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Full Length Research Paper

Control strategy against fungal pathogens of postharvest rot of groundnut (*Arachis hypogaea L.*) using aqueous and ethanol leaf 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 used of synthetic fungicide has a great effect on human consumption, hence the need for safer 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 moulds were associated with postharvest groundnut rot in the seven districts of Hong local government area in the month of July 2016: *Aspergillus niger* (brasilensis), *Aspergilus flavus, Penicillium chrysogenum, Rhizopus stolonifer, Paecilomyces lilacinus, Pseudallescheria boydii, Cylindrocarpon lichenicola* and *Scedosaporium prolificans*. Therefore, the research sought to assess management of rot using plant extract of mahogany. Control trials were carried out using the extracts of leaf of mahogany. Growth of pathogens both *in-vitro* and *in-vivo* were significantly reduced by the plant extracts. Aqueous and ethanol leaf extracts reduced mycelial growth from 72.67 mm to 25.83 mm and 15.33 mm respectively for *Aspergillus niger* (brasilensis) (*in-vitro*) and from 55.00 mm to 28.58 mm by aqueous extracts and to 17.92 mm size of rot by ethanol extracts for *in-vivo* control. Efficacy of extract increased with concentration, while the local genotype was less affected by rot in comparison to Kampala variety. Therefore, mahogany aqueous and ethanol leaf extracts have been found effective against these pathogens and therefore the leaf is recommended for further research in order 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 labour 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 mainly 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 major cash crop produced especially in Hong (Adebayo and Tukur, 1999). Rowland (1999) reported that seed yield in Northern Nigeria is about 3000 Kg/ha. Adamawa Agricultural Development Programme, ADADP (1996) enumerated groundnut genotypes 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 important oil seed crop in Nigeria and is widely grown in the tropics

and sub-tropics (Nigam et al., 1994). It is one of the most important crops that have the ability to thrive 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 per cent of the global groundnut area and 92 per cent of the global production (FAOSTAT, 2011).

Fungi such as *Aspergillus niger* (brasiliensis), *Aspergillus flavus*, *Alterneria anthocola*, *Curvularia lunata*, *Curvulari 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 oil seeds (Chavan and Kakde, 2008). These fungi are associated with heavy loss of seeds, fruits, grains, vegetables and other plant products during picking, transit and storage rendering them unfit for human consumption even by producing mycotoxins and affecting their total nutritive value (Verma et al., 2003). The tropical climate with high temperature and high relative humidity along with poor storage methods adversely affect the storage of cereal grains and oil seed, and this can lead to the total loss of seed quality (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 a residual toxicity to cause environmental pollution (Ajobade and Amusa, 2001), hence the need for alternative safer means of control.

In recent years, much 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 which are considered as cheaper and safer means of mould control (Abdelgaleil et al., 2001). Alternative ways to control seed-borne pathogens, particularly 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 good 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 complex or dissociate (Hughes, 2002). Thus the most commonly used solvents for preliminary investigations of anti-microbial activity in plants are said to be methanol, ethanol and water (Lourens et al., 2004; Parekh et al., 2006).

The aim of the study was to determine the inhibitory effect of aqueous and ethanol leaf 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.

METHODS AND MATERIALS

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

Source of Groundnut Samples

Samples of groundnut seeds of two genotypes commonly found namely Kampala and “Kwathrumthrum” were collected from one major market in each of the seven districts namely Hildi, Kulinyi, Dugwaba, Uba, Gaya, Pella and Hong (Table 1). Fifty of the samples of each genotype were purchased from a seller (two randomly selected sellers/traders within the selected market) in each district making a total of 700 collected from the various district, the samples were conveyed to the laboratory in a dry clean polythene bag. Groundnut samples were labelled according to location and then photographed (Figures 1-5).

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

Sterilization of Inoculation Room and Instruments

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

Figure 1. Sample of Healthy "Kwathrumthrum" Variety Groundnut Seeds

Figure 2. Sample of "Kwathrumthrum" Variety Diseased Groundnut Seeds

Figure 3. Sample of Healthy Kampala Variety Groundnut Seeds

Figure 4. Sample of Diseased Kampala Variety of Groundnut Seeds

Figure 5. *Khaya senegalensis* Leaf

Preparation of Potato Dextrose Agar (PDA)

Thirty nine grams (39 g) of Potato Dextrose Agar (PDA) was dissolved in one 1 L of distilled water, the potato dextrose agar was then poured into two 500 mL conical flask, then plugged with cotton wool and wrapped with aluminium foil before autoclaving at 121˚C for 15 min at 10 lbs. pressure, and 6 mL (0.1%) of streptomycin was added to the litre of sterilized media and swirled gently to mix properly, 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 3) and were allowed to air dry for 7 days, these were then ground using pestle and mortar. Hundred (100) g, sixty (60) g and twenty (20) g were dissolved in sterile distilled water and ethanol in separate conical flasks respectively. These were vigorously shaken and left to stand for 24 h. The samples were then filtered with 3 layers' cheese cloth. The crude aqueous and ethanol extracts were evaporated through heating with 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 effect of the extract on fungal colony growth by creating four equal sections on each plate by drawing two perpendicular lines at the bottom of the plate. The point of intersection indicates the centre of the plates. This was done before dispensing the PDA mixed with the aqueous and ethanol leaf extracts into each of the plates in the different concentrations of 100%, 60%, and 20% (pour plate method) followed by inoculation of the isolate. Control experiment was without 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 placing three healthy seeds before inoculating mycelial/spore suspension of each of the pathogens unto the seeds and also 2 drops of the extracts (aqueous and ethanol) with sterile syringe. Fungal growth inhibition was determined by measuring 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* Aqueous and Ethanol Control

Leaf extract of mahogany effectively controlled the mycelial growth of the pathogens compared to control (Table 2). Both aqueous and ethanol leaf extracts most effectively controlled *Scedosporium prolificans* (colony size of 12.96 mm for aqueous and 11.04 mm for ethanol), *Pseudaiiescheria boydii* with 13.21 mm for aqueous and 9.25 mm for ethanol, *Cylindrocarpon lichenicola* 13.25 mm for aqueous and 11.58 mm for ethanol extract, *Paecilomyces lilacinus* 13.58 mm for aqueous and 11.54 mm for ethanol, *Penicillium chrysogemuni* 18.58 mm for aqueous and 11.96 mm for ethanol, and *Aspergillus brasilensis* with 25.83 mm for aqueous and 15.33 mm for ethanol. There was however no significant difference in efficacy between the two solvents in control of these pathogens. In comparison, leaf extract had less inhibition on the colony expansion of *Rhizopus stolonifer* (28.88 mm for aqueous, 20.58 mm for ethanol), *Aspergillus flavus* (26.63 mm aqueous, 15.46 mm for ethanol). There was a significant variation in growth inhibition (*in-vitro*) by the leaf extract between the solvents on *Aspergillus niger*, *Aspergillus flavus*, *Penicillium chrysogenum* and *Rhizopus stolonifer.*

The *in-vivo* leaf extract of mahogany showed that there was a significant inhibitory effect on all the pathogens of groundnut postharvest rot in Hong Local Government Area of Adamawa State, Nigeria. Similar trend in efficacy was exhibited as in the *in vitro* control. There was less rot in seeds inoculated with the following pathogens: *Scedosporium prolificans* (16.79 mm for aqueous, 15.38 mm for ethanol), *Paecilomyces lilacinus* (17.92 mm for aqueous, 13.63 mm for ethanol), *Pseudaiiescheria boydii*

Table 2. Effect of Aqueous and Ethanol Leaf Extracts of *Khaya senegalensis* on Colony Expansion (mm) and Rot of Fungal Pathogens of Stored Groundnut 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)**								
Solvents								
Aqueous	25.83	26.63	18.58	28.88	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	72.67	68	65.33	88.67	60.67	64	67.33	85.33
LSD	4.82	9	6.46	6.81	6.16	2.74	5.29	4.58
In-vivo								
Aqueous	28.58	35.79	24.5	41.08	16.04	17.92	20.17	16.79
Ethanol	17.92	18.46	12.58	24.13	11.33	13.63	13.17	15.38
Control	55	55	42.5	78.33	34.17	43.33	44.17	42.5
LSD	3.92	3.53	3.92	5.59	2.59	3.31	4.82	3.59

LSD: Least Significant Difference

(16.04 mm for aqueous, 11.33 mm for ethanol), and *Cylindrocarpon lichenicola* (20.17 mm for aqueous and 13.17 mm for ethanol). There was however no significant difference in efficacy between the two solvent extracts of mahogany leaf. The three most notorious pathogen exhibited higher rot in comparison to these other pathogens *Rhizopus stolonifer* (41.08 mm aqueous, 24.13 mm for ethanol), *Aspergillus flavus* (35.79 mm aqueous, 18.46 mm ethanol) and A*spergillus niger (brasilensis)* (28.58 mm for aqueous, 17.92 mm for ethanol). Ethanol leaf extract was more effective in reducing rot incited by these pathogens than the aqueous leaf extract.

The concentration levels in the *in vitro* control showed there was a significant difference among them and the most effective was produced in 100% concentration followed by 60% concentration, least was the 20% concentration (Table 3).

Similarly, the higher the concentration the more effective the extract control on the rot, with 100% concentration having a higher inhibitory effect then 60% concentration followed by 20% concentration (Table 3) in the *in vivo* control trial.

There was a high variation between the Kampala and Local (Kwathrumthrum) genotypes, growth of pathogens was reduced more in the Local (Kwathrumthrum) genotype than the Kampala variety by the extract at 9.99%. The lowest growth was recorded in the Local (Kwathrumthrum) genotype with *Pseudaiiescheria boydii* had 8.75 mm, *Cylindrocrpon lichenicola* (10.17 mm), *Scedosporium prolificans* (10.38 mm), *Penicillium chrsogenum* (10.83 mm), *Paecilomyces lilacinus* (12.17 mm), *Aspergillus niger* (16.00 mm), *Aspergillus flavus* (16.25 mm), and *Rhizopus stolonifera* (20.38 mm) while for the Kampala variety *Pseudaiiescheria boydii* had 18.63 mm, *Paecilomyces lilacinus* (19.38 mm), *Scedosporium prolificans* (21.79 mm), *Cylindrocarpon lichenicola* (23.17 mm), *Penicillium chrysogenum* (26.25 mm), *Aspergillus niger (brasilenesis)* (30.50 mm), *Aspergillus flavus* (38.00 mm) *and Rhizopus stolonifer* (44.83 mm) (Table 4).

DISCUSSION

The study indicates that 'kwathrumthrum' (local genotype) exhibited a higher resistance to all the eight postharvest groundnut rot fungal pathogens. This can be as a result of resistance exhibited by the host which is in agreement with the work of Hasyim et al. (2015) who reported that host plant resistance is considered as one of the most important disease control strategies.

Both aqueous and ethanol leaf 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. This agrees with reports (Lourens et al., 2004, Parekh et al., 2006, Rojas et al.,

Table 3. Inhibitory Effect of Different Concentration of Leaf Extracts on Fungal 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***
***In-vitro* (mycelial growth in mm)**								
Concentration (%)								
20	13.17	15.17	8.67	9.83	5.5	3.75	3	3.17
60	8.5	8.17	6.08	6.75	3	2.08	1.67	1.5
100	5.67	5.83	3.83	4	2.25	1.08	0.83	0.83
LSD	6.82	12.74	9.14	9.63	8.71	3.87	7.48	6.48
In-vivo								
Concentration (%)								
20	19.5	27	15.83	26.25	10.67	10.5	11.5	11
60	12.92	18	11.25	18.25	7.58	6.75	8.42	7.67
100	5.83	8.5	4.58	7.58	2.33	2.5	2.58	3.17
LSD	5.55	4.99	5.54	7.91	3.66	4.68	6.82	5.08

LSD: Least Significant Difference

Table 4. Effect of Leaf Extract on Mycelial Growth of Fungal Pathogens of Stored Groundnut Genotypes (mm) in Hong Local Government Area of Adamawa State, Nigeria.

	Pathogens							
	Aspergillus niger	***Aspergillus flavus***	***Penicillium chrysogenum***	***Rhizopus stolonifer***	***Pseudaiiescheria boydii***	***Paecilomyces lilacinus***	***Cylindrocarpon lichenicola***	***Scedosporium prolificans***
Variety								
Kampala	30.5	38	26.25	44.83	18.63	19.38	23.17	21.79
Local	16	16.25	10.83	20.38	8.75	12.17	10.17	10.38
LSD	3.92	3.53	3.92	5.59	2.59	3.31	4.82	3.59

LSD: Least Significant Difference

2006) that both aqueous and ethanol were effective solvents for preliminary investigations against microbial activity. Meanwhile, ethanol was more effective than aqueous, this could be as result agents present in ethanol capable to dissolve the active component in the plant extract. It also conforms with the work of Shehu et al. (2016) aqueous (water) is an inorganic solvent which may not be effective to dissolved the bioactive compounds responsible for antifungal and antimicrobial activities.

Efficacy of the extracts appreciated with the level of concentration which conforms to report by Green (2004) that higher sample ratio to solvent was ideal for control. The best and ideal concentration of mahogany root extract is 60% since it exhibits similar efficacy with 100%. The result was also in agreement with the work of Abdulsalam et al. (2015) who report that treatment with different concentrations of Mahogany can retard the vegetative growth of fungi responsible for neck rot disease of onions and also in line with the work of Liman et al. (2010) who reported the effect of Mahogany plant in the control of fungi and nematodes. The result is also in agreement with the work of Shehu et al. (2016) who reported that leaf extract of the plant had inhibitory activity on mycelia growth of pathogenic fungi regardless of the extraction solvents and the concentrations of the extracts. Shehu et al. (2016) further stated that, the strong antifungal properties could be due to the fact that leaves are considered as the most important life giving part of the plant body, as they carry out the process of photosynthesis, therefore, the leaves are loaded with bioactive materials (Bareja, 2011).

CONCLUSION

The study revealed leaf extract of Mahogany has the potential to reduced fungal rot of groundnut seeds at different concentration and different solvent. Plant extracts are cheaper, safer, affordable to the farmer and environmental friendly, therefore, there is need for more researches into the use of plant extracts by pathologist. Farmers thus have hope for a cheaper and safer alternative control against deteriorating fungal agents of groundnut.

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