



FUNGI - TOXIC EFFECT OF KOLA POD HUSK AND PLANTAIN FRUIT STALK ASHES ON THREE LEAF SPOT PATHOGENS OF *Telfairia occidentalis* (HOOK F.) *in vitro*.

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ABSTRACT

Leaf spot caused by a variety of fungi pathogens is a major disease of fluted pumpkin (*Telfairia occidentalis* Hook F) reducing its yield, quality and market value. Effective management of this disease complex will enhance the yield of the crop, the quality of harvested leaves and hence increase revenue for rural poor farmers. In this study the ability of ashes from Kola (*Cola nitida* (Vent) Schott & Endl) pod husk and plantain (*Musa paradisiaca* L) Fruit stalk to inhibit the mycelia growth of *Phoma sorghina*, *Curvularia* sp. and *Corynespora cassiicola* isolated from infected *T. occidentalis* leaves were evaluated on potato dextrose Agar (PDA). Seven ash concentrations (0.00, 0.01, 0.1, 0.15, 0.25, 0.5, and 1.00mg/ml) were used with five replicates in a completely randomized design (CRD). Mycelial growth inhibition was evaluated after five days incubation at $26 \pm 2^{\circ}\text{C}$. Data were analysed by analysis of variance (ANVOA) while significant means were compared by Fishers least significant difference (F-LSD). Whereas both ashes significantly ($P \leq 0.05$) inhibited the mycelia of *P. sorghina* and *Curvularia* sp in all concentrations tested, they had no statistical ($P \geq 0.05$) effect on *C. cassiicola* whose growth was enhanced at low ash concentrations. These results show that whereas kola pod husk and plantain fruit stalk ashes may be exploited for the control of leaf spot disease caused by *P. sorghina* and *Curvularia* sp., they may not be effective against *Corynespora* leaf spots.

Key Words: Mycelia inhibitions, Ash, *P. sorghina*, *Curvularia* sp

1. INTRODUCTION

Fluted pumpkin (*Telfaria occidentalis* HOOK F.) is an important leaf and seed vegetable crop grown widely in the forest zone of West and Central Africa (Olaniyi and Oyerele, 2012). In Nigeria, it is mostly grown in the south eastern region where it has ethno botanical significance in the folklore, dietary and cropping systems of the Igbos (Olaniyi and Oyerele, 2012). The succulent young shoot, tender leaves and seeds are consumed by humans for their rich protein, oil, vitamin and minerals. *T. occidentalis* is also utilized in livestock feed (Okoli and Mgbeogu, 1983-Schppers 2002). It is known to have medicinal and industrial values (Oluwale *et al.*, 2003; Horsefall and Spiff, 2009; Fasuyi, 2006). Like other perennial crops, the deep and extensive root system of Fluted pumpkin can “hold soil to prevent erosion, capture dissolved nitrogen before it infects ground and surface water, and outgrows weeds. These help to mitigate global warming by carbon Sequestration” (Wikipedia.org/ wiki/ *Telfaria occidentalis* (Godwin-Egein *et al.*, 2015).

In addition, Philip *et al.*, (2009) reports that *T. occidentalis* keeps providing steady income during the dry season when other crops have seized to produce. Despite these importance, the increasing relevance and high demand for *T. occidentalis*, the production of quality and acceptable leaves is limited by a number of factors including pests and diseases (Nwufu and Ihejirika 2008, Ihejirika *et al.*, 2010).

Among the many diseases of fluted pumpkin, leaf spot disease is one of the most devastating (Godwin-Egein *et al.*, 2015). The leaf spot complex consists of translucent leaf spot caused by *Cercospora citrullina*, *Colletotrichum* leafspot caused by *Colletotirchum gloeosporioides*, *C lindemuthianum* and target spot associated with *Corynespora (Cercospora) Cassiicola* (Maduwesi, 1977; Ihejirika *et al.*, 2010). These pathogens by their activities lead to reduction in the productivity and market value of the crop. Udo *et al* (2013) also associated *Diplocossum spicatum* with leaf spot disease of fluted pumpkin. A number of synthetic fungicides have been reported to control these pathogens (Maduwesi, 1977, Nwufu and Atu 1987, Nwufu, 1992). However, these are

seldom used because of scarcity, and high cost. Besides, development of resistance in chemical based disease management situations, health and environmental concerns necessitate the need for readily available cheap and ecofriendly methods of disease management.

In recent times, the use of micro-organisms, and their metabolites, and plant extracts in the management of plant pathogens both *in vitro* and *in vivo* have been widely reported (Ihejerika *et al.*, 2010, Baka, 2014, Hussein *et al.*, 2014, Santos-Hreddy *et al.*, 2014). However, there is paucity of information on the use of plant ashes for the same purpose (Williams 1986, Eze and Maduewesi 1990., Osai and Ikotun 1996., Asuquo *et al.*, 2002 Nwagbara *et al.*, 2013, Osai *et al.*, 2013). This study reports the management of leaf spot pathogens of fluted pumpkin *in vitro* with ashes from agricultural by products.

2. MATERIALS AND METHODS

The present investigation was carried out in the plant pathology laboratory of the Department of crop science University of Calabar, Calabar Nigeria. Calabar lies within latitude 4° 27' and 5° 32' N and longitude 7° and 9° 28' E.

2.1 Isolation of test pathogens

Isolation of *Phoma sorghina*, *Curvularia sp* and *Corynespora cassiicola* were isolated from infected fluted pumpkin leaves showing symptoms of translucent white and target leaf spots on plots within the Teaching and Research Farm of the Faculty of Agriculture Forestry and Wildlife Resource Management, and maintained on Potato dextrose Agar (PDA) slants. Infected leaves were rinsed under flowing tap water to remove dust and then cut into small pieces of 4 mm at the junction between infected and healthy portions. Cut leaf pieces were then surface sterilized in 10% commercial sodium hypochlorite and rinsed in three changes of sterile distilled water. Sterilized leaf pieces were drained on Whatman No.1 filter paper and placed in PDA plates according to symptom. Four leaf pieces in each symptom group were placed at equidistant positions in a 9 cm diameter Petri dish and incubated at 26±2°C until visible colonies developed. Developing fungi colonies were sub cultured until pure cultures were obtained. Fungi were identified on the basis of morphology, asexual

fruiting body and conidia produced and compared with standard description of Barnett and Hunter (1998) and other identification guides of CMI.

2.2 Preparation of Plant Ashes

Pod husks of Kola nut (*Cola nitida* (Vent) Schott & Endl) and fruit stalk of plantain (*Musa paradisiaca* L) were collected from local farmers in Ikom and Biase local government areas of Cross River State respectively at harvest. The pod husks and fruit stalk were then cut into pieces and sun dried for seven days. Dried Kola pod husks and plantain fruit stalk were separately burnt to yield ashes that were sieved through 2 mm sized mesh and stored in dry air tight containers until used. Mineral element composition of ash were determined following the appropriate procedure of the Association Official Analytical Chemist (A.O.A. C., 2005)

2.3 Evaluation of fungitoxicity of plant ashes

The fungitoxicity of kola pod husks and plantain fruit stalk ashes against the mycelia of *P sorghina*, *Curvularia sp* and *Corynespora cassiicola* was evaluated *in vitro*. Samples of ash from the two test plants were separately weighed and mixed in 100ml of sterile distilled water to give ash concentrations of 0.01, 0.10, 0.15, 0.25, 0.50 and 1.00 mg/ml. One ml of each plant ash concentration was incorporated into molten potato dextrose Agar plate and gently swirled to ensure proper mixing and allowed to solidify. Six replicate plates from each concentration were inoculated at the center with 5mm mycelia discs of each test fungus and incubated at 26±2°C for five days. Similarly inoculated PDA plates without ash were included as control. Mycelia discs were cut from the margin of actively growing culture of the fungus and placed mycelia down in each plate. Incubated plates were observed daily and colony diameter measured along two pre drawn perpendicular lines on the reverse side of the Petri dish. Fungitoxicity was assessed in terms of mycelia growth inhibition.

Thus,

$$\% \text{ MGI} = \frac{\text{MGDC} - \text{MGDA}}{\text{MGDC}} \times 100$$

When MGI=Mycelia growth inhibition.

MGDC= Mean Mycelia growth diameter in control (No ash) plates

MGDA=Mean Mycelia growth diameter in ash amended plates.

The experiments were repeated two times.

2.4 Experimental Design and data analysis

The experiments followed a completely randomized design (CRD) with six replications. Data obtained were subjected to one way Analysis of variance following GENSTAT 8.0 release procedures and means compared by Fishers Least Significant Difference (LSD) at 0.05 probability level.

3. RESULTS

Three fungi were isolated from infected *T. occidentalis* leaves and were identified based on morphology of mycelium, asexual fruiting body and conidia as *Phoma sorghina* (Sacc) Boerema, Dorenbosch and van Kesteraa, *Curvularia sp* and *Corynespora cassiicola* (Berk & M A Curtis) C.T Wei. *P. sorghina* was the highest (69.35%) occurring, followed by *Curvularia sp* (25.65%).

3.1 Mineral Composition and pH of Kola Pod Husk and Plantain Fruit stalk Ashes

Table 1 shows the properties of Kola pod husk and plantain fruit husk ashes used in this study. Kola pod husk ash had slightly higher values of all parameters except Phosphorus which was 0.36mg/g in both ash types. Both ash types were moderately alkaline with pH range of 9.65-10.05 and 9.62-11.00 for kola pod husk and plantain fruit husk respectively. The ashes were low in Sodium (0.07 and 0.06 mg/g) but high in Potassium 3.01 and 2.65mg/g for kola pod husk and plantain fruit stalk respectively. Other Cations present in the ashes were calcium and Magnesium (Table 1).

3.2 In Vitro Fungitoxicity of Plant Ashes

The effect of kola pod husk and plantain fruit stalk ashes on the mycelia growth of *P. sorghina*, *Curvularia sp* and *C. cassiicola* are shown in Tables 2 and 3 and Plates 1-4. Whereas both ashes significantly ($P \leq 0.05$) suppressed the growth of *P. sorghina* and *Curvularia sp* at all levels of concentrations, they enhanced the growth of *C. cassiicola* at low concentrations (Tables 2 and 3).

Table 2 shows that *Curvularia sp* was more sensitive to kola pod husk ash treatment than

P. sorghina. Although total inhibition was not observed in this study, increase in concentration significantly ($P \leq 0.05$) increased mycelia inhibition. The least inhibition against *P. sorghina* and *Curvularia sp* were 5.95% and 9.27% respectively at 0.01mg/ml ash concentration (Tables 2). The highest ash concentration of 1mg/ml gave significantly ($P \leq 0.05$) highest mycelia growth inhibitions against both *P. sorghina* and *Curvularia sp*. Inhibition at this concentration was 78.65 and 80.54% for *P. sorghina* and *Curvularia* respectively. Whereas many concentric growth rings and spores were observed in control plates (no ash) of *Curvularia sp*, only few were seen in ash amended plates after 8 days incubation (Plate 1). Although *P. sorghina* is known to change the colour of the growth medium, the intensity of the red colour increased with concentration (Plate 2). Kola pod husk ash enhanced the mycelia growth of *C. cassiicola* up to 0.15mg/ml, beyond this however, a non-significant ($P \geq 0.05$) growth inhibition of between 5.5 and 12.3 was observed (Table 2).

The mycelia growth inhibition of test fungi due to plantain fruit stalk ashes are shown in Table 3 and Plates 3 & 4. Significant ($P \leq 0.05$) growth inhibition with increase in concentration was also observed with this ash treatment. Whereas *Curvularia sp* was more sensitive to lower doses of plantain ash, the ash was more toxic (68.5% and 82.8%) against *P. sorghina* at 0.50 and 1.00 mg/ml concentrations. Against *Curvularia sp*, percentage growth inhibition was between 12.3% and 78.2% among the test concentrations. The growth of *Curvularia sp* and *P. sorghina* in plantain fruit stalk ash amended media after 10 days growth is shown in Plates 3 & 4 respectively. Whereas there was a more or less uniform growth of *Curvularia sp* in the control plates, the mycelium showed a pseudo-directional growth in ash amended plates (Plate 3). Plantain rachis ash did not significantly ($P \leq 0.05$) reduce the growth of *Corynespora cassiicola* even at 1.00 mg/ml concentration.

4. DISCUSSION

The isolation methods of *Phoma sorghina*, *Curvularia sp* and *Corynespora cassiicola* used in this study and the predominating nature of *P. sorghina* agree with earlier studies (Nwufo, 1992., Osai *et al.*, 2013., Godwin-Egein *et al.*, 2015) which reported *P. sorghina*

as the most prevalent leaf spot pathogen in South eastern Nigeria. Similarly, the consistent mycelial inhibition of *P. sorghina* and *Curvularia sp* by kola pod husk and plantain fruit stalk ashes at the tested concentration corroborates previous reports on the fungitoxicity of plant ashes. (Osai and Ikotun 1996., Asare-Bediako *et al.*, 2007). These authors reported the mycelia growth inhibition of yam minisett rot pathogens by plantain rachis and *Senna siamea* wood ashes respectively. This fungitoxicity is attributable, in part, to the alkaline nature of the ash. The pH of kola pod husk and plantain fruit stalk ash used in the present study was in the range 9.65-10.05 and 9.62-10.00 respectively. Leaf spot fungi are known to thrive in acidic conditions (Jacobson, 1983). The presence of cations such as K⁺ Ca²⁺ and Mg²⁺ may also account for their toxicity. Besides, fungitoxic elements such as sulphur and copper, though not detected in this study have been previously reported from wood ash (Odura *et al.*, 1997). The presence of such elements could equally inhibit or suppress fungal growth. Asuquo *et al.*, (2012) had equally associated phenols with the ash of *Elaeis guineenses* and attributed part of the fungicidal nature of the ash to this group of compounds. The pseudo-directional growth of *Curvularia sp* may be an avoidance strategy meant to escape ash dense areas of the growth medium.

Both kola pod husk and plantain fruit stalk ashes enhanced the colony diameter of *Corynespora cassiicola* at low concentrations. Even at 1.00 mg/ml mycelia growth reduction of this pathogen was not significant. This suggests a tolerance of certain levels of alkalinity and of cation toxicity by *C. cassiicola*.

5. CONCLUSION

The results of this study have shown the fungitoxicity of ashes from kola pod husk and plantain fruit stalk against *P. sorghina* and *Curvularia sp* causing leaf spot in fluted pumpkin. Although the ash were fungistatic rather than fungicidal as total inhibition was not obtained with the test concentrations, they may be utilized in the management of leaf spot complex caused by *P. sorghina* and *Curvularia sp* for improved yield, enhanced quality, acceptability and market value of harvested leaves.

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Table 1: Mineral composition of Kola pod husk and Plantain *fruit stalk* ashes used in this study

Mineral Element	Mineral and pH Composition (mg/g*)	
	Kola Pod Husk	Plantain Fruit stalk
Calcium (Ca)	1.56	1.48
Magnesium (Ng)	1.39	1.28
Potassium (K)	3.01	2.65
Phosphorus (P)	0.36	0.36
Sodium (Na)	0.07	0.06
pH (in water)	9.65 – 10.05	9.62 – 10.00

*Values are means of six replicate plates /concentration.

Table 2: Effect of Kola Pod husk Ash on the Mycelia growth of *P. Sorghina*, *Curvularia sp.* and *Corynespora Cassiicola* after five days incubation at 26 ±2°C

Ash concentration (mg/ml)	Mycelia Inhibition (%*)		
	<i>P. Sorghina</i>	<i>Curvularia sp.</i>	<i>Corynespora Cassiicola</i>
0.00	0.00	0.00	0.00
0.01	5.59 ± 0.77	9.27±1.36	- 10.02±0.56
0.10	9.20 ± 1-04	17.82±1.17	-12.60±1.25
0.15	17-46±0.74	30.02±1.42	- 16.80± 2.11
0.25	33.75 ± 2.15	47.74 ±2.27	5.52±1.00
0.50	64.12 ± 1.01	71.90±1.80	8.72±1.68
1.00	78.65 ± 0.74	80.54±1.20	12.25±1.25
F-LSD (P≤0.05)	0.37	1.08	Ns

*Values are means of six replicate plates /concentration ± standard error.

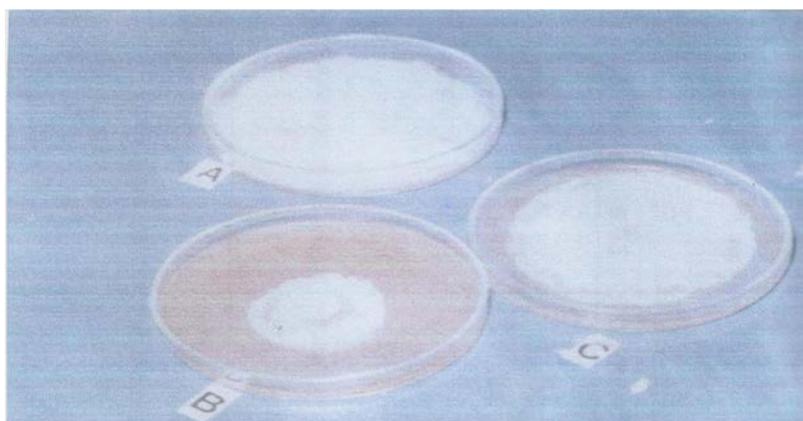


Plate 1 – Effect of different concentrations of kola pod ash on the mycelial growth of *Phoma sorghina* after 10 days incubation at 26±2°C.

A = Control (0.00 mg/ml)

B = 0.25 mg/ml

C = 0.50 mg/ml

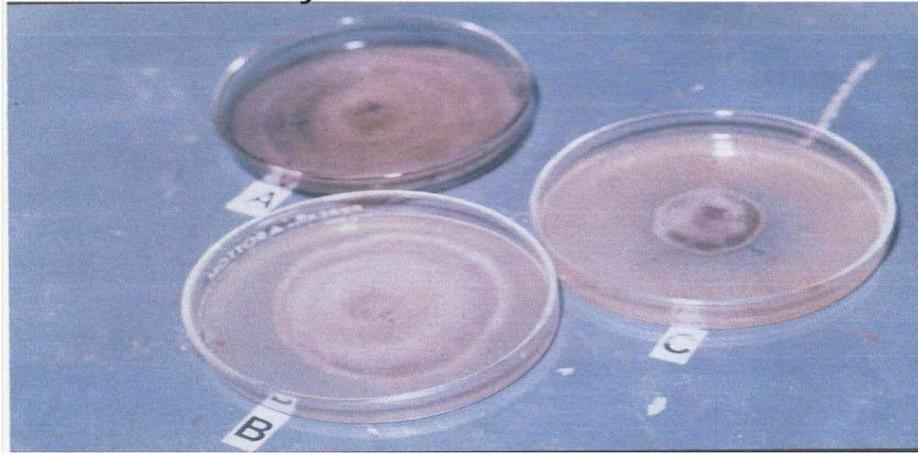


Plate 2: Effect of different concentrations of kola pod ash on the mycelial growth of *Curvularia* sp. After 10 days incubation at $26\pm 2^{\circ}\text{C}$.

A = Control (0.00 mg/ml)

B = 0.25 mg/ml

C = 0.50 mg/ml

Table 3: Effect of Cola Plantain rashes on the Mycelia growth of *P. Sorghina*, *Curvularia* sp. and *Corynespora Cassiicola* after five days incubation at $26 \pm 2^{\circ}\text{C}$

Ash concentration (mg/ml)	Mycelia Inhibition (%*)		
	<i>P. Sorghina</i>	<i>Curvularia</i> sp.	<i>Corynespora Cassiicola</i>
0.00	0.00	0.00	0.00
0.01	7.80 ± 0.42	12.27 ± 0.66	-9.40 ± 0.72
0.10	10.80 ± 0.86	17.56 ± 1.55	10.90 ± 0.48
0.15	19.47 ± 0.75	32.35 ± 2.88	18.82 ± 0.94
0.25	35.86 ± 1.82	49.54 ± 2.62	12.50 ± 1.11
1.50	68.51 ± 0.87	61.98 ± 2.68	8.68 ± 2.56
0.00	82.84 ± 1.21	78.22 ± 1.32	15.60 ± 1.21
F-LSD ($P \leq 0.05$)	1.24	1.11	Ns

*Values are means of six replicate plates /concentration \pm standard error.



Plate 3: Effect of different concentrations of plantain inflorescence ash on the mycelial growth of *Phoma sorghina* after 10 days incubation at $26\pm 2^{\circ}\text{C}$.

A = Control (0.00 mg/ml)

B = 0.25 mg/ml

C = 0.50 mg/ml

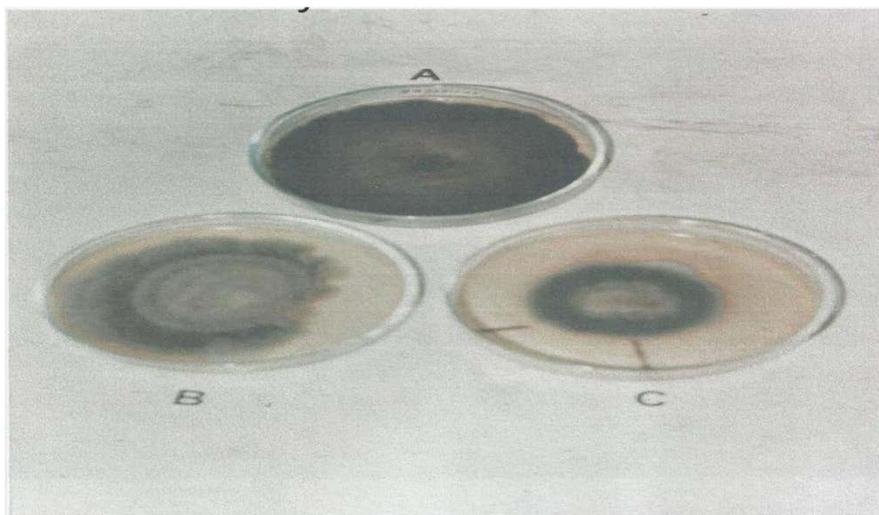


Plate 4: Effect of different concentrations of plantain stalk ash on the mycelial growth of *Curvularia* sp. After 10 days incubation at $28\pm 2^{\circ}\text{C}$.

A = Control (0.00 mg/ml)

B = 0.25 mg/ml

C = 0.50 mg/ml
