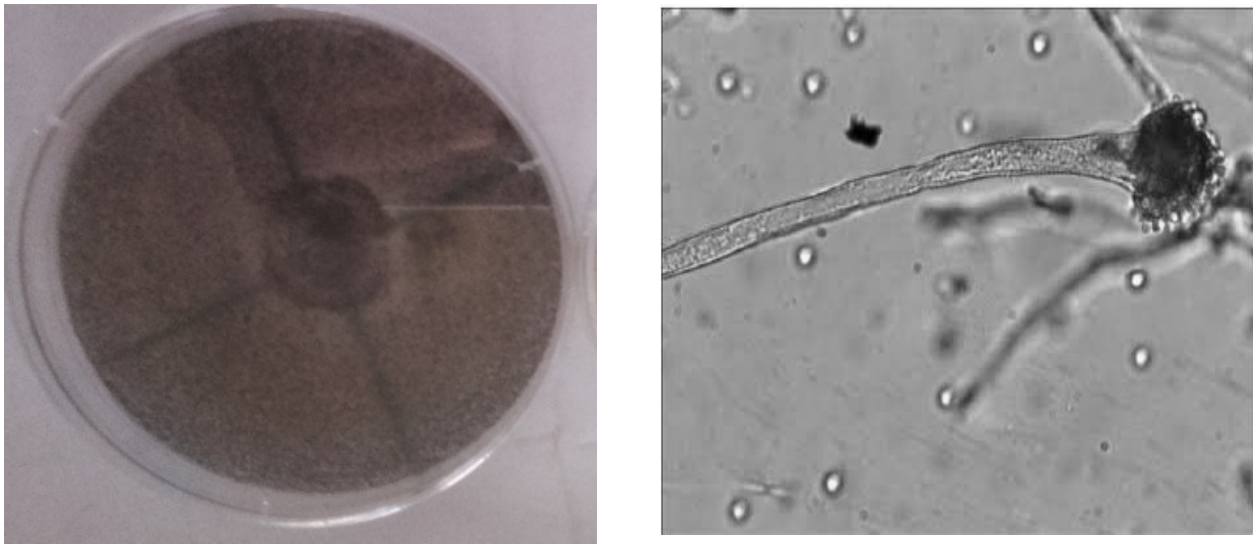
Pieces of diseased white and water yam obtained from different markets in Ibadan were surfaced sterilized and cultured on acidified Petri plates of Potato Dextrose agar (APDA) following standard procedures. Incubation at room temperature was done for 7 days. After pathogenicity tests and preparation of the plant extracts (i.e. leaf and fruits of *Xylopia aethiopica* and *Syzygium aromaticum*), their antifungal assay was examined at three different concentrations viz., 35%, 50%, 75% following standard procedures.

There were two controls i.e. 0% with agar and 0% with ethanol. All experiments were done in triplicates. Incubation was done at 28^{o}C and diametric growth of the fungi were measured at 24 hours interval using meter rule and recorded. The data collected were subjected to analysis of variance (ANOVA) using Generalized Linear Model (GLM) procedure of SAS (version 9.2). Means were separated using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$.

5. RESULTS

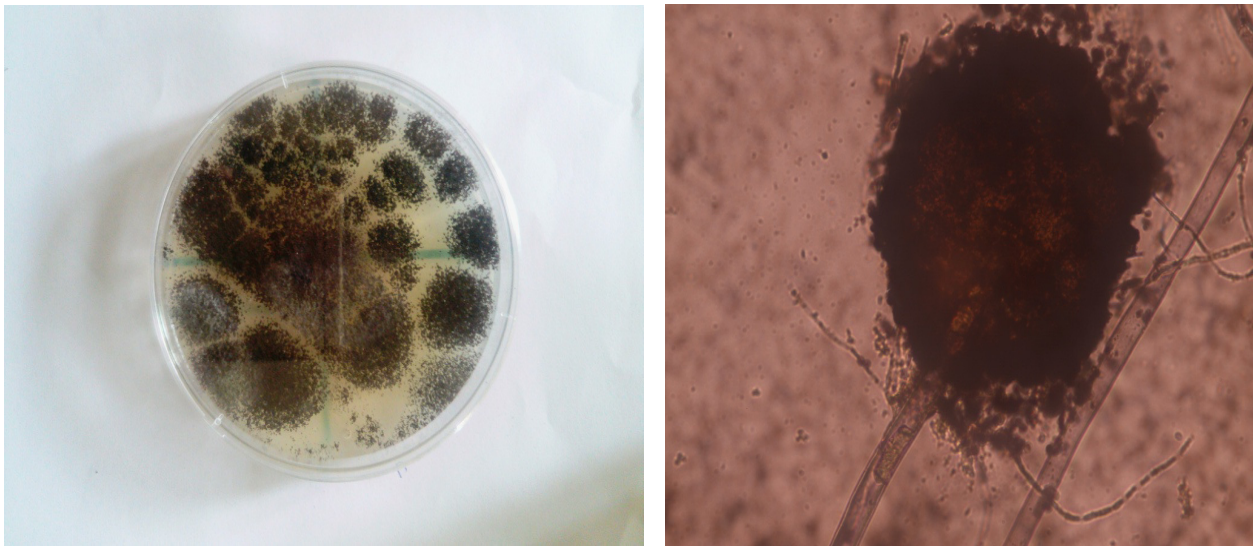
The fungi isolated from the rotting white and water yam tubers include *Aspergillus fumigatus* (Plate 1), *Aspergillus niger* (Plate 2), and *Penicillium chrysogenum* (Plate 3). The pathogenicity test conducted showed that *A. niger*, *A. fumigatus* and *P. chrysogenum* caused rotting on the water and white yam tubers in storage. The result showed that *P. chrysogenum* was more virulent on both yam tubers while the other fungi strains were not as virulent. Growth inhibition of the fungi by leaf and fruit extracts of *Xylopia aethiopica* was significantly higher with ethanol extracts than aqueous extract. (Table 1). Growth reduction by fruit extract was better than that of leaf with significant differences on certain days after inoculation. Growth inhibition of *A. niger* was generally more than that of other two fungi with significant differences on days 5 to 10. Inhibition at all concentrations was significantly better than that in aqueous control. Inhibition at 75% concentration was significantly better than that at other concentrations (Table 1). Growth inhibition of the fungi by fruit extracts of *S. aromaticum* was significantly higher with ethanol extracts than aqueous extract (Table 2).

Generally inhibition of *Penicillium chrysogenum* by the fruit extract was significantly better than that of other two fungi. However, the impact of *S. aromaticum* extracts on growth of *A. niger* was significantly higher than that of *X. aethiopica* while the converse is true for *P. chrysogenum* (Figure 1).



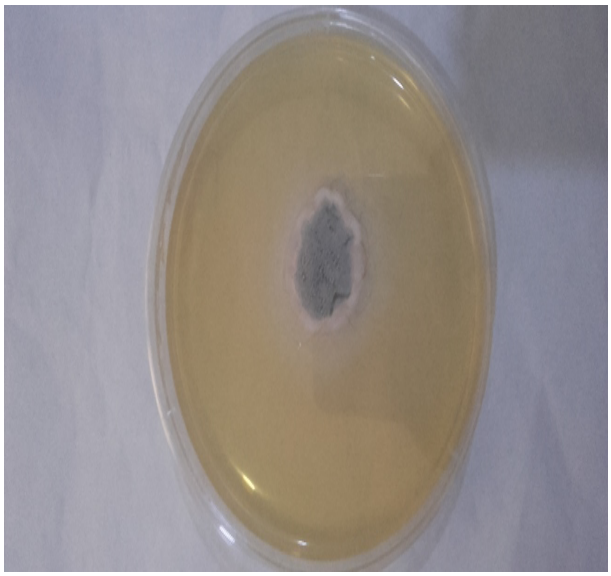
a **b**

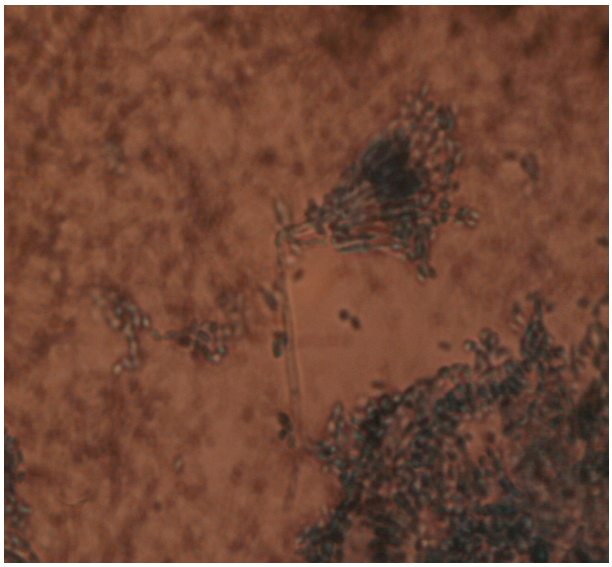
Plate 1: Pure culture (a) and Photomicrograph (b) of *A. fumigatus*



a **b**

Plate 2: Pure culture (a) and Photomicrograph (b) of *A. niger* .





a **b**

Plate 3: Pure culture (a) and Photomicrograph (b) of *P. chrysogenum*

Growth inhibitions of *A. fumigatus* by aqueous leaf extracts of *X. aethiopica* at all concentrations was significantly better than that in the controls (Plate 4). Growth inhibitions of *A. niger* by ethanol fruit extracts of *X. aethiopica* at all concentrations was significantly better than that in the controls (Plate 5). Growth inhibitions of *P. chrysogenum* by ethanol fruit extracts of *X. aethiopica* at all concentrations was significantly better than that in the controls (Plate 6). Growth inhibitions of *P. chrysogenum* by ethanol fruit extracts of *S. aromaticum* at all concentrations was significantly better than that in the controls (Plate 7). Inhibitions at 75% concentration was significantly better than that at other concentrations (Table 2). The F values for model, concentration, fungi, plant part, solvent and days were all highly ($P>0.0001$) significant for the antifungal activities of both *X. aethiopica* and *S. aromaticum*. Different interactions among the variables were also highly significant ($P>0.0001$) (Tables 3 and 4).

Table 1: Growth inhibition of the isolated fungi by *X. aethiopica* (leaf and fruit) extracts at days after incubation

Parameters	Variables	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
	Ethano	0.22 a	0.54 a	0.80a	1.01a	**1.27b**	1.45b	1.63b	**1.85b**	2.01a	**2.28a**
	Aqueous	0.04 b	0.55 a	0.82a	1.13a	1.60a	1.65a	1.78a	2.03c	2.23a	2.43a
Solvents	LSD	0.06	0.14	0.18	0.19	0.24	0.25	0.24	0.25	0.23	0.25
	Leaf	0.19a	0.61a	0.89a	1.13a	1.59a	1.67a	1.78a	2.03a	2.15a	2.36a
	Fruit	0.06b	0.50a	0.73a	**1.01a**	1.27b	1.44b	**1.63a**	**1.85a**	**2.08a**	2.34a
Plant part	LSD	0.06	0.14	0.18	0.19	0.24	0.25	0.24	0.25	0.23	0.25
	A. niger	0.14a	0.54a	0.80a	0.95a	**1.22b**	1.33b	1.42b	**1.56b**	1.67b	**1.85b**
	A. fumigatus	0.19a	0.55a	0.83a	1.11a	1.46ba	1.61ba	1.85a	2.11a	2.29a	2.50a
	P.chrysogenum	**0.06b**	0.55a	0.80a	1.16a	1.61a	1.71a	1.85a	2.16a	2.39a	2.71a
Fung	LSD	0.07	0.17	0.23	0.23	0.3	0.3	0.29	0.3	0.29	0.3
	35%	0.25a	0.95a	1.27a	1.58a	2.09ba	2.27a	2.40a	2.65b	2.92a	3.16b
	50%	0.13b	0.67b	1.00a	1.20b	1.71b	1.76b	1.94b	2.11c	2.27b	2.48c
	75%	**0.03c**	0.38c	0.69b	0.87c	**1.18c**	**1.25c**	1.38c	1.63d	1.69c	**1.90d**
	C1(Agar)	0.23a	0.74b	1.06a	1.67a	2.13a	2.41a	2.69a	3.04a	3.28a	3.61a
	C2(Ethano)	**0.00c**	0.01d	0.03c	0.03d	**0.05d**	0.05d	0.05d	0.28c	0.43d	**0.63e**
Concentration	LSD	0.09	0.21	0.29	0.3	0.38	0.39	0.38	0.39	0.37	0.39

Means with different letters in a column are significantly different ($p \leq 0.05$).

Table 2: Inhibition of the fungi by extracts of *S. aromaticum* fruit at days after incubation

Parameters	Variables	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
	Ethanol	0.10a	0.15b	0.39a	**0.35b**	0.43b	0.53b	**0.61b**	0.76b	0.87b	**1.06b**
	Aqueous	**0.08a**	0.23a	0.26a	0.51a	0.71a	0.87a	1.10a	1.21a	1.35a	1.66a
Solvents	LSD	0.06	0.09	0.14	0.16	0.15	0.17	0.17	0.23	0.22	0.24
	Aspergillus niger	0.15a	0.42a	0.65a	0.77a	0.85a	0.99a	1.09a	1.34a	1.41a	1.59a
	Aspergillus fumigatus	0.12a	0.15b	0.25b	0.35b	0.63b	0.72b	0.85b	1.04b	1.16a	1.46a
	Penicillum chrysogenum	**0.00b**	0.08b	**0.09b**	0.17b	0.30c	**0.40c**	0.47c	0.56c	0.75b	**1.02b**
Fungi	LSD	0.07	0.11	0.17	0.19	0.19	0.21	0.21	0.28	0.27	0.3
Concentration	35%	0.00b	0.12cb	**0.14a**	0.27b	0.43b	0.48b	0.63b	0.97b	1.18b	1.63b
	50%	0.00b	0.16b	0.18b	0.16cb	0.29b	0.29b	0.31c	0.50c	0.61c	0.77c
	75%	0.00b	0.12cb	0.16b	**0.12cb**	0.27b	0.27b	**0.28c**	0.33d	0.33dc	**0.46d**
	C1(Agar)	0.45a	0.68a	0.15b	1.59a	2.00a	2.48a	2.79a	3.11a	3.37a	3.83a
	C2(Ethanol)	**0.00b**	0.00c	**0.00b**	**0.00c**	0.00c	0.00c	**0.00d**	0.00d	0.05d	**0.12e**
	LSD	0.09	0.14	0.22	0.25	0.24	0.27	0.27	0.36	0.35	0.38

Means with different letters in a column are significantly different ($p \leq 0.05$).

Table 3: ANOVA table for antifungal activity of *X. aethiopica* on the fungi isolated from rotting *Dioscorea* spp.

Source	Df	SS	MS	F value	Pr < f
M	321	2528.77	7.88	34.92	0.0001**
C	4	875.52	218.88	970.34	0.0001**
F	2	43.6	21.8	96.65	0.0001**
P	1	6.44	6.44	28.57	0.0001**
D	9	831.67	92.41	409.66	0.0001**
S	1	6.62	6.62	29.37	0.0001**
F *C	8	104.12	13.02	57.7	0.0001**
P *C	4	11.75	2.94	13.02	0.0001**
C *D	36	182.77	5.08	22.51	0.0001**
S *C	4	11.01	2.75	12.2	0.0001**
P *F	2	6.99	3.49	15.49	0.0001**
F *D	18	30.35	1.69	7.47	0.0001**
S *F	2	8.65	4.33	19.18	0.0001**
P *D	9	6.42	0.71	3.16	0.0009**
S *P	1	56.22	56.22	249.21	0.0001**
S *D	9	7.85	0.87	3.86	0.0001**
P *F*C	8	3.71	0.46	2.05	0.0374*
F*C*D	72	33.24	0.46	2.05	0.0001**
S*F*C	8	28.19	3.52	15.62	0.0001**
P *C*D	36	6.7	0.19	0.83	0.7595
S*P*C	4	32.29	8.07	35.79	0.0001**
S*C*D	36	18.17	0.5	2.24	0.0001**
P*F *D	18	2.61	0.14	0.64	0.8687
S*P*F	2	200.33	100.16	444.04	0.0001**
S*F*P	18	6.14	0.34	1.51	0.0766
S*P*D	9	7.41	0.82	3.65	0.0002**
Error	1478	333.39	0.23		
Corrected total	1799	2862.16			
R^2	0.88				

Significant = *: Highly significant= **
Key: **M**- Model, **C-** Concentration**, F**-Fungi, **P**-Plant part, **S-**Solvent**. D –**Days

Table 4: ANOVA table for antifungal activity of *S. aromaticum* extracts on the isolated fungi.

Source	Df	SS	MS	F value	P < f
M	227	998.84	4.4	218.29	0.0001**
C	4	524.07	131.02	6499.53	0.0001**
F	2	43.93	21.97	1089.77	0.0001**
D	9	135.6	15.07	747.45	0.0001**
S	1	19.94	19.94	989.01	0.0001**
F*C	8	23.6	2.95	146.35	0.0001**
C*D	36	139.23	3.87	191.87	0.0001**
S* C	4	31.95	7.99	396.24	0.0001**
F*D	18	5.9	0.33	16.27	0.0001**
S*F	2	19.58	9.79	485.72	0.0001**
S*D	9	7.42	0.82	40.87	0.0001**
F*C*D	72	10.75	0.15	7.4	0.0001**
S*F*C	8	22.23	2.78	137.82	0.0001**
S*C*D	36	9.11	0.25	12.56	0.0001**
S*F*D	18	5.54	0.31	15.26	0.0001**
Error	672	13.55	0.02		
Corrected total	899	1012.39			
R Square	0.99				

Highly significant= ** Key: **M**- Model, **C- Concentration**, **F-Fungi, S-Solvent, D-Days**

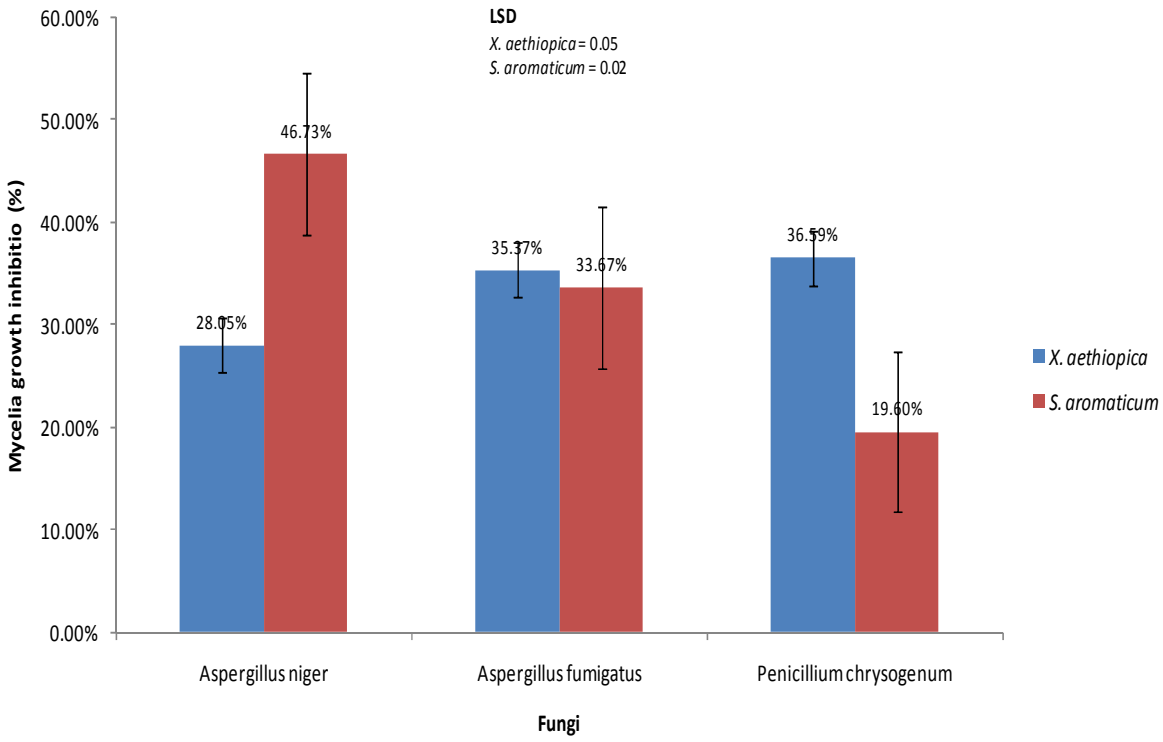
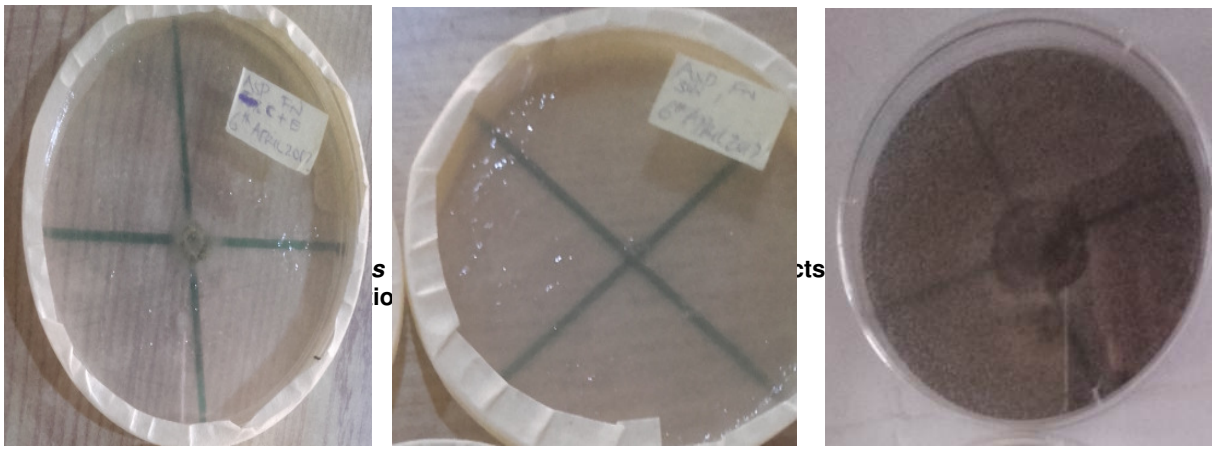
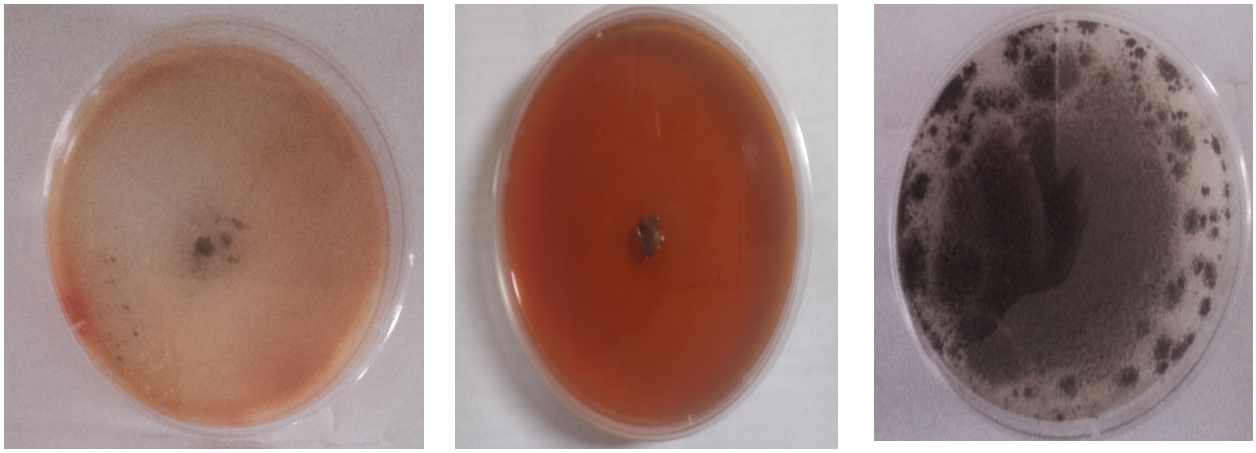


Figure 1: Pooled effect of *X. aethiopica and S. aromaticum* extracts on the isolated fungi.

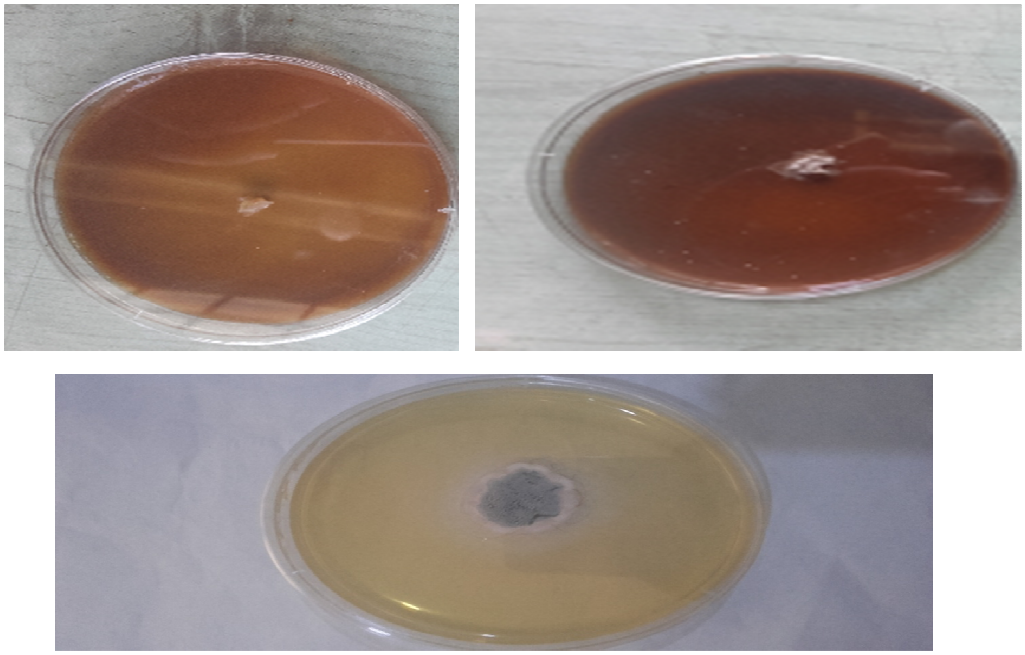




a b c

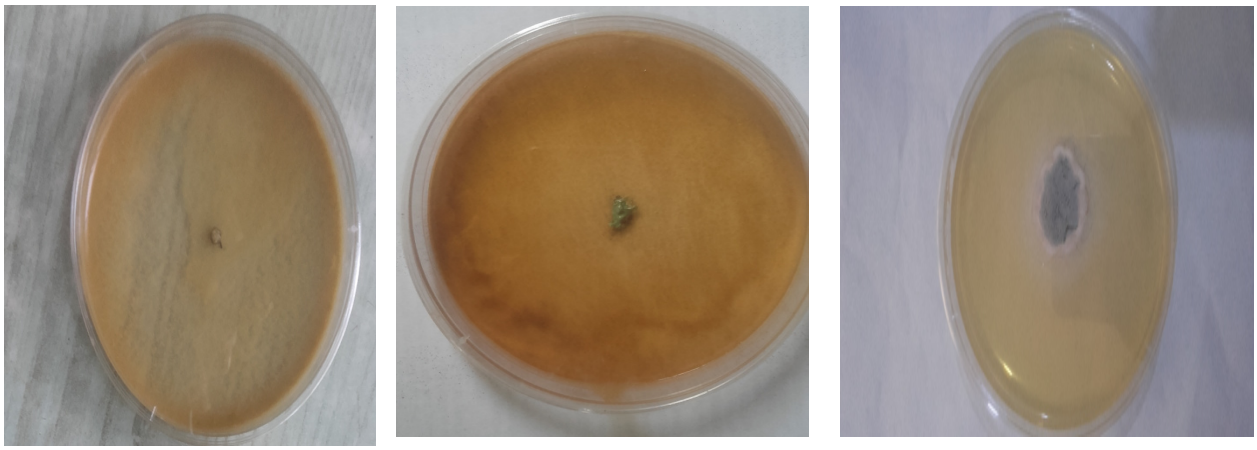
Plate 5: Inhibition of *A. niger by X. aethiopica* ethanol fruit extracts at 35% (a) and 75% concentrations (b) with control (c)

a b



c

Plate 6: Inhibition of *P. chrysogenum by X. aethiopica* ethanol fruit extracts of at 50% (a) 75% concentrations (b) with control (c)



a **b** **c**

Plate 7: Growth inhibition of *P. chrysogenum* by *S. aromaticum* fruit ethanol extracts at 50% (a) and aqueous 75% concentrations (b) with control (c).

6. DISCUSSION

The antimicrobial potentials of *X. aethiopica* and *S. aromaticum* evaluated on *A. niger*, *A. fumigatus* and *P. chrysogenum* obtained from rotting yam tubers (*D. rotundata and D. alata*) showed inhibitory potentials on the mycelial growth of the fungi. *A. niger, A. fumigatus* and *P. chrysogenum* amongst others have been reported to be the causal agents of postharvest rot of yam tubers in storage (Okigbo and Nmeka, 2005). The extracts of *X. aethiopica* and *S. aromaticum* have been reported to have anti-microbial and anti-fungal properties of which their derivatives are of great importance in public health, cosmetics, medicine and agriculture (Coyne *et al*., 2012).

The results obtained with fruit and leaf extracts of *X. aethiopica* is suggestive of higher antifungal potency of the former than the latter. It may thus be advisable to pay more attention on the fruit extract when field experiment is to be done. Extract concentration is also a key consideration for such a field experiment. The highly significant F values (P>0.0001) for models in all the experiments shows their appropriateness or 'goodness of fit'. This means effective growth inhibitions of the three fungi depend to a large extent on the fungi, plant part, concentration and interactions amongst them.

The highly significant F values for concentration, fungi, plant part, solvent, days as well as the various interactions among them in the case of both *X. aethiopica* and *S. aromaticum* are suggestive of the significant impact played by these factors on the antifungal activities of the plant parts. It means the same plant part will most likely exert different antifungal effect on different fungi. This is also corroborated by the results obtained in the pooled effect of *X. aethiopica and S. aromaticum* extracts on the isolated fungi. This agrees with the works of Suleiman and Falaiye, (2013) who reported that extracts from different plant parts are used in controlling different fungi.

The highly significant F values (P > 0.0001) for plant parts may also be suggesting that the different plant parts employed might contain certain phytochemicals that are capable of inhibiting the growth of several fungal pathogens. The highly significant F values (P > 0.0001) for fungi shows that the different fungi had significantly different growth responses in the presence of extracts of *S. aromaticum*. The significant F values (P > 0.0001) for days means that the growth inhibitory effects of the *S. aromaticum* on *A. niger, A. fumigatus* and *P. chrysogenum* among incubation days differed significantly. This is thus suggesting that contact period between plant extracts and the fungi is also critical for effective inhibition.

The highly significant F value (P > 0.0001) for solvent indicates that method of extraction can also impact on the effectiveness of extracts against the fungal growth. This agrees with the work of Azwanida, (2015) who reported that different plant parts require certain extraction methods in order that their antifungal potentials could be obtained. The results obtained with the aqueous and ethanol extracts showed that both solvents are good for extraction of extracts from *X. aethiopica* and *S. aromaticum*.

The highly significant F value (P > 0.0001) for interactions between fungi and concentration means that any particular concentration of extract did not impact similar antifungal effect on any two fungi. This means the antifungal effect of the extracts at any particular concentration differed significantly from one fungus to the other. The highly significant F value (P > 0.0001) for plant part and concentration (P > 0.0001) means that any particular extract concentration of any particular plant part exerted significantly different antifungal effect on two different fungi. In other words the antifungal effect of extract of any particular concentration differed significantly from one fungus to the other. It can thus be said that appreciable growth reduction of the isolated fungi is dependent amongst other factors on the type of extract engaged as well as the concentration of the extracts. It has been reported that *the higher the concentration, the more effective the plant extract on* mycelial growth inhibition.

The highly significant F value (P > 0.0001) for plant part and fungi means that extract from any particular plant part will most likely exert significantly different antifungal effect on two different fungi. The highly significant F value (P > 0.0001) for concentration and day means that two different concentrations of the same extract did not exert similar antifungal impact at the same incubation day. It thus means that the antifungal effects of two different extract concentration on the same incubation day differed significantly. The highly significant F value (P > 0.0001) for plant part and day means that the antifungal impact of extract from any particular plant part differed significantly from one incubation day to the other. This suggests that length of time or contact period between extract and fungus will most likely be key to effective fungal control in field experiment.

The highly significant F value (P > 0.0001) for solvents and fungi means that extracts by different solvents exerted significantly different antifungal impact on the same fungus. Solvent for extraction should therefore be carefully considered for plant extract to be used for antifungal purposes. The significant F value (P>0.0374) for interactions among plant part, fungi and concentration is suggestive. This means effectiveness of any particular concentration of extract of any particular plant part on growth of any fungus does not mean effectiveness on another fungus. Thus the 75% concentration of *X. aethiopica* extract which was most effective against *P. chrysogenum, A. niger and A. fumigatus* may not necessarily be effective against other fungi.

The highly significant F values (P>0.0001) for interactions among fungi, concentration and days means exposure period of any the three fungi to any specific extract concentration played a key role in the effectiveness of such extract (of both *S. aromaticum* and *X. aethiopica*). This fact was also validated by the highly significant F-value (P>0.0001) for interactions among solvent, fungi and days in the case of *S. aromaticum*. It means at any incubation day, a specific extract concentration (of *S. aromaticum* or *X. aethiopica*) exerted significantly different impact on the three isolated fungi.

The highly significant F value (P>0.0001) for interactions among solvent, plant part and concentration shows that the antifungal effectiveness of any particular concentration of a specific *X. aethiopica* plant part was not the same among extraction solvents. It means 75% aqueous and ethanol extracts for instance, of the same plant part (either fruit or leaf) will most likely have significantly different antifungal activities. The highly significant F value (P>0.0001) for interactions among solvent, plant part and fungi shows that *X. aethiopica* extracts of the same part but of different extraction solvent significantly differed in effectiveness on the three fungi. The highly significant F values (P>0.0002) for interactions among solvent, plant part and days means that extract from a specific part of *X. aethiopica* and of a specific extraction solvent exerted different antifungal activity on different days of incubation.

The highly significant F values (P>0.0001) for interactions among solvent, fungi and concentration means that the same concentration of *S. aromaticum* extract of the same extraction solvent had significantly different effectiveness against the three fungi. The antifungal potentials of both *S. aromaticum* and *X. aethiopica* might not be unconnected with certain phytochemicals like tannins, alkaloids, flavonoids, phenols and glycosides contained in them.

Volatile compound known as eugenol which occurred in large quantities in certain fruits has been reported to have antimicrobial activity against some pathogens (Ayoola *et al.,* 2008; Mishra *et al.*, 2014). Fleisher, (2003) submitted that the fruit of certain plants contains higher amounts of flavonoids than the leaves and that it was responsible for the antimicrobial activity of the fruit.

7. CONCLUSION AND RECOMMENDATION

This study has shown that the leaves and fruits of *X. aethiopica* and fruits of *S. aromaticum* have the antimicrobial potentials against fungi associated with rotting in white and water yam tubers especially rot caused by *A. niger, A. fumigatus and P. chrysogenum.* There is need for further study on the phytochemicals of the plants to ascertain those associated with their antimicrobial capabilities before embarking on field experiments.

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