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EFFECT OF *KHAYA SENEGALENSIS* BARK AND OIL ON POST HARVEST FUNGAL AGENTS OF GROUNDNUT SEEDS ROT IN ADAMAWA STATE, NIGERIA

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ABSTRACT

Standardized method of seed treatment is of prime importance in the production of groundnut. The study was to carry out control trial using bark extract (aqueous and ethanol) and oil (seed) of mahogany (Khaya senegalensis) on seven (7) isolated fungi from two groundnut genotypes (peruvian and valencia). The result shows that both mahogany bark and seed extracts are capable of inhibiting mycelial growth of all the isolates. There was no significant variation between the aqueous and ethanol bark extracts in-vitro, however the in-vivo test shows a significant difference between the aqueous and the ethanol bark extract in which the ethanol extract reduced growth of the pathogens more than the aqueous. For all the pathogens except Rhizopus stolonifer there was no growth between 50% to 100% concentration of the Khaya senegalensis oil in-vitro, however in-vivo control at 50% produced scanty to moderate growth for all the pathogens except Rhizopus stolonifer on peruvian, while there was full coverage on the oli seeds of valencia variety with Aspergillus niger and Rhizopus stolonifer having total coverage though Pseudaiiescheria boydii and Cylindrocarpon lichenicola were effectively inhibited and showed no growth at the 50 % and 100 %. Further research to focus on the quantifying the chemical constituents and formulation are suggested.

KEY WORD: groundnut, fungi, mahogany, bark, Oil seed, control.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an annual crop that belongs to Fabiaceae family and is believed to have originated from South America (Wiess, 2000). The crop is the 13th most important food crop source of edible oil and the 3th most important source of vegetable protein (Al-Amod, 2015).

The production of this crop is facing a major constraint which causes losses of healthy seeds, this is as a result of the activities of fungi, bacteria, viruses, nematodes, insects and parasitic weeds (Osman, 2016). However, fungi can be rated as the most harmful microorganism (Al-Amod, 2015). Several fungi were isolated from peanut pods, shells and seeds. These fungi are *Aspergillus niger, Aspergillus flavus, Alternaria dianthicola, Curvularia lunata, Curvularia pellescens, Fusarium oxysporum, Fusarium equiseti, Macrophomina phaseolina, Rhizopus stolonifer, Penicillium digitatum* and *Penicillium chrysogenum* (Elwakil *et al*., 2001; Chavan and Kakde, 2008). Their activities can cause discoloration, rotting, shrinking, seed necrosis, loss in germination capacity and toxification to oilseeds. Al-Amod (2015) reported that the activities of fungi growing on stored groundnut seeds can reduce the germination rate beside the loss of carbohydrate, protein and total oil content, induce increased moisture content, free fatty acid content and enhancing other biochemical changes. Fungi continue to represent a major human health risk throughout the world and particularly in the humid tropics being major spoilage agents of food crops (Olusegun *et al*., 2013).

Manimurugan (2003) reported that disease transmission is usually carried out by seeds because the carry a number of pathogens, which get associated either in the field or in storage. Seed - borne fungi were generally managed by the use of some synthetic chemicals which were also considered to be both efficient and effective (Ahmad and Beg, 2007). The continuous use of this fungicides unravelled its non-biodegradability and thereby having a residual toxicity to cause environmental pollution (Ajobade and Amusa, 2001), therefore there is need for alternative safe means of control. However, attention has been given to the use of non-chemical systems for the treatment of the seed in order to protect it against plant pathogens (Ademola *et al.,* 2004). Plant extracts have played significant role in inhibiting of seed-borne pathogens, improving seed quality and emergence of plant seeds (Abdelgaleil *et al.,* 2004). There is now emphasis on use of botanicals such as the flowers, cloves, leaves, bark, root and seed extracts used

for fungal control (Abdelgaleil *et al.*, 2001) these are considered as cheaper and safer means to the control of moulds. Therefore based on this, the foregoing study was aimed at controlling the fungal pathogens associated with the rot of groundnut seeds in Hong local government area of Adamawa state, Nigeria using mahogany bark and seeds.

METHODOLOGY

The inhibitory effect of *Khaya senegalensis* bark and oil extracts was carried out in the Medical Laboratory of Microbiology Department, Modibbo Adama University of Technology, Yola Adamawa State Nigeria

Source of Isolates

Fungal isolates were isolated from 50 unhealthy groundnut seeds of two genotypes valencia (*fastigiata*) and peruvian (*hirsuta*) collected from one (1) major market in each of the seven (7) districts of Hong local government namely Hildi, Kulinyi, Dugwaba, Uba, Gaya, Pella, and Hong.

Preparation of Extracts

The method of Ijato *et al*. (2011) was used to prepare both aqueous and ethanol extracts. Bark of *Khaya senegalensis* was collected (Figure I) rinsed thoroughly under running tap water and was allowed to air dry for seven (7) days; this was then ground using pestle and mortar. Hundred (100), sixty (60) and twenty (20) grams were dissolved in 100ml of 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.

Seeds of *Khaya senegalensis* plant (figure II) were obtained and dried in a shade to maintain its composition. The dry seeds were pulverised using motar and pestle then boiled and the oil at the surface collected in sterile Mc-Cartny bottles. Glycerol was used to vary the concentrations of the oils, 50%, 60%, 70%, 80%, 90% and 100% of the Glycerol and *Khaya senegalensis* oil each.

Figure I. *Khaya senegalensis* Stem Bark.

Figure II. *Khaya senegalensis* Seeds.

Inhibition Level of Bark Extract and Oil on the Isolates

The *in-vitro* test was carried out using the adopted method of Ijato (2011) to evaluate the inhibition level of the extract on fungal colony growth on 9cm Petri dish. PDA mixed with the aqueous and ethanol leaf extracts was poured separately into each Petri dish in the different concentrations mentioned above, then followed by inoculation of the isolate. A mahogany extract free experiment was set up to serve as control. Radial growth inhibition was recorded using ruler for analysis. The *in-vitro* oil test was carried out by making wells with a 2 mm cork borer on the Potato Dextrose Agar and using a sterile 5mls syringe to inject the various concentrations into the wells. Growth of fungi was assessed through visual observation for lack of growth, scanty growth, moderate growth or luxuriant growth.

The *in-vivo* tests for bark and oil were carried out by placing cotton wool onto the plates then inserting three certified healthy seeds before inoculating a drop of mycelial/spore suspension of each of the pathogens unto the seeds and also two (2) drops of the extracts (aqueous and ethanol) and oil with a sterile syringe. Dimension (inhibition level) of seed rot was recoded using thread and ruler.

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.

RESULT

Effect of Aqueous and Ethanol Extracts of Stem Bark of Mahogany on Fungal Growth

In-vitro control trial using aqueous and ethanol extracts of *Khaya senegalensis* stem bark extract proved effective against the pathogens as there was a significant reduction in mycelial growth compared to control, however there was no significant variation between the aqueous and ethanol stem bark extracts. (Table 1). The *in-vivo* test of *Khaya sengalensis* bark on the pathogens proved to be effective, however there was a significant difference between the aqueous and the ethanol bark extracts in

which the ethanol extract reduced growth of the pathogens more than the aqueous. There was reduction in the mycelial growth of all the pathogens for aqueous and ethanol *in-vitro,* while in the *in-vivo* aqueous growth reduction was more in *Penicillium chrysogenum, Pseudllescheria boydii, Paecilomyces lilacinus* and *Scedosporium prolificans* and as for the ethanol mycelial growth reduction was in *Penicillium chrysogenum, Pseudallescheria boydii, Paecilomyces lilacinus and Scedosporium prolificans* were the ones most effectively controlled.

Concentration of 20% was as effective as that of 100 as there was no significant difference in mycelial growth among the concentrations for the *in-vitro* trial. The concentration levels of the extracts produced variations on the control of the pathogens. The highest was at 100% concentration followed by the 60% concentration then 20 % concentration, there was however no significant difference between 60% and 100% (Table 2).

Efficacy of stem bark extract on the pathogens showed there was a significant difference between the valencia and the peruvian. The local cultivar showed less susceptibility to fungal rot after treatment with the stem bark (Table 3).

Table 1: Aqueous and Ethanol Growth inhibition of Stem-Bark Extracts of *Khaya senegalensis* on Pathogens of Stored Groundnut (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***	***Secdosporium prolificans***
	In-vitro (mycelial growth in mm)							
Solvent								
Aqueous	19.17	17.83	17.00	23.83	15.67	16.58	17.25	22.25
Ethanol	5.56	8.67	8.40	12.09	6.43	7.11	5.59	10.64
Control	72.67	68.00	65.33	88.67	60.67	64.00	67.33	85.33
LSD	23.67	23.58	22.00	28.50	19.08	20.42	20.17	27.75
	In-vivo							
Solvent								
Aqueous	25.83	26.63	18.58	28.86	13.21	13.58	13.25	12.96
Ethanol	15.33	15.46	11.96	20.58	9.25	11.54	11.58	11.04
Control	55.00	55.00	42.50	78.33	34.17	43.33	44.17	42.50
LSD	3.68	3.61	2.81	3.59	2.44	2.54	3.64	2.95

LSD: Least Significant Difference

Table 2: Inhibitory Effect of Concentration of Stem-Bark Extracts on Pathogens (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***	***Secdosporium prolificans***
Concentration (%)	*In-vitro (mycelial growth in mm)*							
20	8.33	9.50	7.33	10.50	5.67	6.17	5.00	9.50
60	3.00	3.33	3.17	3.50	2.18	2.50	1.83	3.67
100	1.67	2.00	2.17	2.00	1.00	1.33	0.67	1.50
LSD	7.86	12.26	11.87	17.10	9.09	10.05	7.91	15.05
Concentration (%)					*In-vivo*			
20	13.17	15.17	8.67	9.83	5.50	3.75	3.00	3.17
60	8.50	8.17	6.08	6.75	3.00	2.08	1.17	1.50
100	5.67	5.83	3.83	4.00	2.25	1.08	0.83	0.83
LSD	5.21	5.11	3.97	5.08	3.45	3.60	5.14	4.18

LSD: Least Significant Difference

Table 3: Effect of Stem-Bark Extract on Pathogen/Groundnut Variety (mm) in Hong Local Government Area of Adamawa State, Nigeria.

	Pathogen							
	Aspergillus brasillensis	***Aspergillus flavus***	***Penicillium chrysogenum***	***Rhizopus stolonifer***	***Pseudaiiescheria boydii***	***Paecilomyces lilacinus***	***Cylindrocarpon lichenicola***	***Scedosporium prolificans***
Variety	*(mycelial growth in mm)*							
Valencia	24.50	28.71	19.96	30.08	16.63	14.21	15.96	15.00
Peruvian	16.67	13.38	10.58	19.38	5.83	10.92	8.88	9.00
LSD	3.09	3.61	2.81	3.59	2.44	2.54	3.64	2.95

LSD: Least Significant Difference

Inhibitory Effect of Oil Extract on the Mycelial Growth of Pathogens

For all the pathogens except *Rhizopus stolonifer* there was no growth between 50 % to 100% concentration of the *Khaya senegalensis* oil, however there was a luxuriant growth for the control that is 0% for the oil. At 50% concentration of the oil *Rhizopus stolonifer* showed luxuriant growth while between 60 – 80 % its growth was reduced to moderate and became scanty between 90 – 100 % (Table 4).

In-vivo control trial with oil of *Khaya senegalensis* at 50 % produced scanty to moderate growth for all the pathogens except *Rhizopus stolonifer* which produced moderate growth at 50% while at 100% growth was scanty to none and control has full coverage on the peruvian variety (Table 5), however all pathogens showed full coverage on the seeds of valencia variety with *Aspergillus niger* and *Rhizopus stolonifer* having total coverage though *Pseudaiiescheria boydii* and *Cylindrocarpon lichenicola* were effectively inhibited and showed no growth at the 50 % and 100 %, there was no coverage at 100% except for *Aspergillus flavus* that was moderate and *Rhizopus stolonifer* with moderate coverage as well, as for the control there was complete coverage on the seeds for all the pathogens (Table 6).

Table 4: Effect of *Khaya senegalensis* Oil on Fungal Pathogens of Groundnut *In -vitro* in Hong Local Government Area of Adamawa State, Nigeria.

Pathogen	Concentrations							
	50%	60%	70%	80%	90%	100% K. s	100% G	Control
Aspergillus brasillensis	+	-	-	-	-	-	-	+++
Aspergillus flavus	+	-	-	-	-	-	-	+++
Penicillium chrysogenum	+	-	-	-	-	-	-	+++
Rhizopus stolonifera	+ + +	+ +	+ +	+ +	+ +	+	+	+++
Pseudaiiescheria boydii	-	-	-	-	-	-	-	+++
Paecilomyces lilacinus	-	-	-	-	-	-	-	+++
Cylindrocarpon lichenicola	-	-	-	-	-	-	-	+++
Scedosporium prolificans	-	-	-	-	-	-	-	+++

Key

- No Growth
+ Scanty Growth
++ Moderate Growth
+++ Luxuriant Growth
K.s *Khaya senegalensis*
G Glycerol

Table 5: Effect of *Khaya senegalensis* Oil on Growth of Pathogen on Groundnut Seed *In-vivo* On Peruvian Variety (%) in Hong Local Government Area of Adamawa State, Nigeria.

Pathogens	Concentrations		
	50%	100 %	Control
Aspergillus brasillensis	20	20	40
Aspergillus flavus	40	20	40
Penicillium chrysogenum	20	-	20
Rhizopus stolonifera	40	20	60
Pseudaiiescheria boydii	-	-	20
Paecilomyces lilacinus	-	-	40
Cylindrocarpon lichenicola	-	-	20
Scedosporium prolificans	-	-	20

Table 6: Effect of *Khaya senegalensis* Oil on Growth of Pathogen *in-vivo* for Valencia Variety (%) in Hong Local Government Area of Adamawa State, Nigeria.

Pathogens	Concentrations		
	50%	100 %	Control
Aspergillus brasilensis	20	20	60
Aspergillus flavus	40	20	40
Penicillium chrysogenum	20	-	40
Rhizopus stolonifera	40	20	70
Pseudaiiescheria boydii	-	-	20
Paecilomyces lilacinus	-	-	20
Cylindrocarpon lichenicola	-	-	20
Scedosporium prolificans	-	-	20

DISCUSSION

Results showed that the mahogany stem-bark and oil were effective in the control of fungal pathogens of groundnut seeds. The results was in agreement with the work of Abdulsalam *et al.* (2015) who reported treatment with different concentration of plant extracts revealed that *Khaya senegalensis* A Juss. extract highly retarded the vegetative growth of the fungi responsible for the neck rot disease of onions. It is similar with the work of Liman *et al*. (2010) but on different organism who confirmed that mahogany extract was highly effective on the control of Root knot disease of tomatoes caused by nematodes. A report by (Montes, 1996) stated that a plant may have different concentrations of a chemical in different vegetal parts; roots, leaves, flowers and fruit and may even be absent in one or more parts. According to Khare *et al*. (2004) plant essential oil is a useful source of anti-fungal compounds and the effectiveness of *Khaya senegalensis* oil in controlling fungal pathogen could probably be due to constituents of secondary metabolite capable of controlling and inhibiting the pathogens. O'Bryne *et al*., (1997) reported that the fresh and dried mahogany bark extracts have also shown strong antimicrobial properties.

This work also conforms to the work of Bamaiyi *et al*. (2006) who have shown that tuber treatment with *Khaya senegalensis* bark extract can be used for controlling potato tuber soft rot disease. Abdelgaleil *et al.* (2004), reported that the extract from the dried bark was more effective than that from the fresh bark. Probably the active compounds are more concentrated in the dried bark than in the fresh bark which contains a higher water content at the time of the extract preparation. The soaking of the fresh bark in water might have further diluted the concentration of the active substance compared to the soaking of the powder from the dried bark. Although, there are few reports on the use of *Khaya senegalensis* products in controlling plant pathogens, extracts from the plant have been extensively used in the control of insect pests of crops, particularly cotton boll worm, apart from the insecticidal properties of *Khaya senegalensis* products, these products have also been reported to possess antifungal and bactericidal properties.

CONCLUSION

This research work has proven that mahogany extract (bark and oil) can suppress the growth of fungal pathogens isolated from groundnut. Therefore continued trails should be carried out on the field and in different locations. Furthermore, research should be conducted to determine the active compound responsible for fungal growth inhibitor.

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