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**ANTI-FUNGAL ACTIVITIES OF AQUEOUS LEAF EXTRACTS OF
MORINGA OLEIFERA LAM. ON *MANGIFERA INDICA* L. POST-
HARVEST FRUIT-ROT PATHOGENS FROM SOME MARKETS IN
YOLA NORTH, ADAMAWA STATE**

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ABSTRACT

Studies on the anti-fungal effect of *Moringa oleifera* Lam leaf extract were determined on causative agents of mango post-harvest fruit rot. A total of seventy two (72) samples belonging to two different varieties ('Bush' and kent) were collected from three different Markets in Yola North of Adamawa State. Different concentration of drum stick leaf extract (20, 40 and 60%) was used. The treatments were laid out in a completely randomized design (CRD) with three replications. *Botrytis cinera*, *Aspergillus flavus* and *Aspergillus niger* were isolated and identified to be responsible for the mango rots. Percentage disease incidence in the markets shows that Jimeta Modern Market had the

highest percentage incidence with 36.82% and the least incidence recorded was in Jimeta Old Market with 27.93%. All concentrations of the tested leaf extract of *Moringa oleifera* significantly ($P < 0.05$) suppressed mycelial growth of the fungi *in-vitro* and fungal rots *in-vivo*. The effect was proportional to the concentration of the leaf extract and suppression was highest at 60% and lowest at 20% concentration. The study revealed that leaf extract of *Moringa oleifera* proved to be effective in the control of post-harvest rots of mango and serves as a good option to synthetic agro-chemicals which are not eco-friendly and biologically safe. Phytochemical study revealed that alkaloids, flavonoids, steroid, phenols, tannins, saponin and glycosides were present in aqueous leaf extracts of *Mangifera indica* while anthraquinone and reducing sugar were absent.

KEYWORDS: Anti-fungal, Post-harvest, fruit-rot, *Mangifera indica*, Leaf extracts.

INTRODUCTION

Fruits of tropical and subtropical regions are appreciated for their nutritional value.^[30] Mango fruits are very nutritious, the peels and seed contained various health-enhancing substances, such as phenolic compounds, carotenoids, vitamin C and dietary fibre.^[3,4,8,24,42] Currently, mango is grown in Africa, Central America, Australia and for a few years in Europe.^[12,23,38,37]

Mango fruit belongs to genus *Mangifera* which consists of numerous species of tropical fruits in the family of Anacardiaceae.^[25] *Mangifera indica* is the most cultivated species and has its origin in India and Myanmar.^[9] Despite adaptation and naturalization of Mango throughout tropics and subtropics, the fruit suffers from a number of diseases, some of which are taking heavy toll on the crop and presenting limiting factors.^[40,15] The potentials of mango as a commercial crop are markedly limited because of its high perishability, which results in considerable wastage.^[29]

Post-harvest losses in fruits can be attributed to several factors, the most important of which is post-harvest diseases. Post-harvest losses of fresh mango fruits are reported to be 25 - 40% in India and 69% in Pakistan and microbial decay which accounts for 17.0 - 26.9% of the total post-harvest losses in Asian countries.^[36] Mango fruits are susceptible to post-harvest diseases, extremes of temperature, and physical injury.^[14] Anthracnose (*Colletotrichum gloeosporioides*), Mango Scap (*Elsinoe mangiferae*), Bacterial black spot (*Xanthomonas campestris*), Alternaria rot (*Alternaria alternata*) Mango malformation (*Fusarium* spp) and stem end rot incited by *Lasiodiplodia theobromae*, *Dothiorella dominicana* are the most common post-harvest diseases of mango.^[43]

Many studies have been conducted regarding the use of drumstick leaf extract in controlling post-harvest fruit rot.^[35] reported that *Moringa oleifera* leaf extract significantly showed activity against *Saccharomyces cerevisiae*.^[7] also reported the antifungal activity of drumstick leaf extract on the growth of *Aspergillus niger*.^[32] reported antifungal activity of crude leaves extracts of *Moringa oleifera* Lam. on *Trichophyton rubrum* and *Microsporium canis*, 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.^[34] Also, the seed was found to have strong coagulative and anti-microbial properties.^[16] Bangalore identified a compound called Pterygospermin from *Moringa* which they reported readily dissociated into two molecules of benzyl isothiocyanate and was clearly understood to have anti-microbial properties.^[26] The use of natural plant extract to control post-harvest fruit

rot would surely have wide public acceptance than the agrochemicals because it is eco-friendly and less harmful, thus, the main aim of the work was to determine the antifungal effect of drumstick leaf extract on mango post-harvest fruit rot. Considering the risk inherent in the use of chemical fungicides ranging from environmental contamination to bio-accumulation of the residue in food chain and living system^[44], it has become imperative to search for residue free and environmentally friendly alternatives. It is in context that the study of this kind is embarked on with a view of determining the efficacy of leave extract of these plants on fungi associated with mango fruit rot. A lot of work has been carried out on the post-harvest mango rot elsewhere, there is no evidence of control on mango fruit rot in Yola North using these plant extracts, hence this research work is aimed at testing the efficacy of these leaf extracts against fungi associated with post-harvest rot of mango fruits *in vitro* and *in vivo*.

MATERIALS AND METHOD

Study Area

The study was conducted in the Department of Plant Sciences, ModibboAdama University of Technology Yola, Adamawa State. Modibbo Adama University of Technology Yola is located in Girei Local Government Area of Adamawa State. Girei lies between Latitudes 8⁰N and 11⁰N and Longitude 11.5⁰E and 13.5⁰E. Girei has a distinct tropical climate marked by dry and wet seasons with temperature and humidity varying with seasons.^[1] The people there are mostly herders and farmers.

Collection of Samples

Mango fruit showing symptoms of deterioration were collected from different markets located in Yola, Adamawa State, Nigeria. Fresh and apparently healthy Mango fruits were also collected and packed into sterilized polythene bags and taken to the Plant Science Laboratory at ModibboAdama University of Technology (MAUTECH), Yola for isolation and further studies.

Plant material used for control was drumstick leaf. Fresh leaves were collected from Sangere and SabonGari village of MAUTECH (Modibbo Adama University of Technology).

Isolation, Incidence and Identification of Fungi from Mango fruit

The two varieties of Mango (Bush and Kent) showing rot symptoms were aseptically cut into sections of approximately 5mm square with a heat sterilized table knife. The sectioned fruits

were surface sterilized, adopting the method of^[13] with 0.1% mercuric chloride for 30 seconds and rinsed with three (3) changes of sterile distilled water to remove surface contaminants. The sterilized sectioned fruits were dried between sterile filter papers and inoculated on already prepared PDA media in 9cm diameter Petri dishes and incubated at room temperature of $25 \pm 2^{\circ}\text{C}$ for 3 days before sub-culturing on fresh sterile PDA media. The fungal organisms obtained were sub-cultured and incubated for 5-7 days and re-sub-cultured repeatedly until pure culture of the isolates were obtained and stored in McCartney bottles in the refrigerator. Identification of the isolated fungi was carried out based on their spore and cultural characteristics on growing media, using the method of.^[20,2] The Percentage market disease incidence was calculated using the formula below;

$$\text{Market Disease Incidence} = \frac{\text{Number of infected fruits per market}}{\text{Total number of fruits collected per market}} \times 100$$

Pathogenicity Test

To ascertain the pathogenicity of the various fungi that were isolated, the approach of^[10] were employed. Healthy partially ripe local and improved variety of mango fruits were surfaced sterilized with 0.5% sodium hypochlorite for 5 minutes and then rinsed in sterile distilled water. With a 5 mm diameter flame-sterilized cork borer, cylindrical cores were removed from each fruit which were then inoculated aseptically with 5 mm diameter disc from the advancing edge of 7-days old fungal culture of each isolate. Vaseline jelly was smeared to completely seal the surface of each of the inoculated fruit to prevent external infection before incubating for 7 days at $25 \pm 2^{\circ}\text{C}$. The controls were inoculated with disc of solidified potato dextrose agar medium. Fruits were inoculated in three replicates. Rot symptoms developed with different fungal isolates were compared to the natural original rot.

Determination of the Effect of Drumstick (*Moringa oleifera*) Leaf Extract on Fungal Mycelial Growth

Leaves of the plant material used were washed and air-dried for two weeks to prevent loss of active component, and ground into fine powder with electric blender. Sterile distilled water was used for extraction. 10 g of the dried leaf powder was placed in 90 ml of sterile distilled water and left to stand at room temperature for 24 hours. The concentrations were gotten by diluting the stock solution (100%), 60 ml of the stock solution was diluted in 40 ml of sterile distilled water to obtain 60% concentration, 40 ml of stock solution diluted with 60 ml of sterile distilled water to obtain 40% concentration and so on.

The approach of^[6,21] was used to evaluate the allelopathic effect of the drumstick leaf extract on fungal growth by creating four equal sections on each plate and drawing two perpendicular lines at the bottom of the plate. The point of intersection indicated the center of the plates. This was done before dispensing PDA into each of the plates. About 60 ml, 40 ml and 20 ml of the leaf extracts of drum stick was separately introduced into the conical flask containing the same quantity of media (250 ml). The amended media were plugged with cotton wool and heated for about 10 minutes to avoid contamination^[27] before dispensing 10 ml each into the Petri-dishes (poisoned food method).^[31] Each Petri dish was inoculated with 5 mm plug of 4 day old pure isolate taken from margins of actively growing culture of pathogen. PDA plates free of the extracts were also prepared as control. Three (3) plates were used as replicates for each particular treatment as well as control. The treatment and control were incubated at $25^{\circ} \pm 2^{\circ}\text{C}$ until fungal growth in control filled the whole Petri-plates, and then all treatments were examined and assessed. Mean radial mycelial growth of each isolate was recorded after two days of inoculation.

Phytochemical Analysis of the Leaf Extracts

The leaf extracts of the and *Moringa oleifera* was subject to phytochemical analysis in the Biochemistry Laboratory of Modibbo Adama 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.^[19,41,33,39] The leaf extracts was screened for alkaloids, flavonoids, steroid, phenols, tannins, saponin, glycosides, anthraquinine and reducing sugar.

Experimental design and statistical analysis

Completely Randomized Design (CRD) as described by^[18] was used with three replicates. Data collected were analyzed statistically using SAS program and the inhibitions of radial mycelial growth were examined using Analysis of Variance (ANOVA). Means that were significant were separated using protected Fisher's Least Significance Difference test (LSD) at $p = 0.05$.

RESULT

**Plate I: Pure culture of *A. niger*.****Plate II: Pure culture of *B. cinerea*.****Plate III: Pure culture of *Aspergillus flavus*.**

The isolated fungi from both the local and improved variety were identified as *Aspergillus niger*, *Aspergillus flavus* and *Botrytis cinera*. The fungi associated with the spoilage of the fruits were identified based on their colonial and morphological characteristics. Percentage disease incidence in the markets is presented in Table 1, of the three markets, Jimeta Modern Market had the highest percentage incidence with 36.82% and the least incidence recorded was in Jimeta Old Market with 27.93%. All the fungal isolates exhibited different degrees of pathogenic effect on the mango fruits. They were not only able to grow on the fruits but were also able to induced some level of fruit rot indicating their virulence, Among the three (3) isolates, *Botrytis cinera* exhibited the highest level of virulence.

Test carried out on the effectiveness of drum stick leaf (*Moringa oleifera*) showed a significant difference with the control at $p < 0.05$. The tested *Moringa oleifera* extracts ($p < 0.05$) reduced the mycelial growth of the fungi *in-vitro* at all concentrations (Table 2). The effectiveness of the drum stick leaf extract significantly ($p < 0.05$) increased with increase in

concentration. Test on the effectiveness of the drum stick leaf *extract in-vivo* on local and improved variety showed a significant difference with the control at $p < 0.05$. The effectiveness increase significantly ($p < 0.05$) with increase in the concentration (Table 3 and 4). The effect was highest at 60% and lowest at 20%. The phytochemical characteristics of the aqueous leaf extracts are summarised in Table 5. The results reveal the presences of chemically active components in the plant. From Table 5, alkaloids, glycoside, terpenoids, flavonoids and phenols were found to be present in. However, Anthraquinone and reducing sugar are absent in *Moringa Oleifera* aqueous extracts respectively.

Table 1: Incidence of Mango Fruits Rot in Jimeta Markets.

Markets	(%) incidence
Modern Market	36.82
Shopping complex	35.25
Jimeta Old Market	27.93
Total	100

Table 2: In-vitro control of Fungal Pathogen using Leaf Extract of *Moringa oleifera*.

Concentration (%)	<i>B.cinera</i>	<i>A.flavus</i>	<i>A.niger</i>
20	2.71	2.22	0.52
40	2.28	1.81	0.25
60	1.81	1.21	0.13
Control	3.42	4.06	0.08
LSD (<0.05)	0.19	0.40	0.43

Table 5: In-vivo Control of Fungal Pathogen using Leaf Extract of *Moringa oleifera* on Mango (Local variety).

Concentration (%)	<i>B.cinera</i>	<i>A.flavus</i>	<i>A.niger</i>
20	0.31	0.15	0.10
40	0.18	0.11	0.05
60	0.11	0.09	0.01
Control	1.31	0.50	0.36
LSD (<0.05)	0.16	0.11	0.10

Table 4: In-vivo Control of Fungal Pathogen using Leaf Extract of *Moringa oleifera* on Mango (Improved variety).

Concentration (%)	<i>B.cinera</i>	<i>A.flavus</i>	<i>A.niger</i>
20	0.16	0.04	0.52
40	0.07	0.02	0.25
60	0.04	0.01	0.13
Control	0.55	0.43	0.08
LSD (<0.05)	0.11	0.11	0.43

Table 5: Qualitative Phytochemistry of Aqueous Leaf Extracts of the Test Plant.

Phytochemicals	<i>Moringa oleifera</i>
Alkaloids	+
Flavonoids	+
Glycoside	+
Phenols	+
Terpenoids	+
Saponins	+
Steroids	+
Tannins	+
Anthraquinine	–
Reducing sugar	–

Key

_ Absent

+ Present

DISCUSSION

The study showed that a number of different fungi are associated with post-harvest rot diseases of mango fruit in Yola North of Adamawa State. The fungi isolated include; *Botrytis cinera*, *Aspergillus flavus* and *Aspergillus niger*, which is in line with reports of^[22] that fungi of the genera *Aspergillus*, *Botrytis* and *Penecillium* are being largely responsible for post-harvest rot of wild mangoes. Previous report on fruit rots pathogens of mango fruits by^[28] in Mubi,^[17,5,45] *Aspergillus* species causes brown-black rot on the fruits and frequently, the white mycelium of the fungus became apparent at the base with black spored surface.^[22] *Botrytis* species caused brownish discoloration of fruits tissue which later became covered with the grey spore producing bodies of the fungi.^[22] From the result of pathogenicity test of the isolates (*Botrytis cinerea*, *Aspergillus flavus* and *Aspergillus niger*), in both local and improved varieties of mango, it has been established that all fungi are pathogenic to the mango varieties used for this study, although the degree of pathogenicity varies. They were not only able to grow on the fruits but also were able to induce some level of fruit rot indicating their virulence. Growth was not evident within the first 24hours after inoculation in all the isolates. Among the isolates, *Aspergillus niger* exhibit the least level of virulence as compared to the other two (2) isolates as reported by.^[22,17] Pathogenicity of *Botrytis cinera* was rated as high (i.e mycelial and/or rot covering between 80% and above of the fruit surface) and this finding was in line with the report of.^[22] The difference in the pathogenicity of fungi isolates from mango fruits might be due to their ability to overcome the natural

defence mechanism of the mango fruits or their ability to induced resistance in the fruits when infected.

From results obtained on antifungal activities, it shows that the rate of mycelial growth of the isolates was effectively controlled by the *Moringa oleifera* leaf extracts. This is also in line with the reports of^[5] in Saudi Arabia on *Aspergillus flavus* and *Aspergillus niger* on mango fruit using leaf extracts of *Moringa oleifera*. The effect of leaf extract of *Moringa oleifera* was also proportional to the concentration and inhibition value at 60% concentration (highest concentration), was higher for *Aspergillus niger*, followed by *Aspergillus flavus* and the lowest inhibition value was observed from *Botrytis cinera*. This finding was in line with^[7] who reported the antifungal activity of drum stick leaf extract on the growth of *Aspergillus niger*. The leaf extract of *Moringa oleifera* proved effective in the control of the pathogens both *in-vitro* and *in-vivo*.

Phytochemical analysis of leaf extracts showed the presences of some active components which include alkaloids, glycoside, terpenoids, flavonoids, phenols, tannins and saponins were present in extract of *Moringa oleifera*. This is in agreement with the works of^[46] who also reported the presence of this phytochemicals in leaf, bark, seed and root extracts of *M. oleifera* in Mubi. The botanical bio-pesticides represent an alternative for control with low environmental impact and high food safety. Several products derived from plants have shown an antimicrobial effect. This is in line with the works of^[11] who states that among the main compounds present in these extracts are: flavonoids, phenols, terpenes, essential oils, alkaloids, lectins and polypeptides. Some plant extracts containing these metabolites has been extracted in water or other solvents, depending on its polarity, and in powder form.^[11]

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