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Control of Fungal Post Harvest Rot Agents of Groundnut (Arachis hypogea l.) Using Leaf Extracts of *Moringa oleifera*

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ABSTRACT

Laboratory studies were carried out on the incidence and in vitro control of groundnut seed rot in Mubi. Rot occurred in all the three location surveyed and the average rot incidences in the market was 20%. MubiKuturu Market showed 23% as the highest percentage, followed by Mubi New Market with 20% and Mubi Old Market with 17%. Four organisms were isolated from the lesions on Potato Dextrose Agar (PDA) as follows; *Aspergillusniger*, *Fusariumsolani*, *Rhizopusstolonifer* and *Aspergillusniger*. The pathogen most frequently occurring was *Rhizopusstolonifer* with 67.22%, followed by *Aspergillusniger* with 15.56%, *Aspergillusflavus* with 15% and the least was *Fusariumsolani* with 2.22%. Pathogenicity test confirmed that all four fungal isolates from groundnut seed were pathogenic although at different level. Plant extracts employed to control the vegetative mycelial growth were from aqueous and ethanolic leaf extracts of drum stick at 20%, 40% and 60% concentration. In vitro application of extracts for the control showed thatethanolic extracts inhibits the mycelial growth better as compared to aqueous extracts. Level of inhibitions was highest in *Aspergillusniger* and least in *Rhizopusstolonifer*. There was a significant difference on vegetative growth of the fungi on both ethanolic and aqueous leaf extracts with increase in concentration at p= 0.0001 as compared with the control. It is recommended that the use of antimicrobials which are commonly found around the habitation of rural areas can be used to reduce the cost of chemicals and over reliance of small scale farmers on agricultural chemical.

Keywords:*Aspergillus niger*,*Fusarium solani*,Groundnut,*Moringa oleifera* and *Rhizopus stolonifer*

INTRODUCTION

Groundnut (*Arachis hypogea* L.) is an annual, self-pollinated, wet season growing plant found in many tropical, subtropical and temperate countries of the world. It is now grown in about 108 countries of the world [28]. Asia with 63 – 6 % land mass produces 71.72% of world groundnut followed by Africa with 18.6% production and North-central America with 7.5% [16].

Groundnut is considered as a valuable legume crop cultivated over an area of 994 hectares in Parkistan with a production of about 1019kg hectare or 101 tones during 2001 – 2002 [4]. Groundnut seed contains 50% edible oil [32]. Seeds are rich in fats, proteins, vitamin B_1, B_2, B_6, nicotinic acid other vitamins [22]. It is found in a wide range of grocery products, its shells are used in the manufacture of plastic, wallboard, abrasives and fuel [9].

Stored food commodities are severely damaged by different groups of fungi including *Aspergillus*spp, *Fusarium*spp, and *Penecillium*spp[32].*Aspergillus* is a common mould in tropical and subtropical countries and causes*aflatoxin*contamination as a result of *moulding* of badly stored commodities such as groundnut, cereal and cotton seeds[32]. Fungi like

Aspergillusniger, *Aspergillusflavus*, *Alterneriadianthocola*, *Curvularialunata*, *Curvulariapellesecens*, *Fusariumoxysporum*, *Fusariumequiseti*, *Microphominaphasiolina*, *Rhizopusstolonifer, Penecilliumdigitatum* and *Penecilliumchrysogenum* cause discolouration, rotting, shrinking, seed necrosis, loss in germination capacity and toxification to oil seeds [7]. 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 [32].

The tropical climate with high temperature and high relative humidity along with unscientific storage condition adversely affect the preservation of cereal grains and oil seeds which led to the total loss of seed quality [5].

MATERIALS AND METHODS

Study Area

The isolation and control of pathogens was carried out in the laboratory and botanical garden of Department of Plant Sciences, from May to July 2015, ModibboAdama University of Technology Yola. According to [1] Adamawa State is located at the North Eastern part of Nigeria and lies between latitudes 7^0 and 11^0 N of the equator and between longitudes 10^0 and 14^0 E of the Greenwich meridian. It shares boundaries with Taraba State in the south and West, Gombe in its Northern Guinea Savanna ecological zone.The mean annual rainfall ranges from 700mm in the North Western part to 1600mm in the Southern part; the length of the rainy season ranges from 120 – 210 days mostly distributed from May to October [1]. The relative humidity peak of 80% is usually in the months of August and September [6].

Sources of Samples and Sample Size

Groundnut seeds ("Ordaaji") with rot symptoms were randomly collected from the market stalls at Mubi Old Market, MubiKuturu Market and Mubi New Market of Mubi North and South Local Government Areas of Adamawa State. Dry groundnut seeds (healthy and diseased) were also collected in a sterilized polythene bag and taken to the laboratory for laboratory analysis. A total of 240 samples were collected from the three different markets with 80 samples from each market using stratified sampling technique and were labelled as MOM, MKM and MNM according to the markets.

Incidence of Rot of Groundnut Seeds in Storage

Incidence and level of rot of groundnut seeds in storage were made. The samples purchased from the markets were sampled out taking the number of spoiled groundnut seeds out of the total number of groundnut seeds purchased from the market. The incidence of groundnut rot were expressed in the percentage using the formula given by [26].

$$\frac{\text{Spoilage groundnut Seeds}}{\text{Total groundnut seeds}} \text{ X } 100\%$$

Isolation of the pathogens

The method of [31] was used. The diseased tissues (DT) from the periphery of the rotten groundnut seeds were sectioned into $5mm^2$pieces using sterilized scalpel after sterilizing the seeds in 0.1 mercuric chloride solution for 30 seconds and washed in three changes of sterile distilled water. Pieces were picked with sterilized forceps.

The sterilized portions were dried between sterile filter papers. With a cold forceps, a sterilized piece of the seed was then plated out on sterile solidified Potato Dextrose Agar (PDA) and incubated at temperature of 27 $\pm$ 2^0C for 5 – 7 days and constant observation for any growth for sub-culturing. Pure isolates of fungal species was obtained by repeated sub-culturing on solidified sterile Dextrose Agar and pure cultures were preserved in agar slants in McCartney bottles. This was appropriately labelled according to organisms. The slants was initially corked loosely to enable the content fungus to grow and then tightly corked and stored at a temperature range of 0 –

10^0C in a refrigerator to serve as stock cultures.

Identification of isolated fungi

Microscopic examination was made after examining the colony characteristics such as colony colour (front and reverse) and growth pattern on media. A sterile needle was used to take a little portion of the hyphae containing spores on to the glass slide which was stained with Lectophenol cotton blue and observed under the light microscope with power objective lens X 40 for the structures of the fungi [8]. Morphological structures such as septation of mycelia and nature of spores was observed under the microscope and compared with the structures in [3].

Pathogenicity Test

Pathogenicity test was carried out using techniques of [20]. Healthy dry groundnut seed was surface sterilized with 0.1% mercuric chloride solution to remove surface contaminant and rinsed in three changes of sterile distilled water and then dried using Whatman No. 1 filter paper. A sterile razor blade was used to make a 2mm cut on the seed and then culture of the isolates was introduced into the open cut surface and to be replaced with the core and sealed with Vaseline jelly. Seeds were incubated in three replicates in a completely randomised design. Fifteen seeds were inoculated for 5 days. On establishment of disease symptoms, inocula from the infected seeds was taken for each isolate and cultured until pure culture were obtained, the morphological and microscopic characteristics of the isolates was compared with the first isolate.

Preparation of Leaf Extracts

The method of [13] was used to prepare both the aqueous and ethanol extracts. Fresh leaves of *Moringaoleifera* plants were collected from Sengere village, Girei Local Government, Adamawa State. The plant was taken to the Plant Sciences Department of ModibboAdama University of Technology, Yola.

The collected plants were washed thoroughly under running tap water and allowed to air dry under shade for 7 days. These were ground andthirty grams of the sample was added to 150ml of distilled water in separate conical flasks. This was vigorously shaken and left to stand for 24 hours. The sample was filtered with three layers cheese cloth. The aqueous filtrate was used at 60, 40, and 20 per concentrations. The same procedure was used for 60, 40 and 20 per ethanol extracts.

Effect of extracts on fungal mycelia growth

The approach of [13] was used to evaluate the effect of the extracts on fungal growth by creating four equal sections on each plate by drawing two perpendicular lines at the bottom of the plates. The point of interception indicates the centre of the plates. This was done before dispensing PDA into each of the plates. The extracts were poured into the flask plug with cotton wool and kept at room temperature [15]. About 2ml of extracts of *Moringaoleifera* was separately introduced into the Petri-dish containing the media and pure isolates (poisoned food method). Control experiment was without addition of any plant extract but sterile distilled water. Disc was used to cutfrom 7 day old culture of fungi using cork borer and was incubated at room temperature for 4 days in which radial growth of the fungi were taken daily, starting from the 2nd day of inoculation[35].

Phytochemical Analysis (Qualitative phytochemical analysis)

The leaf extracts of plant used (*Moringaoleifera*) were subject to phytochemical analysis in the Biochemistry Laboratory of ModibboAdama University of Technology Yola in which the chemical composition of these extracts were determined. This was done to know the active component present in the leaf extracts. The qualitative phytochemical screening of samples was carried out as described by [12], [27], [19] and[25]. The leaf extracts was screened for alkaloids, flavonoids, steroid, phenols, tannins, saponin, glycosides, anthraquinine and reducing sugar.

Experimental Design and Data Analysis

The experimental layout was completely randomized design testing two plant extracts. Each plant extract was at one concentration. The experiment was replicated three times. All the data was analysed using one-way and two-way analysis of variance (ANOVA) according to [10]. Least Significant Difference (LSD) according to [23] was used to separate the means where there was significant difference. The statistical package used to analyse the result was Statistical Analysis Software (SAS) version 7.

RESULTS

Incidence of Ground nut Rot in Mubi

Rot of groundnut seed was observed in all locations. The incidence of rot showed that MubiKuturu Market with the highest percentage of infected samples (23%), followed by Mubi New Market with 20% and Mubi Old Market with the lowest (17%), and the average rot was 20% (Table 1).

Identification and Description of Fungi

Four fungi were isolated from groundnut seed rot and identified as *Aspergillusniger, Fusariumsolani, Rhizopusstolonifer and Aspergillusflavus* (Plate 1-4). Pathogenicity test showed that the four isolates were pathogenic to groundnut seeds. *Aspergillusniger* conidiophores hyaline or pale brown, erect, simple, thick-walled, with foot cells basally, inflated at the apex forming globose vesicles, bearing conidial heads split into over 4 loose conidial columns with over 4 fragment apically, composed of catenulate conidia (over 15 condia/chain) borne on uniseriate or biseriatephialides on pale brown, globose vesicles and phialides acutely tapered at apex. Conidia phialosporous, brown, black in mass, globose, minutely echinulate (Plate 1).
Fusariumsolani produces spores to abundant, white cream mycelium. Macroconidia have 3 to 4 septa on average are slightly curved, are rather wide and thick-walled, and may have slightly blunted apical end. Microconidia are abundant, oval to kidney shaped, and formed in false heads on very long monophialideschlamydospores are abundant (Plate 2).
*Rhizopusstolonifer*sporangiosphores erect, simple or branched, yellowish to dark brown, rhizoidal, connected directly to sporangiosphores, bearing sporangia terminally. Sporangia globose, dark brown to black, minutely spiny, apparently subglobose after maturity, columellate on dehiscence; columellaeglobose, brown.Sporangiasphoressubglobose to subbellipsoidal, pale brown, with bluish stripes (lines) (Plate 3).*Aspergillusflavus*conidiosphores hyaline, simple, short, bearing single conidia apically. Conidia aleuriosporous, brown, ovate or ellipsoidal, muriform, usually composed of 3 transverse septa and 1-2 longitudinal septa, smooth marginally, constricted at or near cross septa (Plate 4).

Table 1: Incidence of Rot of Groundnut in Mubi Markets

Source of Samples	Incidence of Rot (%)
Mubi New Market	20
Mubi Old Market	17
MubiKuturu Market	23
Mean	20

Incidence of Groundnut Rot Disease in Mubi

The frequency of fungi isolated from Mubi New Market showed *Rhizopusstolonifer*had the highest frequency of occurrence (60%), followed by *Aspergillus niger* with 26.67% while *Fusariumsolani and Aspergillusflavus*had the least frequency of 6.67% each. The highest frequency of occurrence in Mubi Old Market was *Rhizopusstolonifer*with 75%, followed by *Aspergillus flavus*(25.00%) and there was no occurrence of *Aspergillusniger*and *Fusariumsolani.* In MubiKuturu Market, *Rhizopusstolonifer*had the highest

frequency of occurrence (66.67%), followed by *Aspergillusniger*with 20% and there was no occurrence of *Fusariumsolani.* The overall total showed *Rhizopusstolonifer*with the highest frequency of occurrence (66.67%), followed by *Aspergillusniger*with 16.67%, *Aspergillusflavus*had 14.29% and the least was *Fusariumsolani*with 2.38% (Table 2).

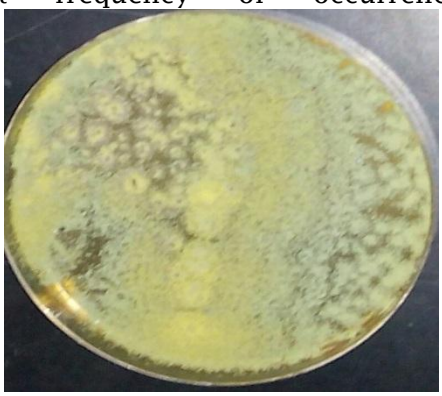

Plate I: Pure culture of *Aspergillusniger*

Plate II: Pure culture of *Fusariumsolani*

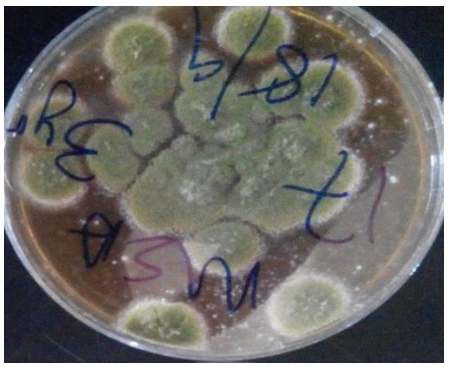

Plate III: Pure culture of *Rhizopusstolonifer* Plate IV: Pure culture of *Aspergillusflavus*Table

2: Frequency of Fungi Isolated from Groundnut Seed in Mubi

Pathogen	Frequency of Pathogens (%)			Average
	MNM	MOM	MKM	
Aspergillusniger	26.67	--	20.00	15.56
Fusariumsolani	6.67	--	--	2.22
Rhizopusstolonifer	60.00	75.00	66.67	67.22
Aspergillusflavus	6.67	25.00	13.33	15.00
Total	100	100	100	100

Key:

MNM= Mubi New Market

MOM= Mubi Old Market

MKM= MubiKuturu Market

Effect of Plant Extract on Fungi Mycelial Growth

Analysis of variance for the *in vitro* control of the pathogens using aqueous and ethanol leaf extracts of drum stick shows that there was a significant difference between mycelial growth of the treatments (extract had lower mycelial growth compared to control at p=0.0001). The effect of concentration of the aqueous extract on mycelial growth of fungal isolates showed significant differences at p=0.0001. As the level of concentration of aqueous extracts increases, the level of growth inhibition also increased. There was a minimum level of inhibition on *Aspergillusniger* at 20% (3.60mm) while, at 60% showed maximum level of inhibition (1.06mm) as compared with control (72.14). Treatment of*Rhizopus*at 20ml had the mean of 2.81mm while at 60% had 0.91mm as compared with control (72.43mm). On *Fusariumsolani*, at 20% had the mean of 1.88mm, while at 60% had 0.93mm as compared to control (16.21mm). In the case of *Aspergillusflavus,* at 20% had mean of 1.99mm, while at 60% had 1.13mm as compared with control (18.40mm) (Table 3).

The mean effect of concentration of the ethanol extracts on mycelial growth of fungal isolates showed significant differences at p=0.0001. As the level of concentration with ethanol increases, the level of inhibition also increased. There was a minimum level of growth inhibition on *Aspergillusniger* at 20% (1.60mm) while, at 60% showed maximum level of inhibition (1.20mm) as compared with control (66.43mm).Treatment with *Rhizopus* at 20% had the mean growth of 3.75mm while at 60% had 2.05mm as compared with control (72.43mm). On *Fusariumsolani*, at 20% had the mean growth of 3.41mm, while at 60% had 1.80mm as compared to control (16.43mm). In the case of *Aspergillusflavus*, at 20% had mean growth of 3.60mm, while at 60% had 0.85mm as compared with control (17.29mm) (Table 4).The treatment of fungi mycelial growth with plant material (*Moringaoleifera*) supressed the growth of pathogens at different

concentration. As the level of concentration with Moringa increases, then the level of inhibition also increases on *Aspergillusniger* and *Fusariumsolani*. There was a minimum level of inhibition on *Aspergillusnige*r at 20% (1.81mm) while at 60% showed maximum level of inhibition (0.71mm) as compared with control(69.29mm).Control of *Rhizopus* at 20% had the mean growth of 1.20mm while at 60% had 0.79mm as compared with control (74.43mm).

Table 3: Mean Effect of Concentration of Aqueous Leaf Extracts on the Mycelial Growth of Pathogens

Concentration (%)	Mean effect (mm)			
	Aspergillus niger	*Fusarium solani*	*Rhizopus stolonifer*	*Aspergillus flavus*
20	3.60	1.88	2.81	1.99
40	3.13	1.79	1.29	1.45
60	1.06	0.93	0.91	1.13
Control	72.14	16.21	72.43	18.40
LSD (p=0.0001)	2.39	0.60	2.36	0.46

Table 4: Mean Effect of concentration of Ethanol Leaf Extracts on the Mycelial Growth of Pathogens

Concentration (%)	Mean effect (mm)			
	Aspergillus niger	*Fusarium solani*	*Rhizopus stolonifer*	*Aspergillus flavus*
20	1.60	3.41	3.75	3.60
40	1.37	2.33	3.35	1.05
60	1.20	1.80	2.05	0.83
Control	66.43	16.43	72.43	17.29
LSD (p=0.0001)	2.39	0.60	2.36	0.46

On *Fusariumsolani* at 20% had the mean growth of 1.43mm, while at 60% had 0.75mm as compared to control (16.32mm) in the case of *Aspergillusflavus* at 20% had the mean of 1.08mm, while at 60% had 0.52mm as compared with control (17.84mm) (Table 5).

The interaction of fungi mycelial growth with leaf extracts and solvent (aqueous) minimized the growth of pathogens. The mean effect of *Moringaoleifera*with aqueous on *Aspergillusniger* is 2.14mm and with neem is 3.05mm in relation with the control 72.14mm. In terms of *Fusariumsolani*, *Moringaoleifera* with

aqueous, the mean is 1.65mm while neem with aqueous is 1.42mm as in line with control 16.21mm. As of *Rhizopusstolonifer*, *Moringaoleifera*with aqueous, the mean is 1.26mm and that of neem is 2.07mm compared with control (72.43mm). Aspergillusflavus, *Moringaoleifera* with aqueous, is 1.41mm and neem is 1.64mm compared with control (18.40mm) (Table 6).

The interaction of fungi mycelial growth with leaf extracts and solvent (ethanol) minimized the growth of pathogens. The mean effect of *Moringaoleifera*with ethanol on *Aspergillusniger* is 0.24mm and the control 66.43mm. In terms of *Fusariumsolani*, *Moringaoleifera*with ethanol, the mean is 0.50mm while the control is 16.43mm. As of *Rhizopusstolonifer*, *Moringaoleifera* with ethanol, the mean is 0.87mm and that of neem is 5.23mm compared with control (72.43mm).

Aspergillusflavus,*Moringaoleifera* with ethanol, is 0.33mm and neem is 3.32mm compared with control (17.29mm) (Table 6). Results of the present investigation show that the growths of fungi were inhibited with the crude aqueous and more with ethanolicleaf extracts of *Moringaoleifera*.

From the result, it is evident that the inhibition of growth of fungi was more pronounced with ethanolic leaf extracts on *Aspergillusniger* as compared to aqueous leaf extracts, while *Fusariumsolani, Rhizopusstolonifer*and *Aspergillusflavus* is vice-versa with the solvent.Significant inhibition of growth observed in the artificial culture media. In all concentration of the aqueous and ethanolic leaf extracts, it was observed that the higher the concentration of leaf extracts, the more effective on growth inhibition of fungi exhibit and it was also noted that from early period of incubation inhibition of growth occurred.

Table 5: Mean Effect of Concentration of *Moringaoleifera* on the Mycelial Growth of the Fungal Isolates

Concentration (%)	Mean effect (mm)			
	Aspergillus niger	*Fusarium solani*	*Rhizopus stolonifer*	*Aspergillus flavus*
20	1.81	1.43	1.20	1.08
40	1.05	1.05	1.20	1.00
60	0.71	0.75	0.79	0.52
Control	69.29	16.32	72.43	17.84
LSD (p=0.0001)	2.39	0.60	2.36	0.46

Table 6: Mean Effect of *Moringaoleifera* Leaf Extracts (Aqueous and ethanolic) on the Mycelial Growth of Fungal Isolates

Pathogens	Mycelial growth (mm)	
	Aqueous	Ethanol
Aspergillusniger	2.14	0.24
Fusariumsolani	1.65	0.50
Rhizopusstolonifer	1.26	0.87
Aspergillusflavus	1.41	0.33
Control	44.90	44.90
LSD (p=0.0001)	1.26	1.26

The Phytochemical Characteristics of the plants investigated

The phytochemical characteristics of the two plants used are summarised in Table 10. The results reveal the presence of chemically active components in the plant studied. From table 7, alkaloids, glycoside, flavonoids and phenols were present in the plant. However Anthraquinone and reducing sugars are absent in *MoringaOleifera* aqueous extracts respectively.

Table 7: Qualitative Determination of Phytochemical Groups of Aqueous Extract of Test Plant Leaves

Phytochemicals	*Moringa oleifera* Leaf Extracts
Alkaloids	+
Flavonoids	+
Glycoside	+
Phenols	+
Saponins	+
Steriods	+
Tannins	+
Anthraquinine	–
Reducing sugar	–

Key
– Absent
+ Present

DISSCUSSION

Fungi are major pathogens that cause post-harvest rot of groundnut. The incidence of rot showedMubiKuturu Market with the highest percentage of infected samples (23 %), followed by Mubi New Market with 20 % and Mubi Old Market with the lowest (17 %), and the total percentage of infected sample is 20 % (Table 1). [2] reported that groundnut stored in different storage facilities are susceptible to attack by fungi, insects and other micro-organism under favourable conditions.The difference in storage potential of seeds at different locations may be due to the difference in initial seed quality under different environmental conditions and invasion of fungi [17].

Four fungi were isolated from groundnut and identified as *Aspergillusniger*, *Fusariumsolani*, *Rhizopusstolonifer* and *Aspergillusflavus* and pathogenicity test showed that the four isolates were pathogenic to groundnut. This result was also in correspondent with the result obtained by [24] who found 28 species of fungi to be associated with groundnut rot in India. [33] isolated nine species of fungi from seeds of different groundnut during storage. [7] found that the species of *Aspergillus, Penicillium, Fusarium, Rhizopus* and *Alternaria* were commonly occurring post-harvest moulds in storage condition. According to [30], groundnut seeds are highly susceptible to disease because they are rich in nutrients useful for numerous fungi such as *Rhizopus*spp, *Penicillium*spp, *Aspergillusniger* and *Aspergillusflavus*. The frequency of fungi isolated from Mubi New market showed *Rhizopusstolonifer* had the highest frequency of occurrence (60%), followed by *Aspergillusniger* with 26.67% while*Fusariumsolani*and *Aspergillusflavus* had the least frequency of 6.67% each (Table 2).

The results exhibit the radial growth of fungibeing inhibited *in vitro* by aqueous and ethanolic leaf extracts of *Moringaoleifera.Moringaoleifera*exhibits antifungal properties justify their traditional use as medicinal plants. This may be due to synergistic effect of several compounds that are in various proportions in a *M. oleifera*which constitute an important source of microbicides, pesticides and many pharmaceutical drugs such as saponin, steroids, tannin, glycosides, alkaloids and flavonoids in the extracts [34]. The aqueous leaf extract was more effective than the ethanol extracts of neem. The level of inhibition was determined by the level of concentration. As the concentration increase, so also the level of inhibition increased. [36] reported leaf extracts of *M. oleifera* had antifungal activity on *Aspergillusniger* and *Aspergillusflavus* isolated from two different mango species in Yola North. [18] reportedantifungal activity of crude leaves extracts of *Moringaoleifera* Lam. on *Trichophytonrubrum* and *Microsporiumcanis*, fungi causing superficial infection on human body. The compound anthronine found in the roots, barks and leaves of the plant is said to be highly toxic on many strains of bacteria [21]. In the late 1940's, and the early 1950'5, a team from University of Bombary, Travan University and the Department of Biochemistry at the India Institute of Science in Bangalore identified a compound called Pterygospermin from *Moringaoleifera*which they reported readily dissociated into two molecules of benzyl isothiocyanate and was clearly understood to have anti-microbial properties [14]. The antibiotic principles are actually the defensive mechanism of the plants against different pathogens [11]. [29] reported that drum stick as medicinal plants, possess potential anti-fungal properties which inhabit fungal mycelila growth of *Aspergillusviridae and Peniciliumdiigitatu*m.

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