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Comparative efficacy of three plant extracts for the control of leaf spot disease in fluted pumpkin (*Telfairia occidentalis* Hook F.)

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Abstract
The production and leaf quality of Fluted pumpkin (*Telfairia occidentalis* hook f) in Nigeria are threatened by leaf spot and blight. The comparative efficacy of Siam weed (*Chromolaena odorata (*L.), Utazi leaf (*Gongronema latifolium* and Bitter leaf (*Vernonia amygdalina*) and a synthetic fungicide (Forcelet) against the mycelia growth of the leaf spot pathogen (*Phoma sorghina*), leaf spot disease, growth and yield of fluted pumpkin (*Telfairia occidentalis* hook f) was studied *in vitro* and *in vivo*. In 2013 and 2014. The experiments design for the *in vitro* experiment was Completely Randomize Design (CRD) with six replicates and Randomize Complete Block Design (RCBD) with four replications in the in vitro study. All plant extracts consistently inhibited the growth of *P. sorghina in vitro*, and suppressed leaf spot development by between 26.2 and 53.54% in 2013 and by between 26.3 and 51.79% in 2014 under field conditions. Leaf and pod yield were also enhanced, *C. odorata* leaf extract was significantly ($p \leq 0.05$) superior to all other treatments followed by *V. amygdalina* whose effect did not differ significantly ($P \geq 0.05$) from Forcelet in all the parameters measured. These results show that leaf extracts of *C.odorata and V. amygdalina* could be used for the control of leaf spot disease and enhanced yield in fluted pumpkin.
Keyword: Efficacy, Plant extract, Control, Leaf spot disease, Yield.

1. Introduction

Fluted pumpkin (*Telfairia occidentalis* HOOK F) is one of the most widely cultivated leaf vegetables especially in Southeastern Nigeria where it is of ethnobotanical importance in the folklore, dietary and cropping system of the people (Olaniyi and Oyerele, 2012). Its succulent young shoot, tender leaves and seeds are consumed by humans and have high nutritional, medicinal and industrial values (Akoroda, 1990, Oluwale *et al.*, 2003, Horsfall and Spiff, 2005, Fasuyi, 2006). They are a good source of organic acids, mineral salts, oils, vitamins, proteins, and carbohydrates. Earlier works (Schippers, 2002; Grubben and Berilen, 2004; Awodun, 2007, Akanbi *et al.*, 2007, Olaniyi and Oyerele, 2012) have reported the relative proportion of these substances and factors that affect them. Additionally, fluted pumpkin plays an important role in the economy of the producing areas where it is a high income earner for farmers (Udo *et al.*, 2013).

Despite its importance, increasing relevance and high demand, the production, quality and market value of fluted pumpkin is affected by a number of pests and diseases (Umoetok *et al.*, 2009; Osai *et al.*, 2013). Of these, leaf spot caused by a variety of fungi (Nwufo, 1992; Nwufo and Ihejirika, 2008; Madunagu *et al.*, 2008; Akan, 2010; Godwin- Egein *et al.*, 2015) is the most prevalent. Leaf spot may be white, brown, gray, translucent or concentric. In the humid environment of Calabar area, the translucent leaf spot caused by *Phoma sorghina* (Sacc.) Boerema, Dorenbisch Sc Van Kesteren is most prevalent and severe especially in early (April) season crops (Osai *et al.*, 2013). Biweekly spray of synthetic fungicides has been reported to reduce leaf spot severity (Nwufo, 1992; Nwufo and Ihejirika, 2008). However scarcity and high cost limit their use. Besides, chemical control of diseases requires expertise, and is hazardous to man, non target beneficial organisms and the environment in which it leaves non biodegradable residues (Baka, 2014a). These drawbacks and the growing desire for organic produced food devoid of synthetic chemicals necessitate the need for alternative disease control that are available, cheap, safe and environment friendly.

In recent times, bioactive secondary metabolites of plant and microbial origin have found application in plant disease management and are becoming important components of integrated pest management (Baka, 2014a; Hussain *et al*., 2014). Such plant metabolites include alkaloids, flavoniods, glycosides, saponins, tannins and terpernoids among other compounds (Owolabi *et al*., 2010; Ilondu, 2013; Ali and Ibiam, 2014; Baka, 2014a&b; Udochukwu, 2015) found among several angiosperms in virtually every locality. The use of plant extracts is not only cheap and readily available but requires low input technologies and hence affordable to resource poor farmers that occupy an important place in vegetable production. This study reports the comparative efficacy of

three plant extracts (*Vernonia amygdalina (L) Delile, Chromolaena odorata (*L.) King and H.E.Robins and *Gongronema latifolium (L.Endl*), Decne, with known antimicrobial properties (Owolabi *et al.,* 2010; Ilondu, 2013; Alemu *et al.,* 2014; Orji *et al.,* 2015) against the mycelia growth of *Phoma sorghina* (Sacc.) Boerema, Dorenbisch Sc Van Kesteren and for the control of translucent leaf spot disease in *Telfairia occidentalis.*

2. Materials and methods

Laboratory and field experiments were conducted in the University of Calabar, Calabar Southeast Nigeria in 2013 and 2014 to evaluate the effect of three plant extracts: bitter leaf (*V. amygdalina),* siam weed (*C. odorata)* and "utazi" (*G. latifolium)* on the mycelia growth of the leaf spot pathogen (*Phoma sorghina),* leaf spot diseases control and yield of fluted pumpkin. Calabar is located between latitude $4^0 27^1$ and $5^0 32^1$N, longitude $7^0 15^1$ and $9^0 28^1$E and and lies 99m above sea level in the rainforest zone. It has an annual rainfall of between 2500 and 3000mm, relative humidity of 70-80% and minimum and maximum temperature of 23^0C and 30^0C respectively (CRSNMANR, 1989)

2.1. Isolation of test pathogen

Phoma sorghina isolate used in this study was isolated from infected fluted pumpkin plants showing translucent leaf spot symptom in mono-cropped plots within the Crop Research Farm of the Department of Crop Science, University of Calabar and maintained in potato dextrose agar (PDA) slants. Infected leaves were washed under flowing tap water, and cut into 4mm pieces at the interface between the infected and healthy portions with sterile blades. Cut leaf sections were surface sterilised with 10% $NaOCl_3$ solution for 1 min and immediately washed in three changes of sterilised distilled water, dried on Whatman No 1 filter paper and then placed in streptomycin (0.1%)-PDA plates. Four leaf pieces were placed per 9cm (diameter) Petri dish. Inoculated plates were incubated at 28 $\pm 2^0$C for five days and developing fungi colonies were sub cultured until axenic culture of the fungus was obtained. Identification was based on Barnett and Hunter (1998) and identification guide of the Commonwealth Mycological Institute, Kew. Pathogenicity of fungal isolate followed the procedure of Osai *et al* (2013). Twenty healthy leaves from four weeks old plants were detached, surface sterilised by swabbing with 70% ethanol and spray inoculated with blended mycelial suspension (Maduewesi, 1977) of the fungus. Inoculated leaves were placed in sterile polythene bags containing cotton wool moistened with sterilised distill water and incubated at ambient temperature for 7 days. Developing lesions were compared with those observed in the field.

2.2. Preparation of Plant extracts

Fresh leaves of Bitter leaf, Siam weed, and "Utazi" were harvested from the Botanical Garden of the Department of Botany, University of Calabar, Calabar. The leaf samples were rinsed under running tap water to remove dirt; air dried for 48 hours and pulverized using a Mortar and pestle. 200 g of each pulverized material was then homogenized in 200 ml of distilled water for 10 min and strained through two layers of cheese-cloth, filtered through Whatman No 1 filter paper and the filtrate was finally centrifuged at 500 rpm for 10 min (Praveen *et al*., 2014). The supernatant liquid was collected and used as stock solution.

2.3. Effect of plant extracts on mycelia growth of P. sorghina

Stock solution of each extract above was diluted with sterile distilled water to obtain the following test concentrations 1 %, 10 %, 25 %, 50 % and 100 %. One ml of each extract concentration was incorporated into sterile molten streptomycin- PDA in Petri - plates, gently swirled to ensure proper mixing and allowed to gel. Six replicate plates from each concentration of each extract were inoculated at the centre with 5 mm diameter mycelia disc cut from actively growing culture of *P. sorghina*. Inoculated plates without extracts served as control. All plates were sealed with paraffin tape and incubated at 28 ± 2^0C. Observation on the mycelia growth was recorded daily for five days of incubation. Colony diameter was determined by measuring the average radial growth along two perpendicular lines on the reverse side of the Petri dishes. The percentage inhibition in growth was computed thus:

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

Where MGI % = Mycelia Growth Inhibition.

MGDC = Average Mycelia growth diameter in control

MGDE = Average Mycelia growth diameter in Extract incorporated plates.

2.4. Effects of plant extracts on leaf spot incidence and severity and yield of T. occidentalis

Field experiments were conducted during the early (April) planting season of 2013 and 2014 to assess the effects of bitter leaf, siam weed and "utazi" leaf extracts on the incidence and severity of leaf spot disease, as well as on

the growth and yield of treated plants in the Crop Research Farm of the University of Calabar, Calabar. The soil is sandy loam ultisol (Esu, 2010).

The experimental field (30 m X 19 m) was cleared with machete and tilled with spade and field was thereafter marked out into plots of 5 m X 4 m in four blocks. Plots were separated by 1m while blocks were 2 m apart. Poultry manure was spread uniformly on the plots and incorporated into the soil at 4 t ha^{-1} the soil was left for two weeks before planting. Seeds of a local variety of fluted pumpkin "Afia-obong" were purchased from the local market, air dried for 24 hrs and planted one seed per hole 2-3 cm deep and spaced 1 m X 1 m (10,000 plants ha). There were four rows of crops in each plot. Hand weeding was done with a hand hoe at 4 and 8 weeks after planting (WAP). Plant extracts and Forcelet (carbendazim 50 % WP) were applied to designated plots at the first appearance of disease symptoms (about 4 weeks after planting) using a 5 L hand held sprayer. Extracts were prepared as previously described and applied at 200g L^{-1} concentration while forcelet (Carbendazim) was applied at 3g a.i. L^{-1}.

2.5. *Experimental design and data analysis*

The *in vitro* experiment followed a completely randomized design with six replicates per treatment, whereas the field experiment was laid out in a Randomized Complete Block design with five treatments (bitter leaf, Siam weed, "Utazi" leaf extracts, Forcelet and no treatment (control)) replicated four times. Observations on disease incidence and severity were made from three weeks after application of plant extracts by visual observation of the symptoms. Plants from the two middle rows in each treatment were selected and count was taken on the number of plants showing symptoms of leaf spot and expressed in percentage.

Disease severity was measured as the portion of diseased leaves per plant (Onuegbu and Bello, 2010). Agronomic data collected included the length of the primary vine, number of leaves per plant, fresh weight of leaves, number of pods/plot and weight of pod at harvest.
Fresh shoot weight was extrapolated for a hectare using the formula:

$$\text{Fresh yield / ha} = \frac{\text{Fresh yield / plot X 10000}}{\text{Plot size X 1000}}$$

All data were subjected to analysis of variance (ANOVA) and significant means were compared by Fisher's Least Significant Difference (F-L SD) at 5% probability level. Following GENSTAT 7.0 release.

3. Results

Phoma Sorghina was consistently isolated from *T. occidentalis* leaves with translucent leaf spot symptom. It produced characteristic dense, hyaline mycelia with dictyochlamydospores on the tip of hyphal strands and intercalary hyphal cells.

3.1. Effect of plant extracts on mycelia growth of P.sorghina.

Data in Tables 1 and 2 shows the growth response of *P. Sorghina to water extracts of C. odorata, G. latifolium and V. amygdalina.* Whereas all the extracts inhibited the colony growth of the test fungus, none was fungicidal as complete inhibition was not achieved in any of the concentrations. Mycelia inhibition was concentration dependent with inhibition level increasing with concentration in all the tested plant extracts. The highest growth suppression of 41.67, 64.0 and 73.33 % was associated with 100 % concentration in *G. latifolium, V. amygdalina* and *C. odorata* respective (Table 1). Among the concentrations of *C. odorata* extract, there was no statistical ($P \leq 0.05$) difference in inhibition among 25, 50 and 100 % and among 10, 25 and 50 %. Similarly growth inhibition in *G. latifolium* and *V. amygdalina* was not significantly different among 10, 25, 50 and 100 % concentrations. On the whole, *C. odorata* extract had a significantly higher inhibitory effect than *V. amygdalina* and *G. latifolium* extracts (Table 2).
Although all plant extracts significantly ($p \leq 0.005$) inhibited the growth of *P. Sorghina* throughout the study period, differences in inhibition between *V amygdalina* and *G latifolium* extract are not statistically significant at 24 hrs of incubation. There was also a reduction in inhibition by *G latifolium* at 120 hrs of incubation (Table 2).

Table 1: Mycelia growth inhibition of *Phoma sorghina* by different concentrations of *C. odorata, G. latifolium* and *V. amygdalina* extracts after 5 days incubation at 28±2^{0}C.

Conc (%)	***C. odorata***		***G. latifolium***		***V. amygdalina***	
	Mycelia Growth (cm)	*Inhibition (%)*	*Mycelia Growth (cm)*	*Inhibition (%)*	*Mycelia Growth (cm)*	*Inhibition (%)*
0	9.00	-	9.00	-	9.00	-
1	4.60	48.89	6.36	29.33	4.75	47.22
10	3.62	59.98	6.12	32.00	3.88	56.88
25	2.93	67.44	5.48	39.11	3.82	57.56
50	2.68	70.22	5.40	40.00	3.50	61.11
100	2.40	73.33	5.25	41.67	3.24	64.00
LSD $_{(p\leq0.05)}$	1.15		1.02		0.64	

Table 2: Comparative effect of the water extract of *C. odorata, G. latifolium* and *V. amygdalina* on the colony growth of *P. sorghina* after different hours of incubation at 28±2^{0}C

Hours of incubation						
	24		**72**		**120**	
Plant Extract	Colony growth (cm)	Inhibition (%	Colony growth (cm)	Inhibition (%)	Colony growth (cm)	Inhibition (%)
C. odorata	1.68	66.93	2.33	73.37	2.40	73.33
V. amygdalina	2.30	54.72	3.38	61.89	3.24	64.00
G. latifolium	2.35	53.74	4.95	44.13	5.25	41.67
Control	5.08	-	8.86	-	9.0	-
LSD $_{p\leq0.05}$	0.78		1.02		1.15	

3.2. Effect of aqueous plant extracts on leaf spot development

Aqueous extracts of *C. odorata G. latifolium* and *V. amygdalina* and Forcelet (Carbendazin) significantly ($p\leq0.050$) suppressed leaf spot disease under field conditions (Table 3). In both years, disease severity was significantly least (11.5% and 10.8%) with *C. odorata* leaf extract with corresponding disease suppression of 54.54% and 50.79% in 2013 and 2014 respectively. Variations in disease severity and disease suppression were not statistical ($p\geq0.050$) between *V. amygdalina* and Forcelet. These were, however, significantly ($p\geq0.050$) lower than in *G. latifolium* treated plots. Disease severity was 18.3% and 16.5% in 2013 and 2014 respectively while disease reduction was 26.23% and 26.24% for both years (Table 3).

Table 3: Effect of aqueous extract of *C. odorata, G. latifolium and V.amygdalina* and Forcelet (carbendazim) on leaf spot disease of *T. occidentalis*

Leaf spot (%) and season (yr)s

Treatments	2013			2014		
	Incidence	*Severity	Suppression	Incidence	Severity	Suppression
C. Odorata	15	11.5	54.51	19	10.8	50.79
Forcelet	17	13.8	44.48	16	12.2	45.63
V.amygdalina	30	14.8	41.44	28	12.8	43.86
G. latifolium	48	18.3	26.23	43	16.5	26.34
Control	68	24.8	-	56	22.4	-
FLSD ($p \leq 0.05$)		2.24			1.86	

***** Disease severity was based on proportion of infected leaves on 6 central plants in the two middle rows of each plot

3.3 Effect of plant extracts on yield parameters of T. occidentalis

Data in Table 4 show that vine length and number of leaves per plants were not significantly different among plant extracts and Forcelet. Fresh marketable yield was significantly ($P \leq 0.03$) highest with *C odorata* spray. The least yield of 141.80Kg/ha was recorded in non treated control plants. In these plants, infected leaves were smaller in size and shot holes were visible on the lamina of heavily infected leaves. Similarly, number of pods was significantly highest in plants sprayed with extracts of *C. odorata* and least with *G. latifolium* treated and non-treated plots (Table 4). Between Forcelet and V. *amygdalina* treatments, number of pods was not significantly ($P \geq 0.05$) different. Weight of pods was statistically similar among plants treated with Forcelets, *V. amygdalina* and *G latifolium* extracts. Pod weight was highest among plants sprayed with extract of *C. odorata.*

Table 4: Effects of aqueous plant extracts and Forcelet (Carbendazim) on yield parameters of *T. occidentalis*

Treatments	Yield parameters[a]				
	Vine length (cm)[b]	No of leaves/plant[b]	Fresh leaf yield (kg/ha)	No. of pods[c]	Pod weight (kg)
C. Odorata	148.40	18.60	236.30	8.24	10.13
Forcelet	150.80	17.52	229.00	5.40	6.74
G. latifolium	146.30	17.00	205.40	2.20	5.62
V. amygdalina	163.52	17.84	231.60	5.00	6.25
Control	144.10	16.80	141.80	2.20	3.50
FLSD ($p \leq 0.005$)	NS	NS	4.32	2.86	3.15

[a] Data in each parameter are across the two years (2013 and 2014) of the study.
[b] Data are means of vine length and leaf count in six central plants of the two middle roles in each plot at ten weeks after planting.
[C] Mean number of pods per plot

4. Discussion

Water extracts of *C. odorata*, *V. amygdalina* and *G. latifolium* consistently suppressed the mycelia growth of *P. sorghina in vitro.* They also suppressed leaf spot disease and enhanced fresh leaf and pod yield. These results agree with previous works that associated fungal growth inhibition and disease suppression with a variety of plant bioactive compounds (Oluma and Elaigwe, 2006; Begum *et al.*, 2009; Al-Samarrai *et al.*, 2012; Baka,

2014b). Some of these compounds (alkaloids, polyphenols, biurates, saponins, terpernoids) have been reported in all the plants used in this study (Ilondu, 2013; Orji *et al.,* 2015). According to Ilondu (2013) the inhibition of radial growth of *C. linatus* and *Cercosporell apersica* isolates of groundnut leaf spot disease by ethanolic extracts of *V. ambigua, V. amygdalina and V. cinerea* is attributable to mixtures of bioactive compounds such as alkaloids, saponins, tannins and flavournoids. Similarly the biotoxic activity of *G. latifolium* against *Colletotrichum* isolate from tomatoes is attributable to alkaloids, saponins, tannins and flavournoids as well as glycosides (Orji *et al.,* 2015). Although Nwachukwu and Umechuruba (2001) reported the use of *V. amygdalina* in suppressing plant diseases, it was less effective in comparison with *C. odorata* in this study. This may be explained by variations in the concentrations of various bioactive ingredients among varieties of *V. amygdalina* (Ilondu, 2013). Its effect was however comparable with that produced by the synthetic fungicide Forcelet (carbendazim 50 WP). Again, the reduction in mycelia growth suppression by *G. latifolium* may be explained by volatility of some of its active compounds (Orji *et al., 2015).* The higher fresh shoot yield obtained in this study may be related to disease suppression. Umoetok *et al (*2009) had reported that suppression of the population *Ootheca metabilis* by neem extracts reduced defoliation and increased marketable vines and leaves of *T. occidentalis*.

5. CONCLUSION

In conclusion, significantly lower leaf spot disease and enhanced marketable leaf yield of fluted pumpkin were recorded with the application of water extracts of *C. odorata*, *V. amygdalina* and *G. latifolium*. These plant materials which are readily available and eco-friendly may therefore be utilised to reduce leaf spot disease and enhance leaf and pod yield in *T. occidentalis*, and thus improve the income of resource poor farmers involved in the cultivation of fluted pumpkin.

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