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Full Length Research Paper

Preservation of seeds against fungi using wood-ash of some tropical forest trees in Nigeria

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Fresh visually healthy seeds of *Zea mays* (maize), *Cucumeropsis mannii* (melon) and *Phaseolus vulgaris* (bean) were stored under three conditions, wood ash of some tropical forest trees in Nigeria namely; *Khaya grandifoliola, Nauclea diderrichii, Piptadeniastrum africanum, Mangifera indica, Mansonia altissima, Triplochiton scleroxylon, Ceiba pentandra, Terminalias superba, Terminalia ivorensis*). Seeds treated with benlate, an orthodox fungicide and seeds without any treatment to serve as the control of the experiment. These were set-up at two different locations on the campus (the laboratory and the botanic garden) for six months. The seeds stored with ashes of *Nauclea diderrichii and Piptadeniastrum africanum* were the most effective, stopping fungal growth and eliminating weevils compared to those seeds stored with benlate which is only effective against fungal growth. *P. vulgaris* (bean) seeds are the best stored of the three seeds probably due to the low moisture content of the seed. Four pathogenic fungi were isolated from the seeds (maize, melon and bean) at both locations and these include; *Aspergillus niger, Aspergillus flavus*, *Aspergillus fumigatus* and *Rhizopus racemosa*. Preliminary phytochemical screening revealed that some of these ashes contained a number of active compounds which enable them (ashes extracts) to inhibit the growth of the pathogenic fungi.

Key words: Seeds, storage fungi and wood-ash.

INTRODUCTION

Seeds like maize, beans and melons are of the world's staple food which provides energy and proteins for many populations. Seeds are effectively preserved in order to secure the availability of food all year round and to ensure the supply of good seeds for planting programs whenever needed (Oyeniran, 1980).

Seeds when improperly stored deteriorate and spoil during preservation for two reasons, either as a result of insufficient drying of the seeds before storage or as a result of the presence of small quantities of spores of storage fungi may be present on seeds going into storage (Van Egmond et al., 2007).

The term "storage fungi" describe a group of fungi adapted to growth in an environment of relatively low moisture content and high osmotic pressure that are involved in and responsible for deterioration of stored seeds (Alexopoulos et al., 2007). Storage fungi include a few species of *Aspergillus, Penicillium* and a few still unidentified species of fungi imperfection. Some storage fungi produce toxic compounds called mycotoxins which are harmful to human and livestock (Agrios, 1997). Some storage fungi invade seeds to such an extent as to render the seeds unfit for human and animal consumption (Dowd, 1994). The use of chemicals on seed in store is an effective storage method is however complicated by the fact that some of these chemicals have been found to have serious limitation of one sort or another, such as toxicity to animals, excessive cost, difficulty of application, undesirable on the processing quality of seeds and the odor and flavor of some chemical compounds on the storage seeds. This probably makes foods processed from it unacceptable to most people (Level, 1990). Wood-ash is composed of the organic and inorganic residue remaining after the combustion of wood, this is used as a means of biological control which is generally favored as a method of storing seeds because it does not have any of those disadvantages of chemicals and tend to be more durable in its effect (Adekunle and Uma, 2005).

This work investigated the efficacy and justifies

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scientifically the use of wood-ash use in seed storage.

MATERIALS AND METHODS

Sample collection

Fresh visually healthy seeds of *Zea mays* (maize), *Phaseolus vulgaris* (Bean) and *Cucumeropsis mannii* (Melon) were collected from the local markets in Bariga Lagos. Diseased seeds were separated from the visually health ones. Seventy-two pieces of plastic bowls were purchased from same market for the storage. The woods (*Khaya grandifoliola*, *Nauclea diderrichii, Piptadeniastrum africanum, Mangifera Indica, Mansonia altissima, Triplochiton scleroxylon, Ceiba pentandra, Terminalia superba* and *Terminalia ivorensis*) were brought from a wood merchant at Okobaba, Ebute-meta, Lagos.

Wood-ash preparation

Two hundred grammes of each of wood was burnt into ash, the quantity of ash produced varies, but was approximately five hundred grams each. The cool dried ashes were parked into sterile nylon bags with label containing the information on each ash respectively.

Calculation of the seeds moisture content

The moisture content of the seeds was determined at the beginning and the end of the storage period. Few seeds were in clean glass Petri-dishes and weighed, the weight served as the initial weight (g). The seeds, in glass Petri-dishes were put in an oven at 60°C for 48 h to allow the moisture to escape. The seeds were re-weighed to deduce the final weight (Agrawal, 1980).

$$\% \text{ moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

Seed preservation

Two storage locations on campus; The Botany laboratory and the glass house in Botanical garden were used. At each storage location, there were thirty-three disinfected plastic bowls which were divided into three groups (eleven bowls for each type of seed). Each bowl contained 1000 healthy seeds weighing about 200 g. The seeds were mixed with the wood-ashes (in ratio 1:2), benlate and one bowl of each type of seed serves as the control that is without any treatment. The mixture of seeds and wood-ashes were then stored in bowls with tight lids with labels containing information like the storage date, seed name, preservative agent and the location of storage. During storage period, seeds were collected randomly from each storage bowl from both locations of storage monthly. Also physical changes in each bowl were noted. The collected seeds were then used in determining the fungi occurrence in each storage bowl.

*Potato Dextrose Agar (PDA) was used in this work as the culturing medium.

Isolation and identification of fungi

Preliminary survey of storage fungi invading seeds was carried out in three different markets (Bariga, Oyingbo and Yaba) in Lagos state for three consecutive months in order to get the frequency of these fungi. Four of each diseased seeds collected from market survey were placed on plates of PDA after been surface sterilized with 40% of sodium hypochlorite. All the plates were incubated at 26°C for 3 to 5 days. Developing colonies of fungi were sub-cultured to obtain pure culture. The fungi cultures were examined both macroscopically and microscopically for colony, mycelia and spore characteristics. The characteristics were compared with those in a standard mycology text (Vashishta and Sinha, 2005). The fungi were kept on PDA slants in the refrigerator prior use.

Extraction from the wood-ashes

Fifty grammes of each of the wood-ashes were soaked in 150 ml of sterile distilled water and 70% ethanol for 24 h; the solutions were then filtered into beaker with the aid of a Whatman No. 1 filter paper. The extracts were concentrated by evaporating it in rotator evaporator at a moderate high speed. The extracts were stored in the refrigerator until use.

Control preparation

Three types of control were employed, that is, Griseofulvin, Benlate and water. 125 mg of griseofulvin was dissolved in 10 ml of sterile distilled water in a beaker; 5 g of benlate was dissolved in 10 ml of sterile distilled water in another beaker and 10 ml of sterile distilled water in a separate beaker.

Antifungal activity test

Four disc of sterilized perforated Whatman filter paper disc were soaked in each extracts and the prepared controls for six hours. The fungi obtained from the monthly market survey and those from the monthly sampling from the storage seeds at both locations were sub-cultured to get the “working cultures” unto which sterile distilled water was added to produce the microbial suspension. Two drops of each of the fungus suspension was spread on PDA plates and four perforated disc soaked in the extracts and the controls were carefully dropped on these PDA plates with the aid of sterilized forceps.

The PDA plates were labeled and incubated at 29°C. Measurement and recording of zone of inhibition were taken at every 24 h for 5 days (Parker, 1979).

Phytochemical analysis

Phytochemical analysis studies were carried on each of the wood-ash using the method described by Harborne (1998). Both alcohol and water extracts of the nine wood-ashes were tested for the presence of alkaloids, tannins, saponins, flavonoids, phlobatanins, cardiac-glycosides and anthraquinone.

RESULTS

At the end of the first month of storage, no change occurred. In the second, some changes were noted, examples of such changes include weevils (*Callosobruchus maculatus*) infestation and microbial growth. And at the end of the storage period, more changes had occurred (Tables 1 and 2).

The diversity of fungi isolated from the stored seeds

Table 1. Physical observation and occurrence of fungi on the three seed stored with various ashes, benlate, and the control at two different locations of storage (garden).

Seed type	Storage means	Physical observation				Fungi species isolated from the seed															
						Aspergillus flavus				*Aspergillus niger*				*Aspergillus fumigatus*				*Rhizopus*			
		1st Month	2nd Month	3rd Month	4th Month	1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th
Maize	*Khaya grandifoliola*	Fewer weevils	Few weevils	Many weevils / color changed	Many weevils / color changed	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
	Nauclea diderrichii	No weevil	No weevil	Fewer weevils	Fewer weevils	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	Piptadeniastrum africanum	No weevil	No weevil	Fewer weevils	Fewer weevils	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mangifera Indica	No weevil	Fewer weevils	Fewer weevils	Fewer weevils	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+
	Mansonia altissima	Fewer weevils	Few weevils	Many weevils	Many weevils	-	-	+	+	-	+	-	-	-	-	-	-	-	-	+	+
	Triplochiton scleroxylon	Fewer weevils	Few weevils	Many weevils	Many weevils	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+
	Ceiba pentandra	Fewer weevils	Few weevils	Many weevils	Many weevils	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	
	Terminalia superba	Fewer weevils	Few weevils	Many weevils / color changed	Many weevils / color changed	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
	Terminalia ivorensis	Fewer weevils	Few weevils	Many weevils / color changed	Many weevils / color changed	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
	Benlate	Fewer weevils	Few weevils	Many weevils	Many weevils	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Control	Few weevils	Many weevils	Much weevils	Much weevils	-	-	+	+	-	+	-	-	-	-	+	+	-	-	+	+
Beans	*Khaya grandifoliola*	No weevil	No weevil	No weevil	No weevil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Nauclea diderrichii	No weevil	No weevil	No weevil	No weevil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 1. Contd.

	Piptadeniastrum africanum	No weevil	No weevil	No weevil	No weevil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mangifera Indica	No weevil	No weevil	No weevil	No weevil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mansonia altissima	No weevil	No weevil	No weevil	No weevil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Triplochiton scleroxylon	No weevil	No weevil	No weevil	No weevil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Ceiba pentandra	No weevil	No weevil	No weevil	No weevil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Terminalia superba	No weevil	No weevil	No weevil	No weevil	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
	Terminalia ivorensis	No weevil	No weevil	No weevil	No weevil	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
	Benlate	No weevil	Few weevils	Few weevils	Few weevils	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Control	few weevils	Few weevils	Many weevils	Many weevils	-	+	+	+	-	-	+	+	-	-	-	-	-	+	-	-
Melon	*Khaya grandifoliola*	No weevil	No weevil	No weevil	No weevil	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-
	Nauclea diderrichii	No weevil	No weevil	No weevil	No weevil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Piptadeniastrum africanum	No weevil	No weevil	No weevil	No weevil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mangifera Indica	No weevil	No weevil	No weevil	No weevil	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
	Mansonia altissima	No weevil	No weevil	No weevil	No weevil	-	+	-	+	-	-	+	+	-	-	+	+	-	-	-	-
	Triplochiton scleroxylon	No weevil	No weevil	No weevil	No weevil	-	-	+	+	-	+	-	-	-	-	+	+	-	-	-	-

varied each month with *Aspergillus niger* as the most frequent and *Aspergillus flavus* as the least (Figure 1).

The wood-ashes of *Nauclea diderrichii* and *Piptadeniastrum africanum* proved to be effective in preserving the seeds, eliminating the fungi and preventing the manifestation of weevils (*C. maculatus*). Benlate, an orthodox fungicide proved to be effective against the fungi attack but does not prevent the weevils (*C. maculatus*) invasion.

Monthly market survey carried out at the three different markets in Lagos for three consecutive months showed five pathogenic fungi to be involved with seed storage. These are *Macrophomina* species*, Sclerotium rolfsii, Aspergillus niger, Aspergillus fumigatus,* and *Rhizopus racemosa*. *Rhizopus racemosa* was the most frequent; *S. rolfsii* was the least frequent

Table 2. Physical observation and occurrence of fungi on the three seed stored with various ashes, benlate and the control at two different location od storage (laboratory).

		Physical observation				Fungi species isolated from the seed															
						Aspergillus flavus				*Aspergillus niger*				*Aspergillus fumigatus*				*Rhizopus*			
Seed type	**Storage means**	**1st Month**	**2nd Month**	**3rd Month**	**4th Month**	**1st**	**2nd**	**3rd**	**4th**	**1st**	**2nd**	**3rd**	**4th**	**1st**	**2nd**	**3rd**	**4th**	**1st**	**2nd**	**3rd**	**4th**
Maize	*Khaya grandifoliola*	Fewer weevils	Few weevils	Many weevils	Many weevils / color changed	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
	Nauclea diderrichii	No weevil	No weevil	Fewer weevils	Fewer weevils	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	Piptadeniastrum africanum	No weevil	No weevil	Fewer weevils	Fewer weevils	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mangifera Indica	No weevil	Fewer weevils	Fewer weevils	Fewer weevils	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+
	Mansonia altissima	Fewer weevils	Few weevils	Many weevils	Many weevils	-	-	+	+	-	+	-	-	-	-	-	-	-	-	+	+
	Triplochiton scleroxylon	Fewer weevils	Few weevils	Many weevils	Many weevils	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+
	Ceiba pentandra	Fewer weevils	Few weevils	Many weevils	Many weevils	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+
	Terminalia superba	Fewer weevils	Few weevils	Many weevils	Many weevils / color changed	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
	Terminalia ivorensis	Fewer weevils	Few weevils	Many weevils	Many weevils / color changed	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
	Benlate	Fewer weevils	Few weevils	Many weevils	Many weevils	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Control	Few weevils	Many weevils	Much weevils	Much weevils	-	-	+	+	-	+	-	-	-	-	+	+	-	-	+	+

Table 2. Contd.

Beans	*Khaya grandifoliola*	No weevil	No weevil	No weevil	No weevil	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Nauclea diderrichii	No weevil	No weevil	No weevil	No weevil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Piptadeniastrum africanum	No weevil	No weevil	No weevil	No weevil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mangifera Indica	No weevil	No weevil	No weevil	No weevil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mansonia altissima	No weevil	No weevil	No weevil	No weevil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Triplochiton scleroxylon	No weevil	No weevil	No weevil	No weevil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Ceiba pentandra	No weevil	No weevil	No weevil	No weevil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Terminalia superba	No weevil	No weevil	No weevil	No weevil	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
	Terminalia ivorensis	No weevil	No weevil	No weevil	No weevil	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
	Benlate	No weevil	Few weevils	Few weevils	Few weevils	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Control	No weevil	Few weevils	Many weevils	Many weevils	-	+	+	+	-	-	+	+	-	-	-	-	-	+	-	-
Melon	*Khaya grandifoliola*	No weevil	No weevil	No weevil	No weevil	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-
	Nauclea diderrichii	No weevil	No weevil	No weevil	No weevil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Piptadeniastrum africanum	No weevil	No weevil	No weevil	No weevil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mangifera Indica	No weevil	No weevil	No weevil	No weevil	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
	Mansonia altissima	No weevil	No weevil	No weevil	No weevil	-	+	-	+	-	-	+	+	-	-	+	+	-	-	-	-
	Triplochiton scleroxylon	No weevil	No weevil	No weevil	No weevil	-	-	+	+	-	+	-	-	-	-	+	+	-	-	-	-
	Ceiba pentandra	No weevil	No weevil	No weevil	No weevil	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
	Terminalia superba	No weevil	No weevil	No weevil	No weevil	-	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-
	Terminalia ivorensis	No weevil	No weevil	No weevil	No weevil	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-
	Benlate	No weevil	No weevil	No weevil	No weevil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Control	No weevil	No weevil	No weevil	No weevil	-	+	+	+	-	+	+	+	-	+	+	+	-	-	-	-

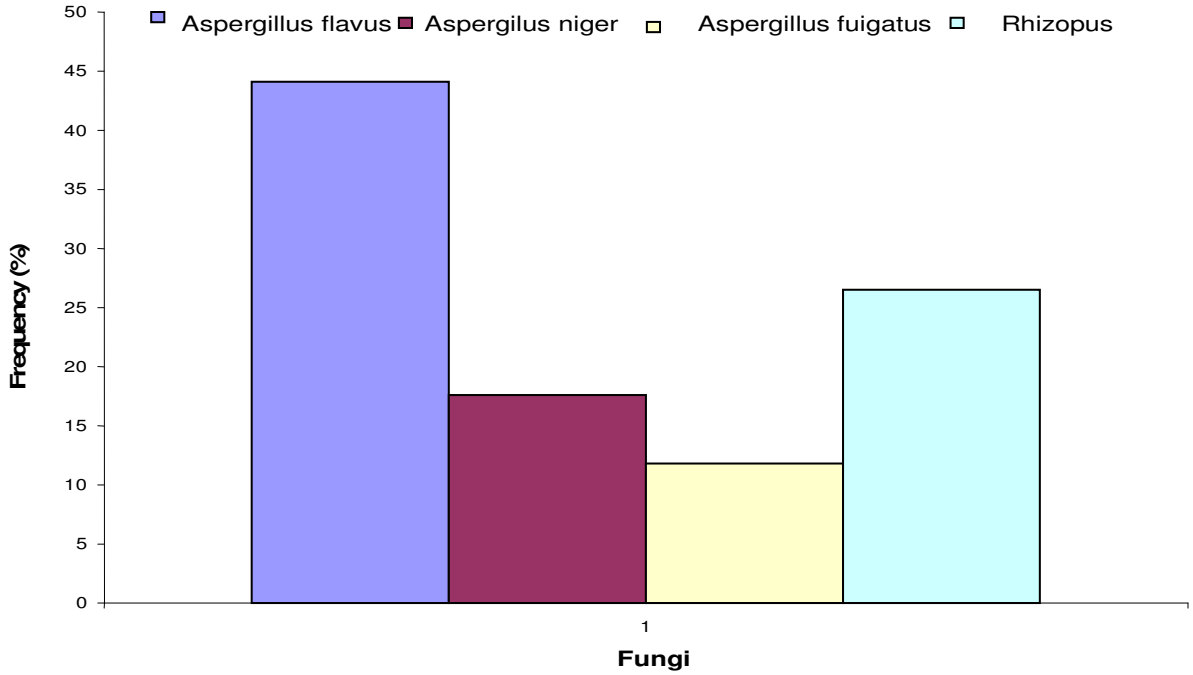

Figure 1. Frequency of pathogenic fungi over six months period of seed preservation.

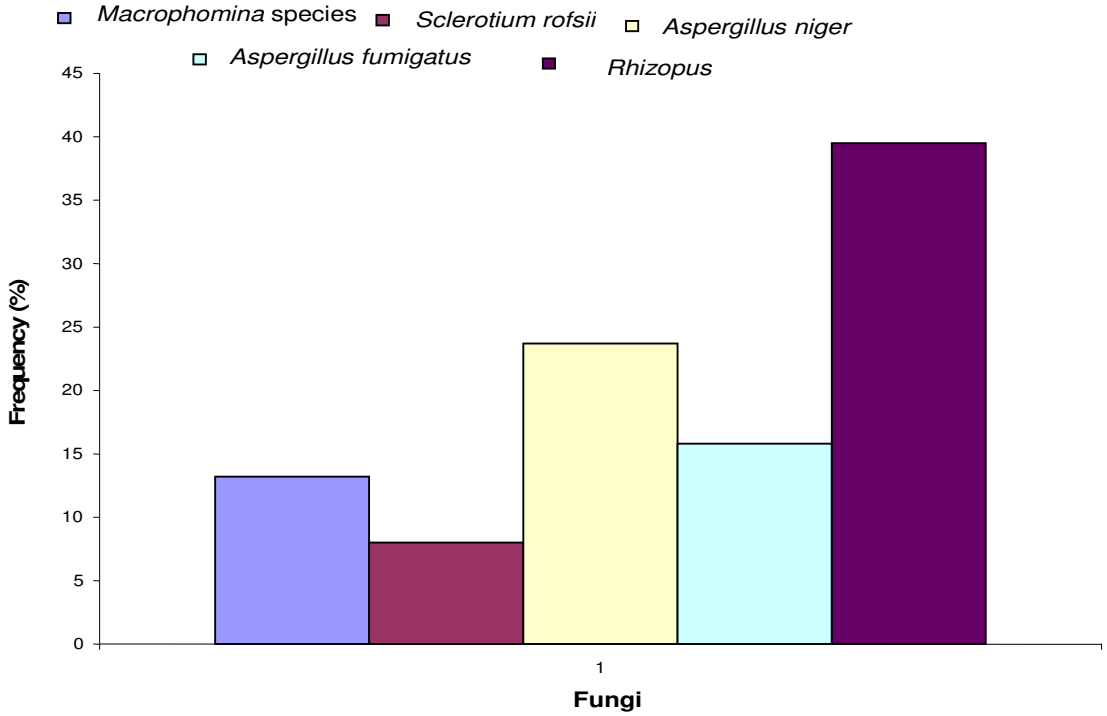

Figure 2. Frequency of pathogenic fungi obtained from monthly markets survey for three consecutive months.

(Figure 2).

There was reduction in the moisture contents of the three seeds stored with wood-ashes at both locations at the end of the storage period except for the control samples which tend to increased a little.

Activity test using Benlate extract had the highest zone of inhibition against all the isolated pathogenic fungi. Both ethanol and water extracts of *N. diderrichii* and

Table 3. Antifungal activity of the nine ashes.

Sample	Zone of inhibition (Mean + S. E. (mm)) fungi				
Ethanol Extract of:	***Macrophomina species***	***Sclerotium rofsii***	***Aspergillus niger***	***Aspergillus fumigatus***	***Rhizopus***
Khaya grandifoliola	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Nauclea diderrichii	14.75 ± 0.54	14.50 ± 0.25	14.00 ± 0.25	14.00 ± 0.25	12.00 ± 0.00
Piptadeniastrum africanum	15.25 ± 0.22	12.00 ± 0.00	11.00 ± 0.00	13.00 ± 0.00	12.00 ± 0.00
Mangifera Indica	0.00 ± 0.00	13.50 ± 0.25	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mansonia altissima	0.00 ± 0.00	0.00 ±0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Triplochiton scleroxylon	0.00 ±0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Ceiba pentandra	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Terminalia superba	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Terminalia ivorensis	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Water Extract of:					
Khaya grandifoliola	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Nauclea diderrichii	15.50 ± 0.25	13.50 ± 0.75	13.50 ± 0.75	14.00 ± 0.00	13.00 ± 0.00
Piptadeniastrum africanum	15.00 ± 0.00	12.00 ± 0.00	11.50± 0.25	11.00 ± 0.00	10.00 ± 0.00
Mangifera Indica	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mansonia altissima	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Triplochiton scleroxylon	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Ceiba pentandra	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Terminalia superba	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Terminalia ivorensis	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Control (Distilled water)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Benlate	15.50 ± 0.25	16.5 ± 0.25	26.00 ± 0.00	21.00 ± 0.00	13.50 ± 0.25
Fulcin	0.00 ± 0.00	0.00 ± 0.00	12.75 ± 0.22	7.75 ± 0.22	09.00 ± 0.00

P. africanum also showed inhibition against all the pathogenic fungi. Only ethanol extract of *Mangifera indica* inhibited the growth of *S. rolfsii*. Fulcin an orthodox animal fungicide showed inhibition against *A. niger, A. fumigatus* and *R. racemosa* only. Other extracts showed zero inhibition (Table 3).

Preliminary photochemical screening for active compounds (alkaloids, flavonoids, tannins, cardiacglycosides, saponins and phlobatanins) in the extracts of the nine ashes showed that both water and ethanol extracts of *N. diderrichii* contained flavonoids and tannins, however, flavonoids was found to be in higher concentration in the ethanol extract (Table 4). No alkaloid, cardiacglycosides, saponins or phlobatanins was found in both the water and ethanol extracts of *N. diderrichii.* Water and ethanol extracts of *P. africanum* showed the presence of alkaloids, flavonoids and tannins with flavonoids concentrated in its ethanol extract. Cardiacglycosides, saponins and phlobatanins were absent in the extraction of *P. africanum.* Both extracts water and ethanol of *M. indica* were found to contain alkaloids and flavonoids, with alkaloids concentrated in the ethanol extract of it. Alkaloids were also present in both extracts (water and ethanol) of *Triplochiton scleroxylon*. The extracts (water and ethanol) of *Terminalia superba* and *Terminalia ivorensis* showed the presence of tannins (Table 4).

DISCUSSION AND CONCLUSION

This work showed that the wood-ash of *N. diderrichii* and *P. africanum* were capable of preventing a number of pathogenic fungi due to the presence of some active compounds in them. Six pathogenic fungi were isolated from storage seeds over the period of six months, which included *S. rolfsii, A. niger, A. flavus, A. fumigatus, Rhizopus* and *Macrophomina* species The various experiments conducted in this work showed that wood-ash have fungicidal effects which probably explain why the ashes of *N. diderrichii* and *P. africanum* were able to prevent the invasion of pests and microbial growth effectively. Nigerian natives use wood ash to preserve seeds against pests and microbes. They claim that the wood-ash was able to preserve the seeds by absorbing

Table 4. Result of phytochemical test for active compounds in the nine ashes.

Extracts	Alkaloids	Flavonoid	Tannis	Cardiac glycosides	Saponins	Phlobatanins
Khaya grandifoliola (Ethanol extract)	-	-	-	-	-	-
Khaya grandifoliola (Water extract)	-	-	-	-	-	-
Nauclea diderrichii (Ethanol extract)	-	++	+	-	-	-
Nauclea diderrichii (Water extract)	-	+	+	-	-	-
Piptadeniastrum africanum (Ethanol extract)	+	++	+	-	-	-
Piptadeniastrum africanum (Water extract)	+	+	+	-	-	-
Mangifera Indica (Ethanol extract)	++	++	-	-	-	-
Mangifera Indica (Water extract)	+	+	-	-	-	-
Mansonia altissima (Ethanol extract	-	-	-	-	-	-
Mansonia altissima (Water extract)	-	-	-	-	-	-
Triplochiton scleroxylon (Ethanol extract)	+	-	-	-	-	-
Triplochiton scleroxylon (Water extract)	+	-	-	-	-	-
Ceiba pentandra (Ethanol extract)	-	-	-	-	-	-
Ceiba pentandra (Water extract)	-	-	-	-	-	-
Terminalia superba (Ethanol extract)	-	-	+	-	-	-
Terminalia superba (Water extract)	-	-	+	-	-	-
Terminalia ivorensis (Ethanol extract)	-	-	+	-	-	-
Terminalia ivorensis (Water Extract)	-	-	+	-	-	-
Control (distilled water)	-	-	-	-	-	-

+ = Present. + + = concentrated when present. = Absent.

the moisture in the seeds. Environmental conditions like temperature, humidity and the moisture in the seeds might have a role to play due to the slight differences in the occurrence of the weevils in the seeds located at the garden and that of the laboratory (Hagstrum and Flinn, 1993). Seeds of *P. vulgaris* had the lowest moisture

content and tend to be the best preserved seeds probably due to the low moisture content of the seeds Adekunle and Uma (1997).

Based on sensitivity test, it was obvious that both extracts (water and ethanol extracts) of *N. diderrichii, P. africanum* and *M. indica* contain substance with fungicidal effect Adekunle (2001).

The preliminary screening of some extracts showed some ashes to contain more active compounds than others. The potency of the extracts may be a function of the number and concentration of substances they contain.

N. diderrichii contained flavonoids and tannins with ethanol extract showing concentration of flavonoids. *P. africanum* contained alkaloids, flavonoids and tannins. The ethanol extract showed concentration of flavonoids. *M. indica* contained alkaloids and flavonoids while the ethanol extract showed alkaloids. The extracts of other ashes contained only one active compound except for *Mansonia altissima* and *Ceiba pentrandra* with all which substances absent in their extracts. The inhibitory effects of *N. diderrichi* and *P. africanum* against all the pathogenic fungi may be due to the presence of flavonoids and tannins in their extracts while only the ethanol extract of *M. indica* showed inhibitory effect against *S. rolfsii* and this was found to contain flavonoids and alkaloids. The fact that ethanol extracts were found to be more active or more potent than their aqueous counterpart may be as a result of the fact ethanol diffuse faster than water due to its volatility.

Benlate an orthodox plant fungicide showed the highest inhibitory effect against all the pathogenic fungi. The difference between the extracts of the ashes and the benlate might be due to the fact that the active ingredients in the ashes were not purified while benlate is already a purified bioactive substance. Benlate tends to be a better preservative agent against microbial growth but not effective enough to prevent the insects invasion. It was also noticed that the benlate tightly bound to the seeds unlike the ashes that could be easily sieved away from the seeds. Fulcin (a synthetic human fungicide drug) showed inhibitory effect against *A. niger, A. fumigatus* and *R. racemosas* only.

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