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Control of Fungal Pathogens of Postharvest Rot of Groundnut (*Arachys hypogea L.*) using Aqueous and Ethanol Root Extracts of Mahogany (*Khaya senegalensis*) in Hong Local Government Area of Adamawa State Nigeria

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Abstract: Fungi are associated with heavy losses of seeds, fruits, grains, vegetables, and other plant products in transit and storage rendering them unfit for human consumption. The effect of synthetic fungicides on humans is hazardous, hence the need to find a safer means of control. A research was conducted in Hong Local Government Area of Adamawa State of Nigeria (the most prominent groundnut farming community in the state). The following molds were associated with postharvest groundnut rot in the seven districts of Hong local government area in July 2016: *Aspergillus niger, Aspergilus flavus, Penicillium chrysogenum, Rhizopus stolonifer, Paecilomyces lilacinus, Pseudallescheria boydii, Cylindrocarpon lichenicola, and Scedosaporium prolificans*. Therefore, the research sought to assess the management of rot using plant extracts of mahogany. Control trials were carried out using the extracts of root of mahogany. The growth of pathogens both *in-vitro* and *in-vivo* was significantly reduced by the plant extracts. Aqueous root and ethanol extracts reduced mycelial growth from 72.67 mm to 21.00 mm and 20.50 mm respectively (*in-vitro*) and from 55.00 mm to 23.45 mm by aqueous extracts and 15.92 mm by ethanol extracts for *in-vivo* control, thus, mahogany aqueous and ethanol root extracts have been found effective against these pathogens, hence, root is recommended for further research in other to formulate a control strategy for these pathogens.

Keywords: Control, Groundnut, Fungi, Mahogany

Introduction

The roles of agriculture remain significant in the Nigerian economy despite the strategic importance of the oil sector, agriculture still provides primary means of employment for Nigeria and accounting for more than one-third of total gross domestic product (GDP) and labor force (Ayoade, 2012).

The major food crops of Adamawa State according to Adebayo (1997) are mainly cereals, legumes, and root crops, while the cash crops are mostly cotton, groundnut and sugar cane. The variable climatic and edaphic factors of the state as well as cultural and socio-economic factors, are reportedly responsible for the distribution of food and cash crops in the State.

In the North-East zone of Adamawa State, groundnut is a key cash crop produced especially in Hong (Adebayo and Tukur, 1997). Rowland (1999) reported that seed yield in Northern Nigeria is about 3000Kg/ha. Adamawa Agricultural Development Programme, ADADP (1996) enumerated groundnut genotypes were commonly grown in Adamawa State to include; "Ordaaji"; (2 nuts/shell), "Kwamakuni"; (3 nuts/shell), "Kwathrumthrum"; (2 nuts/shell larger), "Kwanyambi" or Ex Dakar and Kampala (brown/white striped nuts).

Groundnut (*Arachis hypogaea* L.) is an essential oilseed crop in Nigeria and is widely grown in the tropics and sub-tropics (Nigam *et al.*, 1994). It is one of the most significant crops that can flourish on newly reclaimed sandy soils as a legume of high nutritive value as well as being a source of edible oil (Spears *et al.*, 2002). The major groundnut producing countries from the world are China, India, Nigeria, Argentina, USA, Indonesia, and Sudan. Developing countries account for 96 percent of the global groundnut area and 92 percent of the world production (FAOSTAT, 2011).

Fungi such as *Aspergillus niger*, *Aspergillus flavus*, *Alterneria anthocola*, *Curvularia lunata*, *Curvulari*

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apellesecens, *Fusarium oxysporum*, *Fusarium equiseti*, *Microphomina phaseolina*, *Rhizopus stolonifer*, *Penicillium digitatum* and *Penecillium chrysogenum* cause severe damage to stored commodities resulting in discolouration, rotting, shrinking, seed necrosis, loss in germination capacity and toxification to oilseeds according to Chavan and Kakde (2008). Verma *et al.* (2003) reported that, the action of these fungi resulted to loss of seeds, fruits, grains, vegetables and other plant products during picking, transit and storage rendering them unhealthy for human consumption even by producing mycotoxins and also reduce the total nutritive value. Tropical climate with high temperature and high relative humidity in addition to poor storage methods adversely affect the quality of cereal grains and oilseed, and this can lead to the total deterioration of seed (Bhattacharya and Raha, 2002). Groundnut seed is susceptible to a wide range of pathogens and pests which cause a lot of damage to the crop, thereby reducing yield (Weiss, 2000).

Therefore, many of the seed - borne fungi were generally managed by the use of some synthetic chemicals which were also considered to be both efficient and effective (Ahmed *et al.*, 2012). The continuous use of this fungicides unraveled its non-biodegradability and leaving residual toxicity to cause environmental pollution (Ajobade and Amusa, 2001), hence the need for alternative safer means of control.

Due to awareness of the danger of chemical control in recent years, attention has shifted to the use of non-chemical systems for the treatment of the seed to protect it against plant pathogens (Ademola *et al.*, 2004). Plant extracts have played a significant role in inhibiting of seed-borne pathogens, improving seed quality and the emergence of plant seeds (Abdelgaleil *et al.*, 2004). There is now an emphasis on the use of botanicals such as the flowers, cloves, leaves, bark, root and seed extracts which are considered as cheaper and safer means of mold control (Abdelgaleil *et al.*, 2001). Alternative ways to control seed- borne pathogens, mainly using extracts of medicinal plants are novel, phytochemically and pharmacologically (Sofowora *et al*., 2013), *Khaya senegalensis* as a source of bio-pesticides in tropical and subtropical Africa, is perhaps the most promising because it possesses nearly all characteristics of an ideal bio-pesticides agent currently attracting research interest worldwide.

A preferred solvent in plant extraction should be of low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action and inability to cause the extract to form complex or dissociate (Hughes, 2002). Thus, the commonly used solvents for preliminary research of anti-microbial activity in plants are said to be methanol, ethanol, and water (Lourens *et al*., 2004; Parekh *et al*., 2006).

This study sought to determine the inhibitory effect of aqueous and ethanol root extracts of *Khaya senegalensis* on post-harvest fungal pathogens of groundnut rot obtained from the seven districts of Hong Local Government Area of Adamawa state.

Materials and Method

The control with root extracts was carrived out in the Medical Laboratory of Microbiology Department, Modibbo Adama University of Technology (MAUTECH) Yola, from 18th July 2016 to 24th October 2016.

Source of Samples

Samples of groundnut seeds of two genotypes commonly found are Valencia (Kampala) and Peruvian (Kwathrumthrum) were collected from one (1) major market in each of the seven (7) districts namely Hildi, Kulinyi, Dugwaba, Uba, Gaya, Pella, and Hong. Fifty (50) of the samples of each genotype were purchased from a seller (two randomly selected sellers/ traders within the chosen market) in each district making a total of 700 collected from the various locations; the samples were carried to the laboratory in a dry clean polythene bag. Groundnut samples were labeled according to location and then photographed (Figure I A, I B and II A, II B).

Table 1: Groundnut Varieties used for the Study

No	Subspecies	Variety	Botanical types	Seed coat colour	Pod sizes
1	*fastigiata*	Kampala	Valencia	Brown -white (var)	3 – 4 cm
2	*hirsuta*	Kwathrumthrum	Peruvian	Brown	3 – 4 cm

Figure I A: Sample of Healthy "Kwathrumthrum" (Local) Variety Groundnut Seeds

Figure I B: Sample of "Kwathrumthrum" (Local) Variety Diseased Groundnut Seeds

Figure II A: Sample of Healthy Kampala Variety Groundnut Seeds

Figure II B: Sample of Diseased Kampala Variety of Groundnut Seeds

Figure III. *Khaya senegalensis* Root

Sterilization of Inoculation Room and Instruments

Sterilization of the laboratory environment was carried out to avoid contamination. The bench and tables used were swapped clean using 95% ethanol, and UV light switched on for 30 minutes. Petri-dishes were sterilized at 160^0 C for 1 hour in the oven, forceps and needles used for inoculation were sterilized by flaming on a Bunsen burner flame and dipping into the methylated spirit to cool.

Preparation of Potato Dextrose Agar (PDA)

Thirty-nine grams (39 g) of Potato Dextrose Agar (PDA) was dissolved in one (1) liter of distilled water; the PDA was then poured into two 500ml conical flask, then plugged with cotton wool and wrapped with aluminium foil before autoclaving at 121^0 C for 15 minutes at 10 lbs. Pressure, and 6 ml (0.1%) of streptomycin was added to the liter of sterilized media and swirled gently to mix appropriately, just before pouring into Petri dishes to prevent bacterial growth and allowed to cool and

solidify according to the method of Suleiman and Michael (2013).

Collection and Preparation of Extracts

The method of Ijato *et al*. (2011) was used to prepare both aqueous and ethanol extracts. Fresh leaves of *Khaya senegalensis* were collected from General Murtala Mohammed College Jimeta - Yola, Adamawa State. The collected leaves were rinsed thoroughly under running tap water (Figure III) and were allowed to air dry for seven (7) days; these were then ground using pestle and mortar. Hundred (100), sixty (60) and twenty (20) grams were dissolved in sterile distilled water and ethanol in separate conical flasks respectively. These were vigorously shaken and left to stand for 24 hours. The samples were then filtered with three layers' cheese cloth. The crude aqueous and ethanol extracts were evaporated through heating with a hot plate to complete dryness and concentrations of 100%, 60% and 20% were used.

Effect of Leaf Extract on the Isolates

The *in-vitro* test was carried out using the approach of Ijato (2011) to evaluate the growth inhibition level of the extract on fungal colony growth by creating four equal sections on the bottom of each Petri dish. The point of intersection indicates the center of the plates. This was done before dispensing the PDA mixed with the aqueous and ethanol leaf extracts into each of the Petri dish in the different concentrations of 100, 60, and 20% (pour plate method) followed by inoculation of the isolate. The control experiment was without the addition of any mahogany leaf extract. Growth inhibition was determined by ruler measurements of radial colonial expansion.

The *in-vivo* test was carried out by placing cotton wool onto the plates then inserting three certified seeds before inoculating mycelial/spore suspension of each of the pathogens unto the seeds and also two (2) drops of the extracts (aqueous and ethanol) with a sterile syringe. Fungal growth inhibition was determined by measuring the growth of fungus with measuring ruler (mm).

Statistical Analysis

All the data were analyzed using analysis of variance (ANOVA) according to Gomez and Gomez (1984). Least Significant Difference (LSD) according to Scheff (1953) was used to separate the means that were significantly different. Statistical Analysis Software (SAS) Version 9.1 was used to analyze the results.

Results

In-vitro and in-vivo mold inhibition by mahogany root aqueous and ethanol extracts

In-vitro evaluation of aqueous and ethanol root extracts of *Khaya senegalensis* on mycelial growth of the pathogens proved effective. However, there was no significant difference between the two solvents. The lowest growth of the pathogens recorded i*n-vitro* was in *Pseudallescheria boydii* (17.82mm), *Paecilomyces lilacinus* (18.08mm) for ethanol and *Penicillium chrysogenum* (18.33mm), *Cylindrocarpon lichenicola* (18.42mm) and *Pseudallescheria boydii* for aqueous (Table 1). For the *in-vivo* control trial, the aqueous root extract was more effective (lowest growth) on *Pseudallescheria boydii* **(**11.96mm), *Scedosporium prolificans* (15.29mm), while that of the ethanol root extract was more effective on *Pseudallescheris boydii* (9.83mm), *Scedosporium prolificans* (11.42mm) (Table 2).

In-vivo analysis of variance for the root extract of *Khaya senegalensis* showed a significant difference among the isolates though there was no significant difference among *Pseudaiiescheria boydii*, *Cylindrocarpon lichenicola*, and *Scedosporium prolificans*, however the aqueous and ethanol root extract of *Khaya senegalensis* were effective in controlling the pathogens as compared with the control, the most effective control (ethanol extract) was on *Pseudescheria boydii* (9.83mm), *Scedosporium prolificans* (11.42mm), *Paecilomyces lilacinus* (11.54mm) followed by *Penicillium chrysogemun* (12.04mm), *Cylindrocarpon lichenicola* (12.13mm), *Aspergillus flavus* (15.76mm), *Aspergillus niger* (15.92mm) and *Rhizopus stolonifer* (20.92mm), while for aqueous extracts the lowest was recorded in *Pseudaiiescheria boydii* (11.96mm) followed by *Scedosporium prolificans* (15.29mm), *Cylindrocarpon lichenicola* (15.42mm), *Paecilomyces lilacinus* (17.54mm), *Penicillium chrysogenum* (18.33mm), *Aspergillus niger* (23.42mm), *Aspergillus flavus* (26.63mm) and *Rhizopus stolonifer* (38.50mm) (Table 2).

Table 2: Aqueous and Ethanol Growth Inhibition of Root Extracts of *Khaya senegalensis* on Pathogens of Stored Groundnut (mm) in Hong Local Government Area of Adamawa State, Nigeria.

	Pathogens							
	Aspergillus brasilensis	*Aspergillus flavus*	*Penicilliu m chrysogen um*	*Rhizop us stolonif er*	*Pseudaiiesch eria boydii*	*Paecilomy ces lilacinus*	*Cylindrocar pon lichenicola*	*Secdospori um prolificans*
	In-vitro (mycelial growth in mm)							
Solvent								
Aqueous	21.00	21.17	18.58	26.833	19.00	20.42	20.33	25.50
Ethanol	20.50	19.25	18.33	26.08	17.83	18.08	18.42	23.33
Control	72.67	68.00	65.33	88.67	60.67	64.00	67.33	85.33
LSD	3.09	6.29	4.50	10.69	6.13	7.98	7.14	10.01
	In-vivo							
Solvent								
Aqueous	23.42	26.63	18.33	38.50	11.96	17.54	15.42	15.29
Ethanol	15.92	15.79	12.04	20.92	9.83	11.54	12.13	11.42
Control	55.00	55.00	42.50	78.33	34.17	43.33	44.17	42.50
LSD	4.30	4.66	2.88	5.25	2.93	3.74	3.47	4.76

LSD: Least Significant Difference

Efficacy of root extract as a control agent on the pathogens improved as concentration increased from 20% – 100 %. However, 60% – 100% exhibits similar inhibitory effects on the pathogens for both i*n-vitro and in-vivo*. The root extract of *Khaya senegalensis* concentration effect at 100% *in-vitro* proved to effectively control *Aspergillus niger* 0.50mm, *Pseudaiiescheria boydii* 0.67mm, *Aspergillus flavus*, and *Cylindrocarpon lichenicola* both had 0.83mm, *Rhizopus stolonifer* 1.00mm, *Scedosporium prolificans* and *Penicillium chrysogenum* both had 1.17mm and *Paecilomyces lilacinus* 1.50mm (Table 3). The root extract of *Khaya senegalensis* concentration effect at 100% *in-vivo* proved to effectively control *Pseudaiiescheria boydii* 1.47mm, *Cylindrocarpon lichenicola* 1.75mm, *Scedosporium prolificans* 2.25mm, *Paecilomyces lilacinus* 3.33mm, *Aspergillus niger* and *Penicillium chrysogenum* both had 3.50mm, *Aspergillus flavus* 4.25mm and *Rhizopus stolonifer* 7.58mm (Table 3). The most effective concentration was the 100% concentration followed by 60% then 20%.

Table 3: Inhibitory Effect of Concentration of Root Extracts on Pathogens in Hong Local Government Area of Adamawa State, Nigeria.

	Pathogens							
	Aspergillus niger	*Aspergillus flavus*	*Penicillium chrysogenum*	*Rhizopus stolonifer*	*Pseudaiiescheria boydii*	*Paecilomyces lilacinus*	*Cylindrocarpon lichenicola*	*Secdosporium prolificans*
Concentration (%)	*In-vitro (mycelial growth in mm)*							
20	6.00	8.33	3.83	11.17	10.17	7.50	6.00	8.00
60	3.83	3.67	3.50	5.00	2.17	4.00	3.33	3.17
100	0.50	0.83	1.17	1.00	0.67	1.50	0.83	1.17
LSD	4.37	2.45	6.36	15.12	8.67	11.29	10.09	14.16
	In-vivo							
Concentration (%)								
20	13.08	16.50	8.58	20.50	5.33	6.92	5.75	5.50
60	7.08	9.08	6.17	12.42	2.67	4.58	3.42	3.17
100	3.50	4.25	3.50	7.58	1.47	3.33	1.75	2.25
LSD	6.08	6.59	4.07	7.43	4.15	5.29	4.91	6.73

LSD: Least Significant Difference

There was a significant difference between the Valencia and the Peruvian variety, however, the Peruvian showed it has more resistance than the Valencia variety (Table 4).

Table 4: Effect of Root Extract on Pathogen/Groundnut Variety (mm) in Hong Local Government Area of Adamawa State, Nigeria.

	Pathogens							
	Aspergillus brasilensis	*Aspergillus flavus*	*Penicillium chrysogenum*	*Rhizopus stolonifer*	*Pseudaiiescheria boydii*	*Paecilomyces lilacinus*	*Cylindrocarpon lichenicola*	*Scedosporium prolificans*
Variety								
Kampala	25.79	28.79	21.83	37.71	15.04	18.17	17.50	17.29
Local	13.54	13.63	8.54	21.71	6.75	10.92	10.04	9.42
LSD	4.30	4.66	2.88	5.25	2.93	3.74	3.47	4.76

LSD: Least Significant Difference

Discussion

Both aqueous and ethanol root extracts of mahogany are effective control agents on all the postharvest fungal pathogens of groundnuts both *in vitro* and *in vivo*, though efficacy varied with pathogens. There was, however, no variation between the aqueous and ethanol solvents. This agrees with reports (Lourens *et al*., 2004, Parekh *et al*., 2006, Rojas *et al*., 2006) that both water and ethanol were effective solvents for preliminary investigations against the microbial activity.

Efficacy of the extracts appreciated along with the concentration (solvent to sample ration) which conforms to an earlier report by Green (2004) observed that higher sample ratio to solvent was ideal. The best and ideal concentration of mahogany root extract is 60% since it exhibits similar efficacy.

The 'kwathrumthrum' (local genotype) exhibited higher resistance to all the eight postharvest groundnut rot fungal pathogens. Host plant resistance is considered one of the most essential disease control strategies (Hasyim *et al*.,2014).

Conclusion

The research revealed the root extract of Mahogany (aqueous and ethanol) has the potential to reduced fungal rot of groundnut seeds at different concentration. Plant extracts are cheaper, safer,

affordable to the farmer and environmentally friendly, therefore, there is a need for more researches into the use of plant extracts by the pathologist. Farmers thus have hope for a cheaper and safer alternative control against deteriorating fungal agents of groundnut.

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