

THE EFFICACY OF PLANTAIN INFLORESCENCE ASH IN THE CONTROL OF TRANSLUSCENT LEAFSPOT DISEASE OF *TELFAIRIA OCCIDENTALIS* (HOOK F.)

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ABSTRACT

Telfairia occidentalis (Hook f.) widely consumed in South-Eastern Nigeria where it is grown for its young vines, leaves and its oil-rich seeds. Its production is often threatened by insect pests and diseases especially leafspot which reduces the quality, market value leaf and pod yields of the crop. Due to their scarcity, high cost and environmental concerns, the use of synthetic fungicides for its control is not often practiced. The use of ash from readily available agricultural by-product (plantain inflorescence) was investigated as a potential control measure for this disease. Different concentrations of plantain rachis ash were tested in-vitro for this effect on the growth, sporulation and spore germination of the causal fungus (*Phoma sorghina*). In a repeated green house experiment using artificial spray inoculation and field experiments using natural infection, plantain rachis ash consistently suppressed the growth and sporulation of the pathogen and reduced leaf spot disease. Pumpkin plants tested with 6gml⁻¹ ash after 3HAI had significantly ($P<0.05$) lowest leafspot incidence and severity than 6 and 12 HAI application and 1.5 and 3gml⁻¹ concentrations. Similarly, 6gml⁻¹ ash applied at symptom expression significantly ($P<0.05$) suppressed leafspot incidence and severity and enhanced yield over lower concentration and control; and was statistically ($P<0.05$) comparable with Carbendazim in this parameters. These results suggest the practical use of plantain inflorescence ash in the control of leafspot disease in the production of fluted pumpkin.

KEYWORDS: Plantain Ash, Leafspot, *Phoma sorghina*, *Telfairia occidentalis*

INTRODUCTION

Telfairia occidentalis (Hook f.) is a popular green leafy vegetable in Nigeria grown for its edible leaves and seeds (Nwufu, 1997). Its nutritional superiority to most other local vegetables has been documented (Adetunji, 1997). Nutritionally, the leaf is a good source of organic acids, mineral salts, oils, vitamins, proteins and carbohydrates and also possesses medicinal values. The leaf composition per 100g of edible portion is water 86g, energy 47kcal, protein 25g, carbohydrate 7.0g and fibre 1.7g (Grubben and Benton, 2004).

According to Tindall (1975) and Williams *et al* (1991), high incidence of pests and diseases reduce the crop yield significantly and affects its marketability. The major diseases of this crop are leafspot caused by *Phoma sorghina* (Nwufu and Atu, 1987) and anthracnose (Udo *et al*, 2008). Iherika and Nwofu (2001) reported that chemical control of diseases such as leafspot is very expensive, requires expertise and is hazardous to man, non-target organisms and the environment. They noted further that for self sufficiency in food production, low input technologies affordable to resource-poor farmers should be encouraged.

This study aimed at determining the fungicidal properties of plantain inflorescence ash which is cheap, readily available and environmentally friendly and its potency in controlling leafspot disease caused by *Phoma sorghina*.

METHODS

Isolation and Identification of the Causal Organism

The infected *Telfaira* leaves were harvested from diseased plants growing in mono-cropped farm situated at the Teaching and Research Farm of the University of Calabar, Calabar, Nigeria. The leaves were rinsed under a flowing tap in the laboratory and thereafter cut into 2cm² sections at the interface between the infected and healthy portions with a sterile knife. Cut-leaf sections were surface sterilized in 70% sodium hypochlorite (bleach) solution for 1 minute and rinsed quickly in 3-changes of sterile distilled water, blotted dry on Whatman's No.1 filter paper and placed on potato dextrose agar (PDA) in Petri dishes. Four (4) leaf sections were inoculated per Petri dish. The plates were sealed with paraffin tapes and incubated at 28±1°C until fungal growth was noticed. After 5days, the different isolates were sub-cultured into freshly prepared PDA plates to obtain and maintain pure cultures of the fungi. Isolated fungi were identified as far as possible using the identification guides of the International Mycological Institute Kew and of Hunter and Barnet (1998). Stock cultures of these fungi were stored in Agar slants bottles for later use.

Pathogenicity tests of the different fungal isolates were carried out and the fungus that produced symptoms as those observed in the field was implicated in the infection.

Ash Preparation

Waste plantain peduncle obtained from local farmers at harvest were cut into small pieces and sun dried for 7days. Dried pieces of these cut sections were burnt and the ash sieved through a 2mm-sized mesh and stored in dry air-tight container for later use (Osai and Ikotun, 1996).

Effect of Ash on Mycelial Growth

Six (6) glass test tubes containing 10ml of distilled water were sterilized in an autoclave at 15 p.s.i. (121°C) for 15mins. and allowed to cool. Five (5) different weights of ash (1.0, 10.0, 15.0, 25.0 and 50.0mg) were separately suspended in 10ml of sterile distilled water to produce final concentrations of 0.1, 1.0, 1.5, 2.5, and 5.0mg/ml⁻¹ while sterile distilled water only, served as the control (Osai, 2009). Aliquot (1ml) of each concentration (treatment) was dispensed into Petri dishes using sterilized 10ml pipette after which some molten potato dextrose agar (PDA) was added, the plates were swirled gently and kept on a sterile laboratory bench to gel. Eight (8) plates from each concentration were then inoculated with 2cm diameter mycelial disc obtained from actively growing culture of the fungus. Agar plates containing 1ml sterile distilled water were similarly seeded and served as control.

All plates were incubated at 28 ± 1°C and mycelial growth assessed periodically.

Measurement of colony diameter was done at 3days after inoculation (3 DAI) using the method of Osai and Ikotun, (1996). Colony diameter was taken as the mean growth along two (2) pre-drawn perpendicular lines on the reverse side of each plate. The percentage inhibition was determined from the mean of eight (8) replicates using the formula below:

$$\% \text{ Growth inhibition} = \frac{\text{Growth in control} - \text{Growth in treated}}{\text{Growth in control}} \times \frac{100}{1}$$

Effect of Ash on Sporulation and Spore Germination

This was done according to Mao and Newman. (1998). 25ml of sterile distilled water was poured on a 7-day old culture growing in plates containing different weights of ash (1.0, 10.0, 15.0, 25.0 and 50.0mg). The spores were carefully brushed off the sporophores with a carmel hair brush. The water and, spore mixture was decanted into centrifuge tubes and

centrifuged at 4,000 x g for 5 min. The supernatant was poured out and, 3 ml of sterile distilled water added. Spore concentration was then estimated for every 1ml of spore mixture with a haemocytometer (Neubauer-improved 0630010 model) under a binocular microscope. To assess effect of ash on spore germination, 1ml of spore suspension (5.0×10^4 spores) was placed in 4 equidistant positions in ash-impregnated PDA plates and incubated at $28 \pm 1^\circ\text{C}$ as in mycelial growth test above. The plates were assessed for germination under the microscope at 2hrs interval.

Screen House Studies

Screen house evaluation of the fungicidal properties of the test ash was investigated using the fungal spores with four (4) ash concentrations of 0.0(control), 1.5, 3.0, and 6.0 mg/ml^{-1}

Production of Spore Suspension

One week (7 days) old culture of the isolate was flooded with 25ml of sterile distilled water and with a Carmel hair brush, the spores were carefully brushed off the sporophores. This was decanted into a sterile Petri dish and another 15ml of sterile water added and filtered with a $0.2 \times 0.2 \text{ mm}$ nylon mesh to get rid of mycelial fragments. This filtrate containing the spores was adjusted to a concentration of 4×10^5 spores per ml through the addition of some quantity of sterile distilled water. Spore measurement was taken with a Haemocytometer (Neubauer-improved 0630010 model).

Spore Inoculation

Telfaira occidentalis plants used for this study were grown in steam pasteurized (160°C for 3hrs) soil put in black polyethylene bags. During the inoculation test, the leaves were rinsed with distilled water, wounded with the help of a sterilized needle then inoculated with spore suspension of the test fungus and treated with the various ash concentrations using a hand operated mist blower until run-off. Ten plants were thereafter separately treated with the different ash concentrations at 3, 6 and 12 hours after inoculation. Another set of 10-plants inoculated with the fungus spore suspension only, served as the control. All inoculated seedlings were covered with black polyethylene for 48 hours to provide a moist chamber.

Disease incidence was assessed at 7, 14 and 21 days after inoculation (DAI) while disease severity was scored on a 1 to 5 point scale as reported by Onuegbu and Dimkpa (2010).

Field Study

The effect of ash treatment on the incidence and severity of leafspot disease under natural infection was studied in field experiment conducted at the Teaching and Research farm of the University of Calabar during the 2010 cropping season. The area is located between latitude $5^\circ 32'$ and $4^\circ 27'$ north and longitude $7^\circ 15'$ and $9^\circ 28'$ east. Total annual rainfall is about 2000-2500mm. The mean annual temperature range between 23°C and 33°C while the relative humidity is 60-90% (CRSNMANR, 1989).

Land preparation involved clearing of weeds, stumping and tilling. Twenty-five plots each measuring $5 \text{ m} \times 4 \text{ m}$ were marked out and sown with fluted pumpkin seeds obtained from a local market in Calabar. Two seeds were planted per stand at a spacing of $1 \text{ m} \times 1 \text{ m}$ and later thinned to one seedling to give a total plant density of 10, 000 plants/ha⁻¹. Plants were separated by 1m alley. Ash concentrations (0.0, 1.5, 3.0 and 6.0 mg/ml) prepared as previously described and Carbendazim, 6.0 mg/ml (3.0 mg a.i./ml) constituted the treatment and were applied using a hand sprayer at symptom appearance (about 1 week after planting). Data on disease incidence, severity and yield were collected from the two (2) central rows in each treatment at 21 days after application.

Experimental Design and Statistical Analysis

The experimental layout for the *in vitro* studies was a Completely Randomized Design (CRD) with eight replicates and six ash concentrations served as the treatments.

Green house experiment was a 2-factor factorial in Randomized Complete Block Design (RCBD) with three replications. The four ash concentration constituted factor A, while the spray intervals constituted factor B. In the field experiment, a Randomized Complete Block Design (RCBD) was adopted with five replications. Data collected were subjected to analysis of variance (ANOVA) and means separated using Fischer's Least Significant Difference (F-LSD) at 5% probability level.

RESULTS

Fungus Isolated from Diseased Telfairia Leaves

The fungus frequently isolated from leaf spot lesions on fluted pumpkin leaves is in the genus *Phoma*. This fungus isolated, produced dense mycelia of white to light green in colour with dictyochlamydo spores at the tip of hyphal strands as well as the intercolony hyphal cells and turns the growth medium (PDA) red and was identified as *Phoma sorghina*.

Effect of Plantain Inflorescence Ash on Mycelial Growth, Sporulation and Spore Germination *In vitro*

Results in Table 1 show that, the inflorescence ash inhibited mycelial growth, sporulation and spore germination with percentage inhibition increasing with increase in concentration. Percentage inhibition of mycelial growth significantly ($p < 0.05$) increased with increase in ash concentration. However, total inhibition was not achieved throughout the study. The highest mycelial inhibition of 53.51% was induced by 5.00 mgml⁻¹, while the least inhibition was 5.80% at 0.10 mgml⁻¹ concentration.

Sporulation and spore germination followed the same trend with 5.00 mgml⁻¹ showing the highest inhibitory effect whereas 0.10 mgml⁻¹ had the least (Table 1).

Effect of Plantain Inflorescence Ash on Leafspot Incidence and Severity *In vivo*

Results of the effect of plantain inflorescence ash and spray interval on leafspot incidence of *P. sorghina* is presented in Table 2. The result revealed that increased plantain inflorescence ash application above 3.00mgml⁻¹ significantly ($p < 0.05$) reduced leaf spot incidence compared with the control. Delayed ash spray up to 6 HAI significantly ($p < 0.05$) increased leafspot incidence relative to early application at 3 HAI on 7 DAI. Interaction between ash concentration and spray interval was however not significant ($p < 0.05$). At 21 DAI, successive increment in ash concentration significantly ($p < 0.05$) reduced leafspot incidence. Plants treated with 6.00 mgml⁻¹ ash concentration at 3 HAI showed no leafspot disease symptoms. Interaction between ash concentration and spray interval was significant ($p < 0.05$).

Furthermore, plantain inflorescence ash applied above 1.50 mgml⁻¹ caused a significant reduction in leafspot severity by *P. sorghina* at 7 DAI (Table 3). Delaying the time of ash spray up to 6 HAI significantly ($p < 0.05$) increased leafspot severity relative to early spray at 3 HAI. Interaction between ash concentration and spray interval was significant ($p < 0.05$). However, at 14 and 21 DAI, increased ash concentration caused a significant ($p < 0.05$) decrease in leafspot severity. Moreover, delay in ash treatment up to 12 HAI significantly ($p < 0.05$) increased leafspot severity relative to 6 HAI ash application.

No leafspot symptom was observed on plants treated with 6.00mgml⁻¹ at 3HAI. Delaying the application of ash up to 6HAI at 3.00mgml⁻¹ and 6.00mgml⁻¹ significantly ($p < 0.05$) increase leafspot disease severity. Delay in ash application tended to have increased disease severity across the different ash concentration.

Effect of Plantain Ash and Carbendazim Spray on Leafspot Incidence, Severity and Yield (Field Study)

Results in Table 4 show the effect of plantain rachis ash and carbendazim spray on leafspot incidence, severity and yield of the test plant. All treatments significantly ($P>0.05$) reduced disease incidence and severity and enhanced leaf yield. Carbendazim at 3.0mg a.i./ml had the least disease incidence, severity and the highest leaf yield. These parameters were however, not significantly better than those in 6.0mg/ml ash.

DISCUSSIONS

The fungus frequently isolated from leafspot lesions was identified as *Phoma sorghina*. This is in agreement with earlier reports by Nwufu and Atu (1987) and Nwufu (1997). The isolated fungus was pathogenic. This also agrees with earlier works by (Maduewesi, 1997, Nwufu, 1997), who reported that *P. sorghina* was pathogenic to *T. occidentalis* in South-eastern Nigeria. Similarly, the result obtained on the effect of plantain inflorescence ash on the percentage inhibition *in vitro*, showed that the ash constantly inhibited the mycelial growth of *P. orghina*. The ash was also effective in inhibited and reducing the incidence and severity of leafspot disease caused by this pathogen *in vivo*. The fungicidal properties of this ash may be traced to its high alkalinity (pH 9.26 - 9.95). Eze and Maduewesi, (1990) and Obgeni, (1995) had earlier linked ash efficiency with alkalinity. The fungicidal properties of the ash observed during this study also agrees with those of Osai and Ikotun, (1996) who reported on the use of plantain ash in controlling rot of yam tuber and cassava stem. The presence of phenols and other elements in plantain ash may also explain its phytotoxicity. Asuquo et al (2002) reported that the fungitoxic superiority of *Elaeis guineenses* ash over other plant extracts in their study was due to higher phenols, sodium, potassium and phosphate levels.

Further more, the result obtained in the effect of plantain inflorescence ash on the incidence and severity of *P. sorghina in vivo*, indicates that the efficacy of the ash increases with an increase in the ash concentration. Delayed ash spray caused an increase in leafspot incidence and severity induced by this fungus. Interaction between ash concentration and spray interval showed that higher ash concentration applied early, reduced leafspot incidence and severity to the lowest. This suggest that time of control as well as concentration, are important in disease suppression using plantain ash.

Table 1: Effect of Plantain Inflorescence Ash on Mycelial Growth, Sporulation and Spore Germination *In-vivo*

Ash Conc. (mgml ⁻¹)	Mycelial Growth (% Inhibition)	Sporulation	Spore Germination
0.00	0.00	2.0x10 ³	5.00x10 ²
0.10	5.80±0.42	3.6x10 ²	3.4x10 ²
1.00	8.80±0.86	2.7x10 ²	3.1x10 ²
1.50	16.47±0.75	1.9x10 ²	2.3x10 ¹
2.50	32.86±2.00	0.5x10 ²	1.4x10 ¹
5.00	53.51±0.87	1.2x10 ¹	0.6x10 ¹
LSD (0.05)	0.37		

Values are means of eight replicates per concentration.

Data was based on mycelial diameter after 3 data of incubation at 28±2°C

Table 2: Effect of Different Concentrations of Plantain Inflorescence Ash and Spray Intervals on Disease Incidence at 7, 14 and 21DAI (*In-Vitro*)

Ash Conc. (mgml ⁻¹)	Spray Intervals			
	3HAI	6HAI	12HAI	Mean
7DAI				
0.00	16.0(3.77)	16.0(3.77)	16.0(3.77)	16.0(3.77)
1.50	8.0(2.44)	12.0(3.00)	16.0(3.77)	12.0(3.00)
3.00	8.0(2.44)	12.0(3.00)	16.0(3.77)	12.0(3.00)

Table 2: Contd.,

6.00	0.0(0.71)	4.0(1.47)	4.0(1.47)	2.7(1.22)
Mean	8.0(2.31)	11.0(2.81)	13.0(3.20)	
14.DAI				
0.00	20.0(4.53)	20.0(4.53)	20.0(4.53)	20.0(4.53)
1.50	8.0(2.44)	12.0(3.00)	16.0(3.77)	12.0(3.00)
3.00	8.0(2.44)	12.0(3.00)	16.0(3.77)	12.0(3.00)
6.00	0.0(0.71)	4.0(1.47)	4.0(1.47)	2.7(1.22)
Mean	9.0(2.43)	12.0(3.00)	14.0(3.39)	
21DAI				
0.00	20.0(4.53)	20.0(4.53)	20.0(4.53)	20.0(4.53)
1.50	8.0(2.44)	16.0(3.77)	20.0(4.53)	14.7(3.51)
3.00	8.0(2.24)	12.0(3.00)	16.0(3.77)	9.0(2.43)
6.00	0.0(0.71)	4.0(1.47)	4.0(1.47)	2.7(1.22)
Mean	11.0(2.81)	13.0(3.20)	15.0(3.56)	

	7DAI	14DAI	21DAI
LSD (0.05) Comparing ash concentration means =	0.52	0.47	0.50
LSD (0.05) Comparing spray intervals means =	0.45	0.31	0.23
LSD (0.05) Comparing ash conc. and spray interval means =	ns	0.34	0.43

Values in parenthesis are square root transformed data to which LSD (0.05) values apply

DAI = Days after inoculation

HAI = Hours after inoculation

ns = Non-significant

Table 3: Effect of Different Concentrations of Plantain Inflorescence Ash and Spray Intervals on Disease Severity at 7, 14 and 21DAI (*in-vitro*)

Ash Conc. (mgml ⁻¹)	Spray Intervals (Hours after Inoculation (HAI))			
	3HAI	6HAI	12HAI	Mean
7DAI (Days after Inoculation)				
0.00	3.20	3.60	3.80	3.53
1.50	3.00	3.40	3.40	3.27
3.00	2.60	2.80	3.00	2.80
6.00	1.00	2.20	2.60	1.93
Mean	2.45	3.00	3.20	
14.DAI				
0.00	3.80	4.00	4.00	3.93
1.50	3.00	3.40	4.00	3.46
3.00	2.60	2.80	3.00	2.80
6.00	1.00	2.20	2.60	1.93
Mean	2.60	3.10	3.40	
21DAI				
0.00	4.00	4.40	4.20	4.20
1.50	3.00	3.60	4.00	3.53
3.00	2.80	2.80	3.00	2.87
6.00	1.00	1.00	2.60	1.53
Mean	2.70	2.95	3.45	

	7DAI	14DAI	21DAI
LSD (0.05) Comparing ash concentration means =	0.62	0.32	0.44
LSD (0.05) Comparing spray intervals means =	0.23	0.28	0.38
LSD (0.05) Comparing ash conc. And spray interval means =	ns	0.55	0.77

Table 4: Effect of Plantain Rachis Ash and Carbendazin Spray on Leafspot Incidence, Severity and Yield of *T. occidentalis* under Field Condition

Ash Conc. (mgml ⁻¹)	Plant Infected (% Incidence)	Severity *	Leaf Yield (t.ha ⁻¹)
0.0	63.4	4.2	2.10
1.5	50.2	3.1	2.81

Table 4: Contd.,

3.0	33.6	3.2	3.08
6.0	27.4	2.4	4.12
Carbendazim (6mg/ml)	22.7	2.2	4.22
LSD (p=0.05)	5.2	0.60	0.82

*Severity was scored on a 1 to 5 point scale after Onuegbu and Dimkpa (2010)
Fifty (50) plants were scored in each treatment

CONCLUSIONS

The consistency of plantain rachis ash in suppressing the growth, sporulation and germination of *Telfairia* leaf spot pathogen in vitro and disease development in green house and field experiments at levels comparable with carbendazim shows that plantain ash may be used by resource poor farmers to suppress leaf spot and increase yield in this crop.

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