FUNGITOXICITY OF ASHES FROM SOME TROPICAL TREES AGAINST SEED-BORNE FUNGI ISOLATED FROM SUNFLOWER (*Helianthus annuus* L.)

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SUMMARY

The ashes of sixteen tropical trees were evaluated *in vitro* for their potential to reduce the mycelial growth of seedborne fungi; *Aspergillus niger, Fusarium oxysporum* and *Fusarium incarnatum*. Sawdust samples of sixteen tropical trees were collected from the saw mill industries and were sun-dried and burnt using a pre-fabricated incinerator (12 – 100kg/hr) to get ash. The ash samples were heat sterilized (Gallenkamp hot air oven, 160°C for 5 h) to eliminate the resident mycobiota. Fungi tested were isolated from sunflower seeds via blotter test or agar plate methods and inoculated on Petri dishes containing Potato dextrose agar media amended with ash while un-amended media plates served as control. Inoculated media were incubated under room temperature at 28 ± 1 °C. Diameter of the fungal colony was measured using a meter rule along two diagonal lines drawn perpendicularly on the reverse side of each Petri dish 7th day after inoculation. *Fiscus exasperata* ash reduced mycelial growth of fungi by an average of 96.93%. Average mycelial growth reduction by *Gmelina arborea* and *Terminalia superb* ashes were 87.65% and 84.74% respectively. These were followed by *Piptadeniastrum africanum* and *Sterculia rhinopetela* ashes with 78.37 and 76.32% mycelial growth reductions respectively. *Bombax* spp. ash had the least fungitoxicity effect as evidenced by only 25.69% in mycelial growth reduction of *F. incarnatum*. This study showed that the prospect of wood ash in the control of seedborne pathogens and a possible alternative to chemical pesticides.

Keywords: *A. niger,* Ash, *Fusarium incarnatum* and *Fusarium oxysporum*

The use of plant products as an alternative to synthetic fungicides in plant disease control is a welcomed option in tropical subsistent farming system. This is due to the high cost of synthetic

fungicides as well as its residual effect, lack of technical know-how about on usage and numerous adulterations of available seed treatment fungicides (Zarafi and Emechebe, 2006). The effectiveness of biopesticides as extracts in controlling several plant pathogens has been widely reported (Egbontan *et al*., 2013; Jimoh *et al*., 2016; Falade *et al*., 2017). However, the potential of plant ash (the product of burning material) has received little research attention despite their availability and ease of preparation Ekpo and Banjoko (1994). Enikuomehin and Kehinde (2007) and Opara and Bassey (2016) reported the fungicidal and bactericidal potentials of some ash samples on maize, wheat and tomato pathogens. Enikuomehin *et al*. (1998) also reported the fungitoxicity of ash samples to *Sclerotium rolfsii*, however, no report of the fungitoxicity of plant ashes to seed-borne fungi of oil seeds is available. The present study was carried out to screen the fungitoxicity of ashes from some tropical plants at varying concentrations used traditionally by farmers against insect pests and diseases to seed-borne fungi of sunflower *in vitro*.

MATERIALS AND METHODS

Sawdust of *Antiaris Africana*, *Bombax* spp., *Brachystegia* spp., *Lophira alata*, *Gmelina Arborea*, *Ficus exasperata*, *Esogordonia papaverifera*, *Piptadeniastrum africanum*, *Ptenitrocarpus soyauxii*, *Sterculia rhnopetela*, *Shorea* spp., *Tectona grandis*, *Terminalia superb*, *Triplochiton scleroxylon*, *Khaya* spp. and *Entandrophragma utile* were collected from saw mill industries. A portion of each sawdust type was sun-dried for 2-4 week and burnt using a pre-fabricated incinerator (12 – 100 kg/hour) according to PATH (2010).

Isolation of the tested fungi

Fungal pathogens (*Fusarium* spp) were isolated via agar plate and *Aspergillus niger*, blotter plate methods from asymptomatic sunflower seeds and the cultures obtained were identified using morphological characters and description recorded in Barnett and *Hunter (1998*). For *Fusarium oxysporum* and *Fusarium incarnatum* isolation, sixty sunflower seeds were surface-treated in 1% NaOCl for 30 seconds, and rinsed in distilled water for 20 seconds and placed on semi-selective

medium peptone Pentachloronitrobenzene (PPA) according to EL-Wakil (2014). The plates were incubated for 5 days under fluorescent light on a 12hr day/night schedule at 22 – 24 °C. After 5 days, 10 randomly selected colonies of *Fusarium* species were transferred to PPA and inoculated for 7 days as described above, after which single spore isolation (purification) were done. *Fusarium* spp were further cultured on Carnation Leaf Agar (CLA) where characteristic features like sporodochia and uniform macroconidia could be vivid, then cultured on Potato Dextrose Agar (PDA) for pigmentation and colony morphology, and incubated for 10-14 days. Morphological identification of *Fungal* species were done according to Nelson *et al.* (1983) and Leslie *et al.* (2005). All the isolated *Fungi* spp were identified using Olympus BX51 Digital Microscopy (Olympus Optical Co., Ltd, Japan).

Preparation of aqueous ash extracts

Ten, 7.5 and 5 g of each ash samples was weighed onto Aluminum foil using sensitive weighing balance (Model: PL203; Surinda and Company, Ambalahttps://www.adamequipment.co.uk/eclipse-analytical-balances) autoclaved at 160°C for 15 min with pressure of 15 to 22 PSI (adjustable) and dissolved in 100 ml of sterile water for 24 hours and sieved through 1 mm cheese cloth to under aseptic condition.

Fungitoxicity determination of aqueous aqueous ash suspension

One milliliter of each ash suspension was dispensed in 9-cm-diameter Petri dishes; 15mL of cooled molten chloramphenicol-modified (60 mg/mL) potato dextrose agar (CPDA) was dispensed into each Petri dish and gently swirled to ensure even dispersion of ash suspension. Each Petri dish with solidified media was centrally inoculated with a 9-mm mycelial disc of 6-day-old culture of the respective fungus. The factorial set of treatments consisting of three levels of ash concentrations and a control (where no extract is added) was arranged in a completely randomized design replicated three times. The inoculated media were incubated at ambient temperature of 28 ± 1 °C. Colony diameter was measured after 6 days along two perpendicular pre-drawn lines on

the reverse side of the plate. Fungitoxicity was expressed as percentage reduction in mycelial growth using the formula adopted from Enikuomehin *et al*. (2002):

$$M_p = \frac{M_1 - M_2}{M_1} \times 100$$

Where M_p is percentage reduction of mycelial growth, M_1 is mycelial growth in Petri dish and M_2 is mycelial growth in Petri dish containing ash.

Statistical analysis

All data collected were subjected to analysis of variance (ANOVA) using "CoStat software" (2005) and mean separation was done using Least Significant Difference (LSD).

RESULTS

Mycelial growth reduction of *A. niger, F. oxysporum* and *F. incarnatum* by ash suspensions

All ash types reduced the mycelial growth of the test fungi (Table 1). *A. africana* ash reduced mycelial growth of *A. niger*, *F. oxysporum* and *F. incarnatum* by 58.39, 59.78 and 62.58% respectively. A. africana ash was more fungitoxic (LSD = 2.62; $P \leq 0.05$) to F. oxysporum than to other fungi. *Bambax* sp. ash was less fungitoxic (LSD = 2.28; $P \leq 0.05$) on mycelial growth of *F. incarnatum* compared with other test fungi. Mycelial growth reduction due to *Bombax* sp. ash ranged between 25.69 and 48.20% across all the test fungi. Mycelial growth reduction due to *Brachystegia* sp. ash ranged between 43.59 and 68.61% across test fungi. However, the ash suspension of the plant was more fungittoxic (LSD = 1.32; $P \leq 0.05$) to *A. niger* than other fungi. *Lophira alata* ash inhibited mycelial growth of *A. niger* by 74.54%, *F. oxysporum* (59.50%) and *F. incarnatum* (65.48%). *A. niger* has a significantly (LSD = 0.98; $P \leq 0.05$) higher mycelial growth reduction compared to other fungi. The mycelial growth reduction due to *G. arborea* ash was *A. niger* (86.03%), *F. oxysporum* (87.53%) and *F. incarnatum* (89.39%). *F. exasperata* ash induced mycelial growth reduction of on test fungi with a range of 95.60 and 98.53%. Against *A.*

niger, *E. papaverifera* ash enhanced mycelial growth reduction by 56.23%, *F. oxysporum* (68.19%) and *F. incarnatum* (83.21%). Reduction in mycelial growth reduction of *F. incarnatum* due to *E. papaverifera* ash was comparable (LSD = 2.31; $P \leq 0.05$) with that of other test fungi. *P. africanum* ash was more fungitoxic (LSD = 2.19; $P \leq 0.05$) to *F. incarnatum* than other *A. niger* but comparable to *F. oxysporum*. Mycelial growth inhibition due to *E. papaverifera* was *A. niger* (67.16%), *F. oxysporum* (81.26%) and *F. incarnatum* (86.69%). Reduction in mycelial growth across the test fungi by *P. sayauxii* ash varied significantly ($P \leq 0.05$) from 57.54 to 78.65%. However, *F. oxysporum* had the lowest mycelial growth inhibition followed by *A. niger* and *F. incarnatum*. Fungitoxicity of *S. rhinopetela* to mycelial growth of *A. niger* (78.47%) and *F. oxysporum* (78/65%) was comparable (LSD = 2.40; $P \leq 0.05$) while significantly ($P \leq 0.05$) lower (71.86%) mycelial growth inhibition was observed with *F. incarnatum*. *Shorea* sp. ash induced significantly ($P \leq 0.05$) lower mycelial growth reduction on *F. incarnatum* (59.54%) compared to *A. niger* (73.18%) and *F. oxysporum* (64.81%). *T. grandis* ash induced highest (LSD = 1.73; $P \leq 0.05$) mycelial growth reduction on *A. niger* (85.81%). However, against *Fusarium* spp., mycelial growth inhibition was between 59.54 and 64.82% as enhanced by *T. grandis* ash. Mycelial growth reduction of *A. niger* by *T. superb* ash was 90.09% and was significantly (LSD = 2.11; $P \leq 0.05$) higher than that of *F. incarnatum* (78.02%) and that of *F. oxysporum* with mycelial growth reduction of 78.02%. *T. scleroxylon* ash induced a comparable mycelial growth inhibition across the test fungi. Fungitoxicity of *Khaya* spp ash was stronger ($t = 2.04$; $P \leq 0.05$) towards mycelial growth of *F. oxysporum* inducing up to 87.08% than towards other test fungi. Ash from *E. utile* sample induced a comparable mycelial growth reduction between *A. niger* (68.14%) and *F. oxysporum* (65.62%). Lower level of mycelial growth reduction (57.15%) of *F. incarnatum* was induced by *E. utile* ash (Table 1).

In all instances, mycelial growth inhibition of test fungi reduced with increase in concentration of the plant ash suspensions. Against *A. niger*, concentration of 10 % (w/v) recorded a significantly (LSD = 2.18; $P \leq 0.05$) higher mycelial growth reduction than at 7.5 and 5 % (w/v). Similarly, with *F. oxysporum*, plant ash suspensions at concentration of 10 % (w/v) induced a significantly

(LSD = 2.82; $P \leq 0.05$) higher mycelial growth reduction than other concentrations which showed a comparative effect on the fungus. However, concentration of 10 % (w/v) of plant ash suspensions was superior (LSD = 0.97; $P \leq 0.05$) to other concentrations in reducing mycelial growth of *F. incarnatum* (Table 1).

Across the test fungi, plant ash suspensions at 5 % (w/v) concentrations reduced mycelial growth of *F. incarnatum* more (LSD = 1.73; $P \leq 0.05$) compared with the other two test fungi. However, at 7 % (w/v) concentration, plant ash suspensions recorded a comparable (LSD = 1.79; $P \leq 0.05$) mycelial growth reduction between *A. niger* and *F. oxysporum.* At concentration of 10 % (w/v), plant ash suspensions induced a comparable mycelial growth reduction on all test fungi (Table 1). Plant ash suspensions at all levels of concentration showed a significant mycelial growth reduction on all the test fungi (Table 1).

Table 1: Mycelial growth reduction of *Aspergillus niger*, *Fusarium oxysporum* and *Fusarium incarnatum* by some tropical trees ash at varying concentration

Ash suspension	Mycelial growth reduction (%)			
	Aspergillus niger	*Fusarium oxysporum*	*Fusarium incarnatum*	LSD
Antiaris africana	55.39	60.02	59.37	2.62
Bombax spp	47.32	39.28	25.69	2.38
Brachystegia spp	68.61	61.39	43.59	1.32
Lophira alata	74.54	59.50	65.48	0.98
Gmelina arborea	86.03	87.53	89.39	1.20
Fiscus exaperata	96.66	95.60	98.53	1.07
Esogordonia papaverifera	56.23	69.19	83.21	2.31
Piptadeniastrum africanum	67.16	81.26	86.69	2.19
Ptenirocarpus sayauxii	70.47	57.54	78.29	1.87
Sterculia rhinopetela	78.47	78.65	71.86	2.40
Shorea spp	73.18	64.81	59.54	1.08
Tectona grandis	85.81	64.82	59.54	1.73
Terminalia superb	90.09	86.11	78.02	2.11
Triplochiton scleroxylon	66.94	60.75	56.63	1.62
Khaya spp	43.21	87.08	60.61	2.04
Entandrophragma utile	68.14	65.62	57.15	1.90
Concentration/level (L)				
5.0	59.25	60.23	70.36	1.73
7.5	68.37	69.90	84.67	1.79
10.0	85.02	90.05	99.93	0.98
LSD	2.18	2.82	0.97	
Interaction				
A x L	S	S	S	

Mean of percentage mycelial growth reduction compared using F-ration test at $P \leq 0.05$; mean separated by Least Significant Difference; S - Significant

DISCUSSION

The reduction of mycelial growth of *A. niger*, *F. oxysporum* and *F. incarnatum* by the ash samples suggests that these ash samples were fungitoxic. There were variations in the toxicity of the ash samples to the different fungal species. However, *F. exasperata* ash exhibited strong fungitoxicity to all the test pathogens. Others, such as *G. arborea* ash was strongly fungitoxic to *F. incarnatum*, and *T. superb* was strongly fungitoxic to *A. niger*. The observed fungitoxicity of ash samples from *F. exasperata*, *G. arborea* and *T. superb* makes these ash samples candidate for further research. Kawamura *et al*. (2005) reported that five constituents of *G. arborea* isolated and identified as (+)-7′-O-ethyl arboreol, (+)-paulownin, (+)-gmelinol, (+)-epieudesmin and (−)-β-sitosterol has been reported to be antifungal in nature. Similarly, antifungal constituents which include ficusamide, furanocoumarins, (*S*)-(_) oxypeucedanin hydrate, (*R*)-(_) oxypeucedanin hydrate, bergapten (5-methoxypsoralen) were isolated from *F. Exasperata* (Adesope *et al*., 2006). Kra *et al*. (2014) reported the antifungal activities and established the antimicrobial virtues of T. superb. The root extract of *G. arborea* have been reported to inhibit the growth of *Aspergillus niger*, *Penicillium notatum* and *Candida albicans* (Hussani and Deeni, 1991). The water-soluble fraction of *G. arborea* completely digested the conidia of many postharvest pathogenic fungi (Barkai-Golan, 2001). Also, extracts of *Ficus* species significantly arrested mycelial growth of *A. flavus*, *A. niger*, *Botryodiploidia theobromae*, *F. oxysporum*, *F. solani*, *Penicillium chrysogenum*, *P. oxalicum* and *Rhizopus stolonifer* (Durugbo *et al*., 2012). Padam *et al*. (2012) attributed the antimicrobial properties/activities in various banana extracts to the presence of alkaloids, flavonoids and cardiac glycosides. According to Adebayo-Tayo and Odeniyi (2012), the mechanism by which isothiocyanates present in *Ficus capensis* inhibit cell division in fungi may involve enzymes by direct reaction with disulfide bond or through thiocyanate anion reaction to inactivate sulphydryl enzymes in cells. It has been demonstrated that this isothiocyanates is a source of natural fungicides (Barkai-Golan, 2001) which is regarded as both safe and effective against various fungal disease. Presence of antifungal agents in wood ash have earlier been reported by Eze and Maduewesi (1990) and fungitoxicity of wood product like sawdust ash by

Enikuomehin *et al*. (1998). This underscores the possibility of that burning may denature the organic compound of these plants and this may have metamorphosed into other fungitoxic compounds.

CONCLUSION

The present study demonstrates the potential of ash samples in the control of these seedborne fungal species. However, further studies are required to determine their practical application as seed treatments in tropical agriculture where the cost of commercial fungicides is prohibitive.

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