Studies of Seed-Borne Pathogens of African Breadfruits (*Treculia africana* Decne)

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ABSTRACT

The seeds of African breadfruit (*Treculia africana*) were obtained from Aluu Local Market in Ikwerre Local Government Area, Rivers State, Nigeria and tested for seed-borne pathogens in the Department of Forestry and Environment Laboratory (Pathology Unit). The following fungi were isolated from the diseased seeds of *T. africana; Aspergillus flavus* (50.6%), *Rhizopus stolonifer* 43.5%) *Aspergillus niger* (32.5%), *Fusarium moniliforme* (30.6%). These fungi were found to have caused significant increase in the seed rots of *I. africana.* Results on the effect of Ascorbic acid at 200mg dosage inhibited the test fungi significantly, while the effect of leaf extract (*Ocimum gratissimum*) on the conidiospores germination, mycelia extension of the isolated fungi showed increased antifungal activity against the fungal pathogens. This research holds promise for the use of botanicals as an alternative means of protecting seeds from pathogen attack especially endangered species like African breadfruit. **Keywords**: African Breadfruit, Seed-borne pathogens, *Ocimum gratissimum* (Scent leaf), Ascorbic acid (Vt.C,), Conidiospore

INTRODUCTION

African Breadfruit *(Treculia africana Decne var. africana)*

This common forest tree is given many names by various localities where its is found, the Yoruba's call it Afon, Binis-Ize, Ibo's – Ukwa, the Hausa – Barafuta and Efik, Ibibios Annangs call it Ediang (Irvine, 1961). The African bread fruit tree is a native of many parts of West and tropical Africa. It can be found in Sudan, Benin, Cameroon, Central African Republic, Congo, Cote d'Ivoire, Democratic Republic of Congo, Equatorial Guinea Gabbon, Nigeria, Mozambique, Angola, Principe and Sao Tome Islands. The African breadfruit belongs to the family *Moraceae* and other three members of the genera *Treculia such as T. africana var. mollis, T. zenkeri* and *T. obodiodea* (Hutchinson and Dalziel, 1954), while Okafor, (1981) classified *Treculia* based on fruit head, *T. africana var. africana* (large fruit heads) and *T.africana var.* mollis (medium heads), *T. africana var. inversa* (small fruit heads). *T. africana* is said to be one of the several food plant species in Nigeria, a large number of these species are collected from the wild while a few occur in home gardens (Akachukwu, 1997). Despite the vital contribution of this forest food plant species in Nigeria, it has been threatened as a result of habitat destruction, loss of seed viability and deforestation of forest reserve (Akachukwu, 1997; World Agroforestry 2006).Among the various factors responsible for low yield of Agricultural crops, diseases play a major role. Incidentally, the most devastating crop diseases are seed-borne, roughly 550million tones of the total crop production in the world per annum is lost due to plant diseases (James,

1980). In Bangladeshi, the total crop lost has been estimated at 10% due to seed-borne diseases (Fakir, 1980).

However, about 90% of all food crops grown on earth are propagated by seeds and these propagules have been shown to be susceptible to different types of seed-borne disease (Neergaard, 1979; Onuegbu, 1995; Richardson 1999). Seed-borne pathogens are not only the sources of disease outbreak but also serve as media for survival of such pathogens for a longer period.

The infected seeds act as means through which pathogens are spread to disease-free zones (Neergaard, 1979; Agarwal, 1981). Seed-bore fungi are serious parasite of seed primordial, maturing seeds and some of the seeds are infected in the field during harvest and in storage (Mc-Gee, 1981). However, micro-organisms especially fungi are known to attack the fruits and kernels (Dalziel, 1937). It is responsible for loss of quality of fruits and seeds, infect may micro-organisms especially fungi and bacteria have been implicated in disease complex of agricultural crops in the store (Nigerian stored product Research Institute, (NSPRI, 1982).

Among the seed-borne pathogens, fungi infection causes seed abortion, shunken seeds, seed rot, sclerotisation, seed necrosis, seed discoloration and reduced germination and vigor. Seeds are known to be attacked by various types of fungi many of which are plant pathogens (Richardson, 1999; Singh *at al, 1*983). These pathogens often colonise seed primordial and maturing seeds and reduce seed yield qualitatively and quantitatively. Singh *et al.* (1983) reported that smut fungi in cereals cause seed abortion, the disease reduces the length of ears as well as number of spikets in the infected heads. Saad *et al.* (1988) found that may of the seed-borne fungi in cowpea (*Vigna unguiclulata*) reduced seed germination and produced symptoms on infected seedlings.

Some of the pathogenic seed-borne fungi colonise the seed coat causing necrotic spots such superficial necrotic lesion are caused by *Ascochyta pisi* in pear *(Pisum sativum).* Raut, *et al.*, (1983) reported that wheat seeds collected from *Alternaria triticina* infected plants were small and shriveled and based on 1000 grain weight showed 46-75% reduction in weight over the infected plants. Similarly Carson (1885, also repeated that *Alternaria* constituted greater problem in seed production in the field than in commercial fields. Fungi such as *Aspergillus spp., Botryodiplodia theobromae, Penicillum spp,. Fusarium spp.* cause seed rot in many crops. (Rao, *et al*., 1985; Vasanthankumar, 1986) while Vishnuavat, *et al*., (1985) observed the maximum loss in the viability of Mustard seeds (Brasica niger) by *Alternaria brassicae* when the seeds were shrivelled and discoloured. Chiarappa and Gambiogi (1986) reported that Zambia lost 60% of its maize crop due to infection by two seed-borne pathogens *Fusarium and Diplodia* while Gata (1989) showed that losses due to *Aphelenchoides besseyi* caused organisms of the "white tip" disease in rice ranged between 12-14% in Tanzania and in Kenya. Onsando (1987) studied crop losses due to black rot in cabbage caused by the bacteriaum, *Xanthosomonas campestris pv campestris.* Also, Idris and Ahmed (1981) showed a loss of 41% in groundnut seeds crop due to peanut mottle virus in Sudan. Therefore, these should be urgent need to collect and preserve the various *Treculia* spp pound within our environment from the attack of seed-borne pathogens.

This research therefore would provide baseline data for seed-borne pathogens of *Treculia africana* while recommending ways of reducing the seed-borne incidence and seventy or the seeds of *T. africana* meant for human consumption. This research therefore aims at;

(i) Isolation and identification of seed-borne pathogens on the seeds of *Treculia africana*.

(ii) Investigating the efficacy of *Ocimum gratissimum* (Scent leaf) extracts and in ascorbic acids reducing seed-borne incidence in the seeds of *T. africana*

MATERIALS AND METHODS

Experimental Site and Materials

The experiment was carried out at the Forestry and Environment Laboratory (Forest Pathology Unit) Faculty of Agriculture, Rivers State University of Science and Technology, Nkpolu-Oroworukwo, Port Harcourt. The biochemical analysis of the diseased and non-diseased seeds of *T. africana* was carried out at the Department of Food Science Laboratory, Rivers State University of Science and Technology, while the plant leaf extract analysis was done at Plant Physiology Laboratory, Department of Plant Science and Biotechnology, University of Port Harcourt, Choba. The diseased and relatively healthy seed of *Treiculia africana* (Ukwa) was obtained from Aluu market in Ikwerre Local Government Area of Rivers State. The seeds were stored in a sterilized container and kept in a refrigerator until when needed.

Preparation of Culture Media

The culture medium used for this study is potato dextrose agar (PDA) which was prepared by weighing 200g of Irish Potato tubers. The potato was boiled in 1 litre of distilled water for 20minutes. The broth was filtered with muslin cloth into an Erlenmeyer flask and made up to 500ml with distilled water. 20g dextrose and 15g agar powder was added to the filtrate and the flask finally plugged with sterile cotton wool and then sterilized in an autoclave at 121^0C and 1.03kg cm^{-3} for 20minutes and later dispensed into sterile Petri-dishes, the content was allowed to solidify before inocultating the fungi pathogens isolated.

Isolation and Identification of Fungi from Diseased Seeds of *T. africana*

The Isolation of fungi associated with the seeds of *T. africana* was carried out using the Standard blotter (Scottie-tissue) method as described by the International Seed Testing Association (ISTA, 1999). Three layers of filter papers (Whatman – 9cm) were soaked in sterile distilled water and placed in sterilized Petridishes.

The diseased seeds of the *T. africana* were surface-sterilized by soaking it in 5% sodium hypochlorite solution for 10minutes and rinsed with sterile distilled water for three consecultive times. Thereafter, the sterilized seeds were plated on the sterilized Petri-dishes and incubated for 7 days on laboratory bench at room temperature (25 ± 2^0C) on the 7^{th} day, the percentage frequency of occurrence of fungi were calculated as;

$$\text{Percentage (\%) frequency of occurrence} = \frac{No \text{ of seeds containg fungi}}{Total \text{ No. of seeds assessed}} x \frac{100}{1}$$

However, fungi were identified on the basis of colour using the light microscope with reference to (Richardson 1990; Mathur Kongsdal, 2003). Pure cultures of the individual fungus were sub-cultured on potatoe dextrose agar (PDA) medium and allowed to sporulate in dark room.

Preparation of Plant Leaf Extracts

The leaves of *Ocimum gratisimum* (scent leaf) was washed in distilled water and left for water to drain and them dried in an oven at 40^0C and then ground with grinder into a fine powder which was made to stand or soaked in distilled water for some hours and the suspension was filtered with filter paper and filtrate collected in a round bottom – flask. The amount or concentrations of plant extract prepared were 20, 40, 60, 80, and 100% (Abudulraman *et al.,* 2004).

Pathogenicity Studies

The ability of the isolated fungi to induce diseases in a relatively healthy seeds of *T. africana* leaves while the uninoculated seeds served as control. The sample used was first washed and surface sterilized with 5% sodium hypochlorite before introducing the identified fungi growing on the PDA medium and then kept under normal room temperature for 14 days and the fungi which manifested was incubated and identified microscopically and later compared

with the initial fungi observed from the initial isolation from the diseased seeds of *T. africana.*

Experimental Design Data Analysis

The design for this study was Completely Randomized Design (CRD) with three replicates. The statistical differences between treatments means were tested using Least Significant Difference (LSD) at 5% Probability.

Results

The results on the percentage occurrence of seed-borne fungal of *Treculia Africana* obtained from Aluu-market are shown in Table 1. The results showed that *Aspergillus flavus* had the highest frequency occurrence of fungal pathogen (50.6%) followed by *Rhizopus stolonifer* (43.5%), *Aspergillus niger (32.5%)* while the least was *Fusarium moniliforme* (30.8%). However, these fungal pathogens have been implicated to be responsible for the rots of *T. africana* fruits.

The results on the effect of Ascorbic acid on the growth of the fungal pathogen, isolated from the diseased fruits of African breadfruits are shown in (Table 2). The results indicated that irrespective of the fungus involved, there was a significant (P > 00.5) reduction on the fungal growth as the dosage of ascorbic acid applied increased from (100-200mg).

The effect of leaf extracts of *O. gratissimum* on the growth of fungal pathogen of African breadfruits were shown in Table 3. The *in vitro* results revealed that *O. grantissimum* has antifungal properties which caused significant (P > 0.05) reduction on the fungal mycelia growth. This progressive and drastic reduction increased as the extract concentration increased from (20 – 100%).

DISCUSSION

Results on the post-harvest fungal diseases isolated form African breadfruit (*T. africanna*) have shown that the most common fungi associated with the seeds are *Aspergillus flavus, Aspergillus niger, Rhizopus stolonifer* and *Fusrium moniliforme*. However, some of these fungi have earlier be implicated as storage organisms of *Seaamum indicum* seeds (Singh, *et al.,* 1972). It is also reported that some of these isolated fungi have caused serious diseases of fruits (Nwufo and Emebiri, 1989; Arinze, 2005).

The effect of ascorbic acid on the *in-vitro* growth of fungal isolated from diseased African breadfruits indicated that inspite of the fungus involved, there was a progressive reduction of fungus growth as the dosage of ascorbic acid increased. Aderiye (1984) had earlier reported that ascorbic acid reduced browning in stored okro fruits which implies that a reduced oxidation by polyphenol oxidase. The result further suggests that ascorbic acid might have reduced the oxidation of fats by the test fungi.

The effect of crude extract of *Ocimum gratissimum* has been proven to contain essential oils which possess pharmacological agents and some important phytochemicals such as eugenol, terpenoids, thymol. Linalool, 1,8 cineol, citral, ethylcinnamate. β-selinene, 1,8 cineole, trans-caryophyllene alkaloids, tannins and flavonoids (Sulistiarini, 1999; Jedlickoya, *et al.,* 1992).

Pharmacological studi`es on the plant have shown by earlier researchers that it has chemotherapeutic and insecticideal properties (Nwosu and Okafor, 1995; Pessoa, *et al*., 2002; Afolabi, *et al.,* 2007).

However, this present research revealed that plant leaf extract of *O. gratissimum* significantly (P > 0.05) reduced the growth of test fungi which confirms the chemotherapeutic and insecticidal properties of scent leaf. (*O. gratissimum)* as antifungal agent. Udo, *et al.,* (2000) in his findings, reported that fungal isolated from sweet potato and yam was inhibited from growth and sporulation on the application of garlic extract.

Conclusion and Recommendation

The study of seed-borne pathogens of African breadfruit (*Treculia africana*) has revealed that *Aspergillus flavus, Aspergillus niger, Rhizopus stolonifer* and *Fusarum moniliforme* have

been implicated with the fruit/seed rot of African Breadfruit. It was further noticed that these fungi altered the composition of the seeds. Results of *Ocimum gratisimum* on fungal growth showed that the extract have some antifungal activity which significantly reduced the fungal growth. In the same manner Ascorbic acid also reduced the growth of fungal in this study.

In Nigeria, not much study has been done on the pathology of African bread fruits. The few work done are restricted to the ecology, uses, nutritional composition of the seeds. However, this dearth of information in the pathology of the seeds calls for this research which is focused on the pathology and disease control parameters capable of reducing deterioration of the seeds. However, the isolation of hazardous fungi such as *Aspergillus flavus* in the seeds of African breadfruit with proven aflatoxigenic and carcinogenic potentials calls for urgent public health concern especially for local consumer.

Table 1: Fungi isolated from diseased seed of Breadfruit (*Treculia africanna)* using blotter method

S/N	Fungal Isolates	(%) Percentage Occurrence
1	*Aspergllus flavus*	50.6
2	*Aspergillus niger*	32.5
3	*Rhizopus stolonifer*	43.5
4	*Fusarium moniliforme*	30.6

Table 2: Effects of Ascorbic Acids in the Growth of Seed-Borne Fungi of African Breadfruit (*T. africanna)*

	Does of Asscorbic Acid (mg)		
Fungal	**0**	**100**	**200**
Aspergillus flavus	0.96	0.54	0.20
A. niger	0.78	0.45	0.23
R.stolonifer	1.20	0.94	0.42
F. moniliforme	0.70	0.35	0.15
LDS (0.05)	0.91	0.12	0.08

Table 3: Effect of leaf extracts (*Ocinum gratissimum*) on the growth of fungal pathogens of African Breadfruit

	Extract concentration (%)			
Fungal Isolates	**0**	**20**	**60**	**100**
Aspergillus flavus	6.70	4.20	2.25	0.00
Aspergillus. niger	7.63	3.50	1.40	0.00
Rhizopus stolonifer	9.00	4.50	2.00	0.25
Fusarium moniliforme	6.80	3.41	1.45	0.20
LDS (0.05)	0.56	0.31	0.21	0.02

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