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Zakawa NN
Department of Bontany, Adamawa State University Mubi, Adamawa, Nigeria

Channya KF
Plant science Department, Modibbo Adama University of Technology Yola, Adamawa, Nigeria

Magga B
Plant science Department, Modibbo Adama University of Technology Yola, Adamawa, Nigeria

Akesa TM
Department of Bontany, Adamawa State University Mubi, Adamawa, Nigeria

Correspondence
Zakawa NN
Department of Bontany, Adamawa State University Mubi, Adamawa, Nigeria

Antifungal effect of neem (*Azadirachta indica*) leaf extracts on mango fruit post-harvest rot agents in Yola, Adamawa state

Zakawa NN, Channya KF, Magga B and Akesa TM

Abstract
A study for the rot of Mango fruit was carried out in three markets of Yola North Local Government of Adamawa State (Jimeta Modern market, Jimeta shopping complex and Jimeta old market) in April, 2015. Also the effect of crude water extracts of neem on the rot control was carried out. The following isolates *Botrytis cinerea, Aspergillus flavus* and *Aspergillus niger* were identified and proven through pathogenicity test to be the pathogenic. Out of a total sample size of 72 fruits (24 fruits/market) there was an average rot incidence of 33.3%. The highest pathogen incidence was in Jimeta Modern market (36.82%) while the least was in Jimeta old market (27.93%). Severity of mango fruit rot in the markets showed that Jimeta Modern Market was moderate (29.03%), Jimeta Shopping complex was also moderate (29.04%) while Jimeta Old market was high (41.93%). Control trials (invitro and invivo) on *Botrytis cinerea, Aspergillus flavus* and *Aspergillus niger* had 2.71mm, 2.30mm, 0.90mm rots (invitro) respectively. The neem leaf extract was highly effective compared to control set up (P=0.0001).

Keywords: *Botrytis cinerea, Aspergillus flavus Aspergillus niger, in vivo, in vitro*

1. Introduction
Mango (*Mangifera indica* L.) is a juicy fruit belonging to the genus *Mangifera*, consisting of numerous tropical fruiting trees, cultivated mostly for edible fruits [27]. The majority of these species are found in nature as wild mangoes. They all belong to the flowering plant family *Anacardi aceae*. The mango is native to South and Southeast Asia, from where it has been distributed worldwide to become one of the most cultivated fruit in the tropics [9]. The high concentration of *mangifera* genus is in the Western part of Malaysia (Sumatra, Java and Borneo) and in Burma and India [12].

Mangifera indica L. The "common Mango" or "Indian mango" is the only mango tree commonly cultivated in many tropical and subtropical regions. It originated in Indian subcontinent (present day India and Pakistan) and Burma [9]. Many hundreds of named mango cultivars exist, in mango orchards; several cultivars are often crossed to improve pollination. Many desired cultivars are monoembryonic and must be propagated vegetatively by grafting or they do not breed true. A common monoembryonic cultivar is "Alphonso", an important export product, considered as "the king of mangoes" [7].

Mango suffers from a number of diseases, some of which are taking heavy toll on the crop and presenting limiting factors [22; 5]. Post- harvest losses in fruits can be attributed to several factors, the most important of which is post-harvest disease. The post-harvest losses of fresh mango fruits are reported to be 25 - 40% in India and 69% in Pakistan; and microbial decay accounts for 17.0 - 26.9% of the total post-harvest losses in Asian countries [17]. The percentage loss of fruit over the marketable period has been reported to be the highest for mango [11]. The potential of mango as a commercial crop is markedly limited because of its high perishability, which results in considerable wastage [12]. In addition, mango fruits are susceptible to post- harvest diseases, extremes of temperature, and physical injury [4]. Mango fruit diseases of major concern to producers are anthracnose caused by *Colletotrichum gloeosporioides* (Penz) and stem-end rot caused by *Botryosphaeria parva* [24]. Several factors affect mango production with post-harvest losses being one of the major constraints [26, 1] found that the most prevalent fungi isolated from Egyptian mango fruits during storage were *Botryodiplodia theobromae*, *Colletotrichum gloeosporioides*, *A. alternata* and *Botrytis cinerea*. Its susceptibility to postharvest diseases increases during storage as a result of physiological changes and senescence which favour pathogen development [18, 19].

2. Materials and Method

2.1 Study Area

The isolation, identification and pathogenicity as well as control trials were carried out in Plant Science laboratory of Modibbo Adama University of Technology, Yola. Mangoes with similar symptoms such as dark brown to black decay spots, malformation of vegetative and floral tissues coated and penetrated deep into the fruits were collected from three different markets in the Local Government Area.

A sample of two different varieties of the commonly found cultivars was collected at random. A total of 72 samples of 2 different cultivars (Bush mango and Kent) were collected from the three different Markets (24 samples from each market) in a sterile polythene bag. The Markets were; the Jimeta Modern Market, the Jimeta Old Market and the Jimeta Shopping Complex (Burnt Market).

2.2 Isolation of Pathogens

The medium for isolation used, was Potato Dextrose Agar (PDA), which was prepared according to [21]. The periphery of the diseased mango tissues were cut and sterilized in 0.1% mercury chloride for 30 seconds [3]. They was rinsed in three changes of sterile distilled water and were plated out on PDA to which they were sub-cultured on to a new set of PDA plates. The sub-culture 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. The isolates were identified by observing their colonial morphology and microscopic characteristics and will be compared with structures of the different species of fungi as shown by [22].

2.3 Determination of Incidence of Rot in the Markets

Samples of the two cultivars of mangoes (Bush mango and Kent) were collected at random from the three markets selected, in which samples were collected from different traders at different locations in the market. The incidence of mangoes fruit rot in the Market was determined by counting the infected Mangoes from the samples collected from each market.

2.4 Identification of the Isolated Pathogens

The isolated fungi from Mango fruits were identified as *Botrytis cinerea, Aspergillus flavus* and *Aspergillus niger*. These were identified based on their colonial and morphological characteristics.

2.5 Control Measures

2.5.1 In vitro

One millilitre portion of the leaf extracts (100%) and a serial dilution of the extracts at 20%, 40% and 60% concentration were separately dispensed into test tubes [15]. The extracts of different concentrations were poured into Petri dishes and the PDA was also poured into the dishes containing the extracts which was allowed to cool and solidified.

The control contains sterile distilled water in place of the leaf 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 4 days in which radial growth of the fungi were taken daily, starting from the 2nd day of inoculation.

2.5.2 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 mango 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±3°C). 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.

3. Result and Discssion

3.1 Identification of Fungal Isolates

The isolated fungi from mango fruits were identified as *Aspergillus niger, Botrytis cinerea,* and *Aspergillus flavus* (Plates I-III) which is in line with reports of [8] that fungi of the genera *Aspergillus, Botrytis* and *Penecillium* are being largely responsible for post-harvest rot of wild mangoes. There has been previous report on fruit rot pathogens of mango fruits by [10] in Mubi [6, 2]. *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 [8]. *Botrytis* species caused brownish discolouration of fruits tissue which later became covered with the grey spore producing bodies of the fungi [8]. These were identified based on their colonial and morphological characteristics.

3.2 Incidence of Rots in the Markets

From (Table 1), Jimeta modern market has the highest average percentage incidence of rotted mongo fruits with 36.82%, followed by Jimeta shopping complex with 35.25% and then lastly Jimeta old market with 27.93%. 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 were 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 [8;6]. Pathogenicity of *Botrytis cinerea* was rated as highly pathogenic (i.e mycelial and/or rot covering between 80% and above of the fruit surface) as reported also by [8]. The difference in the pathogenicity of fungi isolates from mango fruits might be due to their ability to overcome the natural defense mechanism of the mango fruits or their ability to induced resistance in the fruits when infected. *Aspergillus flavus* was rated as having high pathogenic effect in the fruits. Growth and rot cover between 61%-80% of the mango surfaces. This is in agreement with the result obtained by [23], where they found *Aspergillus flavus* to be highly virulent in mango fruits and also by [6], who reported that severity of the fungus was at its peak at 35^0C and 100% relative humidity when inoculated.

Aspergillus niger had a moderate pathogenicity on the mango fruits with radial growth and rot covering 41%-60% surface

of the mango fruits as reported by [26], were he used some selected plant extracts against the phytopathogenic fungi *Aspergillus niger*.

Plate I: Pure culture of *A. niger*

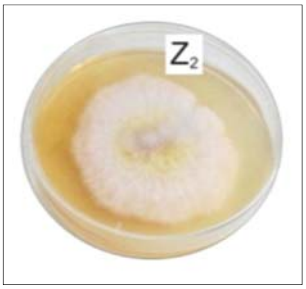

Plate II: Pure culture of *B. cinerea*

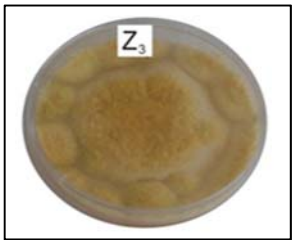

Plate III: Pure culture of *Apergillus flavus*

3.3 Effect of *Azadirachta indica* leaf extracts on the mycelial growth of the fungal pathogens

Tests carried out on the effectiveness/efficacy of the aqueous leaf extracts of *Azadirachta indica in vitro* showed a significant difference with the control at P=0.0001. At organism level, there was a significant difference at p= 0.0001 in which *Azadirachta indica* was found to inhibit the growth of all three organisms but was more effective on mycelial growth of *Aspergillus niger* followed by *Aspergillus flavus* and lastly *Botrytis cinerea* with 0.90mm, 2.30mm and 2.71mm as shown in (Table 2). *In vivo* analysis showed a significant difference between the three pathogens at P= 0.0001 in both the local variety of mango and the improved variety used. The rate of rot development in *Botrytis cinerea* was 0.62mm in the local variety and 0.27mm in the improved, while that of *Aspergillus flavus* was 0.21mm and 0.13mm and *Aspergillus niger* had 0.15mm in the local variety (Table 3). It has been shown that the rate of mycelial growth of the isolates was effectively controlled by the leaf extracts of *Azadirachta indica.* This is also in line with the reports of [2] for *Aspergillus flavus* and *Aspergillus niger* on mango fruit. Experiment shows that extracts from the *Azadirachta indica* leaves inhibited conidial germination of radial mycelia growth of number of pathogenic fungi such as *Aspergillus* spp. *Fusarium* spp. and *Cladosporium* spp. [14]. The *Meliaceae* specially *Azadirachta indica* (Indian neem tree), contains at least 35 biologically active principals of which nimbin and azadirachtin [16] are the most active insecticidal ingradients and are present predominantly in the seeds, leaves and other parts of the neem tree [14].

There was a significant difference along the concentration levels at the probability level of P=0.0001, in which 60% concentration was found to be more effective in inhibition of mycelial growth with mean rot of 1.06 mm, this was followed by 40% with 1.36 mm, 20% with 1.82 mm and the control (0%) was the least to inhibit mycelial growth with 4.00 mm mean fruit rot as shown in Table 4.

Table 1: Incidence (%) of Mango Fruits Rot in the Jimeta

Markets	Average
Modern Market	36.82
Shopping complex	35.25
Jimeta Old Market	27.93
Total	100

Table 2: Mean Effect of Leaf Extracts of *Azadirachta indica* Growth of the Pathogens *In vitro*

	Botrytis cinerea	Aspergillus flavus	Aspergillus niger
Leaf Extracts	2.71	2.30	0.90
Control	4.31	4.94	2.71
LSD (0.0001)	0.42	0.47	0.44

Table 3: Effect of Leaf Extracts of *Azadirachta indica* Growth of the Pathogens *In vivo*

	Botrytis cinerea		*Aspergillus flavus*		*Aspergillus niger*	
	L	I	L	I	L	I
Leaf Extracts	0.62	0.27	0.21	0.13	0.15	--
Control	1.31	0.55	0.50	0.43	0.36	--
LSD (0.0001)	0.13	0.10	0.11	0.12	0.09	--

Key: L: Local Mango Cultivar (Bush mango)
I: Improved Mango Cultivar (Kent)

Table 4: Mean Effect of Concentration of Aqueous extracts of *A. indica* on fruit rot of mango

Concentration (%)	*Azadirachta indica* (mm)
Control	4.00
20	1.82
40	1.36
60	1.06
LSD (p=0.0001)	0.44

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