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ANTIFUNGAL ACTIVITIES OF SEED EXTRACTS OF MAHOGANY ON CARICA PAPAYA (PAWPAW) FRUIT ROT IN MUBI, ADAMAWA STATE

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ABSTRACT

Pawpaw (*Carica papaya* L.) fruit production has become an integral part of daily life as a source of food, vitamins and minerals which is one of the most popular, cheapest, economically important fruit tree grown in Nigeria. The study of the fungi associated with postharvest rot of *Carica papaya* fruit were carried out in three different markets of Mubi North and South Local Government Area of Adamawa State (Kuturu Market, Mubi Main Market and Mubi New Market) from June to November, 2016 to test for both invitro and invivo antifungal efficacy of seed extracts of *Khaya senegalensis*. Sample of papaya fruits were collected, and industrial PDA was used for the isolation of the fungi pathogens. Four fungal pathogens were isolated from papaya fruits and were identified as *Rhizopus stolonifer*, *Botryodiplodia theobromea*, *Aspergillus niger* and *Mucor* spp. Control trials using seed extracts of Khaya senegalensis was conducted at six different concentration levels (0%, 20%, 40%, 60%, 80% and 100%). Incidence of rot showed that Kuturu Market had the highest with 26.77%, followed by Mubi Main Market (20.77) and then Mubi New Market (20.20). Results obtained from the control trials with seed extracts of *Khaya senegalensis* both invitro and invivo showed significant differences ($p>0.001$) and was effective in inhibiting fungal growth although with variation in their effect.

KEYWORDS: Khaya senegalensis, invitro, invivo, Efficacy, Seed extract, Fungal Pathogens.

INTRODUCTION

In Africa, papaya (*Carica papaya* L.) fruit production has become an integral part of daily life as a source of food, vitamins and minerals.[5] It serves as a means of trade and barter, food security and employment, in Nigeria, pawpaw is one of the most popular, cheapest, economically important fruit tree grown and consumed for its nutritional content.[6] It is usually grown as compound fruit crop or semi-wild fruit crop from discarded seeds.[8] The fruit consists of water, vitamins A and C, protein and ash.[5] The fruit can be freshly eaten or cooked and can be used in the preparation of jellies, juice and jams.[21] Papaya has a mild laxatives axtion and the seeds are used medicinally against worms and ulcer.[5] The green leaves, fruits and flowers may be used as cooked vegetables.[12]

Papaya also has several industrial uses due to the presence of the enzymes called papain used in the production of chewing gums, tenderizing meat and drug preparation for various digestive ailments and for the treatment of gangrenous wounds as well as in textile industry an cosmetics.[19] The seeds are used to expel worms and the flowers may be taken in an infusion to induce menstruation.[23] The increasing interest in medicinal herbs has increased scientific scrutiny of their medicinal potential and safety thereby providing physicians with data to help patients make use of them wisely.[24]

Papaya fruit are beset with both field and storage rot problems.[17] identified 22 different fungi in post-harvest decay of papaya fruits which include *Aspergillus niger, Aspergillus flavus, Rhizopus oryzea, Fusarium moniliforme*. Bacterial pathogens involved with the rot of papaya include the species of soft-rotting *Erwinia, Pseudomonas, Xanthomonas, Cytophaga, and Bacillus.*[15] These microorganisms under the influence of environmental factors (temperature, pH, oxygen, moisture content) are serious threat to papaya fruit production. Besides the losses to marketers the rotten fruit also causes a serious health hazards to consumers.[20] had earlier reported that most of microbes infecting plants tissues often produced secondary metabolites in their host, which are known to be hazardous to animals including man. Fumonisin on maize by *Fusarium* spp, aflatoxins and ochratoxins on several plants produced by *Aspergillus* spp.[25] Nigeria is the third largest producer of papaya in the world with 703,800 metric tons.[14]

MATERIALS AND METHODS

Study Area and Sample Collection

The isolation, identification and pathogenicity as well as control trial was carried out in Plant Science laboratory of Modibbo Adama University of Technology Yola from August to October of 2016. Mubi Local Government is found in the Northern Senatorial Zone of Adamawa State. The region experience rainfall from April to October with its peak between August and September.[29] Mubi lies between latitude 10°-15m and 10°-16m North and longitude 13º-14m and 10°-16mEast.[11] The climate of the area is tropical with average temperature of 32°C and a relative humidity ranging from 15% to 68%.[9] Mean annual rainfall is about 1056mm.[3]

Papaya with disease symptoms such as dark brown to black decay spots, malformation of vegetative and floral tissues coated and penetrated deep into the fruits was collected from three different markets in Mubi. A sample of papaya fruit was collected at random. A total of 45 samples was collected from the three different Markets (15 samples from each market) in a sterile polythene bag. The Markets are: Mubi Old Market, Kasuwan Kutoru Market and Mubi New Market.

Determination of Disease Incidence and Disease Severity

Papaya Samples were collected at random from the three markets selected, in which 5 samples were collected from 3 different traders at different location in the market. The incidence of papaya fruit rot in the Market was determined by counting the infected fruits from the samples collected from each market.

$$\frac{\text{Spoilage papaya fruits}}{\text{Total papaya fruits}} \quad \text{X } 100\%$$

Severity of fruit rot was assessed and scored according to modified[13] visual scale of 1-5 in which

1. 1- 20% of papaya fruit infected,
2. 21-40% of papaya fruit infected,
3. 41-60% of papaya fruit infected,
4. 61-80% of papaya fruit infected,
5. More than 80% of papaya fruit infected.

The reactions of the isolates based on the 1-5 visual scale were grouped in the following categories based on the[26] pathogenic potential of grouping of isolates

1-20% - Low virulent group

21-40% - Moderately virulent group

41-60% - High virulent group

61-80% - Very high virulent group

Above 80% - Totally virulent group

Isolation and Identification of Pathogens

The peripheral surface of the diseased papaya tissues were cut and sterilized in 0.1% mercury chloride solution for 30 seconds.[10,30] They were rinsed in three changes of sterile distilled water blotted dried between whatman filter papers and were plated out on PDA to which they were sub-cultured on to a new set of PDA plates. The sub-cultures were incubated for 5-7 days and sub-cultured immediately until a pure culture of the isolates are obtained and stored in McCartney bottles for further use.[30] The isolates were identified by observing their colonial morphology and microscopic characteristics and were compared with structures of the different species of fungi as shown by.[28] This was replicated three (3) times for each of the isolates.

Preparation of Aqueous Seed Extracts of *Khaya senegalensis*

Seeds of *Khaya senegalensis* were collected from Michika, Adamawa State. The seed of *Khaya* were picked and peeled before rinsing it with distilled water then the rinsed seeds were placed on an aluminium foil and milled using mortar and pestle into pulverized mashed form. 10g of each pulverized mashed was soaked in 100mls of cool distilled water for 24 hours. The suspensions were filtered using sterile chesses cloths. The extracts was then poured into a clean sterile conical flasks plugged with cotton and was heated at 100^0C for 10 minutes to avoid contamination. These was allowed to cool, wrapped in aluminium foil and kept until when required.[18]

Determination of the Effect of Aqueous Seed Extracts on Radial Growth of pathogens *In vitro*

One millilitre portion of the seed extracts (100%) and a serial dilution of the extracts at 20%, 40%, 60%, 80% and 100% concentrations were separately dispensed into test tubes. The extracts of different concentrations were poured into Petri dishes and the PDA was poured into the dishes containing the extracts which was allowed to cool and solidified.[30]

The control contains sterile distilled water in place of the seed extracts, three (3) replicates were used for each pathogen with the extract concentration and control. Disc was used to cut from 7 day old culture of fungi using cork borer and was incubated at room temperature for 7 days in which radial growth of the fungi was taken daily, starting from the 2nd day of inoculation. These were replicated three (3) times for each of the four fungal isolates.

In vivo

Fruits were washed and surface-sterilized by dipping in the 0.1% mercuric chloride solution for 30 seconds then washed three (3) times with sterilized distilled water and allowed to dry. Fruits (three replicates for each fungus, each replicate containing 3 fruits) were wounded with sterile cork borer, a 5.0 mm diameter holes were Made on each papaya fruit with cork borer. A disc of each fungus culture (4.0 mm diameter) were soaked for 30 seconds in 1ml of plant extract in sterile Petri-dish; and immediately introduced into the hole, using a sterile mounting needle and forceps; the tissue previously removed from the hole were replaced after about 2.0 mm had been cut off to compensate for the thickness of the inocula. The points of inoculation were sealed with Vaseline and the inoculated mango fruits were incubated on clean laboratory table for 5 days at room temperature (25±3oC). Data from lesion size of pathogens were measured using ruler (mm) to see the effect of the leaf extract on the fungal pathogens. Data was analysed for effect of extract on the rot.

Seed of *Khaya senegalensis*

Diseased Fruits of Papaya

RESULTS

Identification, incidence and virulence of pathogens

The isolated fungi from *Carica papaya* fruits were identified as *Botryodiplodia theobromae, Mucor, Rhizopus stolonifer* and *Aspergillus niger* (Plates 1-4). These were identified based on their colonial and morphological characteristics. All the four isolated fungi were found pathogenic (virulent) on papaya fruit during pathogenicity test.

From Table 1 Mubi new market has the highest average percentage occurrence of rotted papaya with 26.30% followed by Kuturo market with 20.83% and then lastly Mubi Main Market with 20.50%. *Botryodiplodia theobromae* has the highest occurrence in Mubi Main Market with 46.67% followed by *Rhizopus stolonifer* with 40.00% followed by *Aspergillus niger* then lastly by *Mucor* with 26.67%. *Rhizopus stolonifer* occur highest in Mubi New Market with 53.33% followed by *Aspergillus niger* with 46.67%followed by *Botryodiplodia theobromae* then lastly followed by *Mucor* with 20.00%. In Kuturu Market *Mucor* has the highest percentage with 26.67% followed by *Botryodiplodia theobromae* and *Rhizopus stolonifer* with 20.00% and 20.00% respectively then lastly by *Aspergillus niger* with 13.33% as shown in Table 2.

Table 1: Percentage occurrence of Disease Fruits (*Carica papaya*) from the Selected markets in the study area.

Markets	Number of fruits	Number of diseased fruit	% disease fruits
MMM	83	17	20.50
KM	72	15	20.83
MNM	80	21	26.30
Total	235	53	67.63

KEY

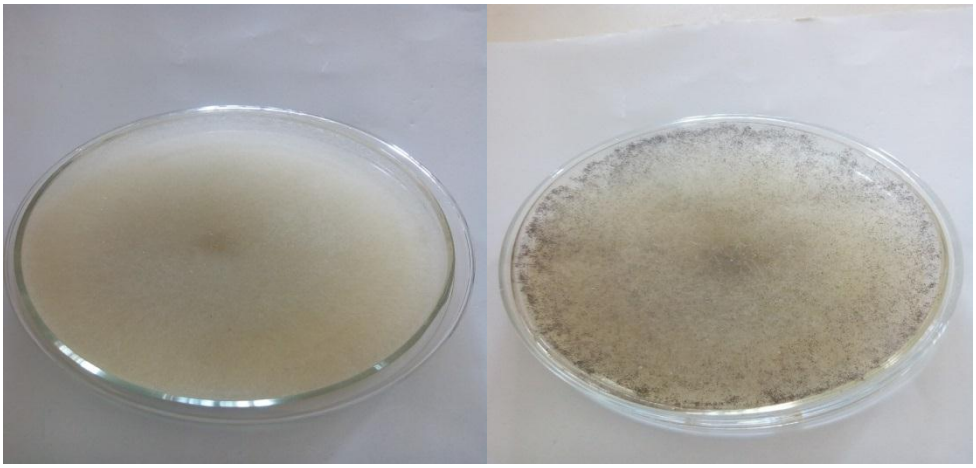
MMM: Mubi Main Market

KM: Kuturo Market

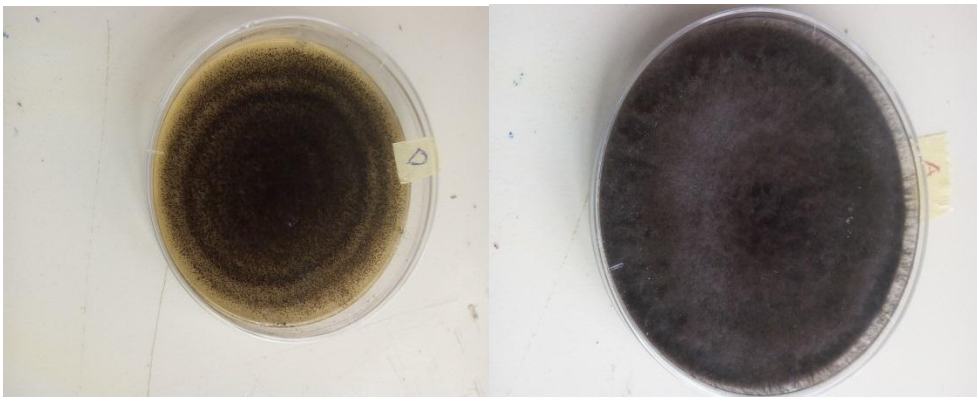
MNM: Mubi New Market

Table 2: Occurrence (%) of Pathogens of papaya Fruit in Mubi Markets.

Markets *theobromae*	*Botryodiplodia*	*Mucor*	*Rhizopus stolonifer*	*Aspergillus niger*
Mubi main market	46.67	26.67	40.00	33.33
Mubi new market	33.33	20.00	53.33	46.67
Kuturo market	20.00	26.67	20.00	13.33
Average	33.33	24.45	37.78	31.11



Mucor circinelloides **Rhizopus stolonifer**



Aspergillus niger **Botryodiplodia thoebromea**

In vivo control of fungal pathogens using seed extracts of *Khaya senegalensis*

Effect of Seed extracts of Khaya seneglansis on the mycelial growth of pathogens

Tests carried out on the efficacy of the aqueous seed extracts of *Khaya seneglansis in vitro* showed a significant difference with the control (3.20) at P=0.0001 as compared with

2.35mm, 2.35mm, 2.00mm and 1.45mm (for *Botryodiplodia theobromae, Mucor, Rhizopus stolonifer* and *Aspergillus niger*) respectively. At organism level there was also a significant difference at p= 0.0001 in which seed extracts of *Khaya seneglansis* was found to inhibit the growth of all four organisms but was more effective on mycelial growth of *Aspergillus niger* followed by *Rhizopus stolonifer* and lastly *Botryodiplodia theobromae* and *Mucor* with 1.45mm, 2.00mm, 2.35mm and 2.35mm respectively as shown in Table 4.

In vivo analysis showed a significant difference with the control (4.26mm) at P=0.0001 and 3.14mm, 0.94mm, 1.86mm and 0.64mm for *Botryodiplodia theobromae, Mucor, Rhizopus stolonifer* and *Aspergillus niger* respectively. Amongst the four pathogens at P=0.0001 the seeds extracts of *Khaya seneglansis* was found to be more effective in inhibiting the mycelial growth of *Aspergillus niger* witth 0.64mm followed by *Mucor* (0.94mm) then followed by *Rhizopus stolonifer* (1.86mm) and was less effective to inhibit the mycelial growth of *Botryodiplodia theobromae* (3.14mm) as shown in Table 5.

In vitro control showed that all the concentration used in the test were found to be effective in inhibiting the mycelial growth of the four pathogens at significant difference (P= 0.0001) to the control 100% concentration was found to more effective in inhibiting the mycelial growth of *Botryodiplodia theobromae* (1.43mm) followed by 80% with 1.77mm and there was no significant difference between the effect of 20% and 40% concentration with 2.79mm and 2.71mm respectively. In inhibiting the mycelial growth of *Mucor* the extracts was found to be more effective at 100% concentration with 1.43mm as against 80% and 60% concentration with1.95mm and 2.11mm respectively, 20% and 40% recorded least effect on the mycelial growth with 2.55mm and 2.72mm respectively with no significant difference. All the concentrations of the extract were also found to control the mycelial growth of *Rhizopus stolonifer* but there was no significant difference between 2.29mm (20%) and 1.90mm (40%) and 1.23mm (60%), 0.94mm (80%) and 0.62mm (100%) which was recorded to be more effective with no significant difference between the effects of the concentrations, against the mycelial growth of *Aspergillus niger* 1.76mm (20%), 1.65mm (40%), 1.29mm (60%), 0.91mm (80%) and 0.68mm (100%) with 80% and 1005 found to be more effective in inhibiting the mycelial growth of the pathogen as compared with the control at significant difference (P= 0.0001) as shown on table 8.

In vivo test showed that all concentrations of the extract were found effective on all pathogens as compared to control at significant difference (P= 0.0001), 4.04mm (20%),

3.51mm (40%), 3.01mm (60%), 2.08mm (80%) and 1.93mm (100%) with 80% and 100% found to be more effective on *Botryodiplodia theobromae*, 1.09mm (20%), 0.89mm (40%), 0.77mm (60%), 0.64mm (80%) and 0.51mm (100%) with no significant difference between the effect of all concentrations on the mycelial growth of *Mucur*. On *Rhizopus stolonifer* 20% has 2.18mm, 40% has 1.96mm effects on the mycelial growth with no significant difference between the two concentrations while 60% (1.57mm), 80% (1.35mm) and 100% (1.19mm) were found to be more effective in inhibiting the growth of the pathogen as compared to the control at significant difference (P= 0.0001). While the concentrations were also found to be effective on *Aspergillus niger* 100% (0.38mm), 80% (0.46mm) and 60% (0.53mm) were found to be more effective as compared to 20% (0.76mm) and 40% (0.67mm) which are considered less effective compared to the control (table 9).

Table 3: Mean effect of aqueous seed extracts of *Khaya Senegalensis* on growth of the pathogens (mm) *In vitro.*

Fungi	*Botryodiplodia theobromae*	*Mucor stolonifer*	*Rhizopus*	*Aspergillusniger*
Seeds Extracts	2.35	2.35	2.00	1.45
Control	3.20	3.32	3.09	2.39
LSD (0.0001)	0.15	0.15	0.36	0.32

Table 4: Mean effect of aqueous seed extracts of *Khaya Senegalensis* on growth of the pathogens (mm) *in vivo.*

Fungi	*Botryodiplodia theobromae*	*Mucor*	*Rhizopus stolonifer*	*Aspergillusniger*
Seeds Extracts	3.14	0.94	1.86	0.64
Control	4.26	1.77	2.91	1.06
LSD (0.0001)	0.29	0.13	0.05	0.13

Table 5: Mean effect of concentrations of aqueous seed extracts of *Khaya senegalensis* on the mycelial growth of the pathogens (mm) *in vitro.*

Concentrations (%) Fungi	*Botryodiplodia theobromae*	*Mucor*	*Rhizopus stolonifer*	*Aspergillusniger*
20	2.79	2.72	2.29	1.76
40	2.71	2.55	1.90	1.65
60	2.21	2.11	1.23	1.29
80	1.77	1.95	0.94	0.91
100	1.43	1.43	0.62	0.68
Control (0)	3.20	3.32	3.09	2.39
LSD (0.0001)	0.25	0.25	0.63	0.23

Table 6: Mean effect of concentrations of aqueous seed extracts of *Khaya senegalensis* on the mycelial growth of the pathogens (mm) *in vivo*.

Concentrations (%) Fungi	*Botryodiplodia theobromae*	*Mucor*	*Rhizopus stolonifer*	*Aspergillus niger*
20	4.04	1.09	2.18	0.46
40	3.51	0.89	0.76	1.19
60	3.01	0.77	1.96	0.38
80	2.8	0.64	0.67	2.91
100	1.93	0.51	1.57	1.06
Control (0)	4.26	1.77	0.53	0.22
LSD (0.0001)	0.51	0.22	1.36	0.09

DISCUSSION

The study showed that a variety of different fungi are associated with post-harvest rot diseases of pwapwa fruit in the study area (Mubi, Adamawa State). Survery in Mubi in 2016 shows that rot occurred in all locations. Mubi new market has the highest average percentage occurrence of rotted papaya with 26.30% followed by Kuturo market with 20.83% and then lastly Mubi main market with 20.50%. Four fungal pathogens were isolated from *Carica papaya* and were confirmed to be pathogens through pathogenicity test. Theses pathogens are; *Botryodiplodia theobromae, Mucor*, *Rhizopus stolonifer* and *Aspergillus niger* which is in agreement with the works of[10] who reported *Botryodiplodia theobromae* and *Rhizopus stolonifer* as pathogens responsible for the rot of papaya fruit in Ibadan. Abdu, 2016 reported *Mucor* spp and *Aspergillus niger* as the fungal pathogens responsible for the fruit rot of *Carica papaya* in Girei and Yola South However, based on organisms, *Botryodiplodia theobromae* had the highest occurrence in Mubi main market (46.67%), *Rhizopus stolonifer* occurred highest in Mubi new market (53.33%) while in Kuturo market, *Mucor* spp occurred highest with 26.67%.

The aqueous seed extracts of *Khaya senegalensis* were tested for antifungal activity against the four (4) isolates from papaya fruits (Botryodiplodia theobromae, Mucor, Rhizopus stolonifer and Aspergillus niger). It was showed that aqueous seed extracts of Khaya senegalensis showed some degree of antifungal activities in all isolated organisms tested. Mahogany products have been reported to possess antifungal properties[1,4,16] found out that the active component in the seed extract of Khaya senegalensis is Khivorin.[7] and[27] reported the seed oil and the powder of the fruit of mahogany (*Khaya senegalensis*) as having some compound the can serve as fumigants and contact insecticides for the control of *Callosobruchus maculatus* and stored product insect.

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