

Antifungal Activity of *M. indica* and *F. platyphylla* Leaf and Stem Bark Extracts against Onion Bulb Rot Fungi: A Comparative Study in North Western Nigeria

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Abstract

In recent decades, there have been concerns of onion bulbs diseases due to microbial infection. Despite advancements in science and technology leading to the discovery and development of several quantitative control strategies, onion rots remain the leading cause of onion spoilage, especially in underdeveloped countries. This study aimed to investigate the antifungal activity of leaf and stem extracts from *M. indica* and *F. platyphylla* against fungi associated with rot diseases in onion in Kano state, Nigeria. Phytochemical analysis was conducted using standard methods to investigate the presence of saponin, glycoside, steroid, and phenol, alkaloid and flavonoid in the selected plants. Also the fungi associated with the rot of onion were isolated and identified using microscopic technique for their respective morphological features. This was followed by determination of inhibitory activity of the extracts against the identified fungi. The survey identified *Aspergillus niger* and *Aspergillus flavus* as the pathogenic fungi responsible for the rot disease and indicated that *M. indica* exhibited higher antifungal efficacy compared to *F. platyphylla*, with *M. indica* showing significant inhibition of both *A. niger* and *A. flavus*. The chloroform extract of *M. indica* leaf displayed the highest effectiveness, with 94.54% inhibition of *A. niger* and 93.63% inhibition of *A. flavus*. Additionally, the methanol extract of *M. indica* stem bark also exhibited notable antifungal activity. In contrast, *F. platyphylla* showed moderate to the least effective inhibition against the tested fungi. The findings suggest that both *M. indica* and *F. platyphylla* possess antifungal activity against *A. niger* and *A. flavus in vitro*.

Keywords: *M. indica*; *F. platyphylla* *allium cepa*; *Aspergillus niger*; *Aspergillus flavus*

Introduction

Onion is an important vegetable cash crop used in diets as seasoning, flavoring and medicinal purposes across cultures. It has since become an indispensable source of income for farmers across the globe. In Nigeria, onion is cultivated in several northwestern part of country including Kano State. However, most of these farmers sell off their product immediately after the harvest. This is majorly due to poor storage facilities resulting into microbial infestation and particularly onion rot caused by fungi contributing more to the damage [1,2]. Traditional and conventional methods to control these infections have not been effective as they have numerous limitations broadly categorized into human health and environmental pollution. Therefore, there is a need for naturally occurring management strategies to replace the inefficient methods currently being applied. Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans including protection of plants from diseases and preserving their physicochemical conditions

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[3]. Phytochemicals have been classified as primary or as secondary metabolites depending on their role in plant metabolism [4]. Various properties have been attributed to plants as reservoirs of bioactive compounds for numerous applications. These bioactive compounds can be derived from any part of the plant including the leaf and stem [5]. Several studies have shown higher biological activities for the crude plant extracts than a single isolated compound due to their synergistic effects (Manoharachary and Nagaraju, 2016).

Plant products have been a part of antifungal compounds since ancient time and thus screening various plants for antifungal activity has been reported by Amos et al. [6]. Antifungal compounds naturally occur in plants, leaves vegetables, and roots with quantitative resistance, possessing defense mechanisms that protect against various plant diseases [7,5]. Antifungal screening of plants has revealed the presence of numerous bioactive compounds ranging from proteinaceous and non-proteinaceous metabolites which include thionine, defensins, flavonoid, phenols, terpenoids etc., [6-8]. These metabolites serve as defense mechanisms against microorganisms particularly for bacteria, parasites and fungi [8]. Therefore, the use of these management strategies have become more popular in the control of many plant diseases due to the widely held belief that it is safe. *Mangifera indica* L. and *Ficus platyphylla* extracts are quantitative resistance that are widely used to treat various plant diseases, particularly those causing onion rotting such as white rot, neck rot, soft rot purple blotch, blast, rust, smudge, leaf spot, pink rot, and basal rot etc., [9,10-12]. Terpenoids and alkaloids exhibit various important antifungal compounds used against onion rots. In view of that, this study aimed at analyzing the presence or absence of different antifungal compounds of *M. indica* and *F. platyphylla* and testing their effect in the control of fungi associated with onion rots in Kano state of Nigeria.

Method and Materials

Study sites

The experiments were conducted in the biological science department laboratory at Yusuf Maitama Sule University, Kano. Kano is located in the dry sub-humid agroecological zone with coordinates 11.58°39'N and 8.33°45'E. The annual rainfall ranges from 696.4mm to 700mm (27.4in) or 58mm (2.3in) per month. The wettest (rainy) weather is in August when an average of 228mm (9in) of rainfall occurs. The driest weather is in January, November and December when an average of 0mm (0in) of rainfall occurs. The hottest month is April with a maximum temperature of 38 °C (100°F), while the coldest month is December with an average maximum temperature of 29 °C (84°F). The mean annual temperature ranges from 26 °C to 32 °C.

Collection and authentication of plant sample

The fresh leaves and barks of *M. indica* and *F. platyphylla* were carefully collected from their natural habitat in the school farm of Yusuf Maitama Sule University. The samples of the plants' leaves and barks in a polyethylene bag were conveyed to the herbarium section of Yusuf Maitama Sule University for taxonomic identification and authentication. The plant materials were brought to the laboratory,

rinsed with water to remove dirt, and dried at room temperature before examination. The leaves and barks were then removed from the dried leaves and crushed into a powder form using a pestle and mortar.

Collection of onion bulb

The samples of the diseased onion bulbs were collected around Yusuf Maitama Sule University Market within Kano metropolis. The diseased onion bulbs were washed with tap water and surface-sterilized in 90% alcohol for 3 to 5 minutes, then rinsed with sterile distilled water before use.

Isolation and identification of onion bulb rot fungi

Onion bulb was used for the detection of the pathogens responsible for the rots on the affected onion bulbs. The bulb was stripped of its outer dry scale and surface-sterilized in 1% commercial bleach for one minute. It was then rinsed in three successive changes of sterile distilled water and blot dried with sterile filter paper. Small segments of tissues (3cm³) from the advancing margins of rotted lesions were cut out with a sterile scalpel and forceps and plated on acidified Potato Dextrose Agar (PDA) in 90mm Petri dishes. The plates were incubated at room temperature (28±°C) for seven days. Developing fungal colonies were sub-cultured continuously on fresh PDA plates to obtain pure cultures of the isolates. Fungal isolates were microscopically examined and identified using a microscope, following identification guides based on cultural and morphological characteristics of the International Mycological Institute.

Preparation of the slide

Slides of the mycelium observed from different isolates were prepared as follows: A drop of lactophenol cotton blue solution was placed in the center of a clean glass slide. A small portion of the unidentified fungi culture was cut out with an inoculating needle; the portion was placed in the lactophenol cotton blue droplet on the slide and teased out with another needle. A cover slip was then lowered over the teased portion.

Identification of isolated fungi

Slides prepared from the individual fungal colonies were examined under the microscope to study their morphological features. The identified fungi were compared with the observed features of the colony descriptions by Robbert and Ellen [13].

Preparation of plant extract

50g of air-dried and ground *Mangifera indica* and *Ficus platyphylla* leaves and stem barks powder were extracted by percolation with 200ml (1:4 ratio) each of methanol and chloroform at room temperature for one week. The contents were filtered using Whatman No. 1 filter paper, and the residue was discarded. Later, the extracts were obtained following evaporation to dryness using a rotary evaporator.

Phytochemical analysis of plant extract

Phytochemical analysis was conducted to qualitatively determine the presence or absent of the following secondary

metabolites that is alkaloid, glycoside, phenol, steroid, saponin, flavonoid, and carbohydrate. Using method outline by (Evans and Trease 1999).

Alkaloids: Using pipette, 3ml of drag end off reagent was added to the extract, forming creamy precipitate indicated the presence of alkaloid as reported by Evans and Trease (1999).

Tannins: Few drops of FeCl_2 solution was added to 3ml of the extracts in a test tube followed by shaking. A result of dirty green coloration confirmed the presence of tannins as demonstrated by Evans and Trease (1999).

Flavonoids: One ml of the extract was treated with 1ml of dilute NaOH. The presence of a cloudy precipitate confirm the presence of flavonoid as described Evans and Trease (1999).

Saponin: Five milliliters(5ml) of distilled water was added to the 2ml of the extract in a test tube and shaken vigorously. The formation of foams or stable thing following the shaking indicated the presence of saponin as demonstrated by Evans and Trease (1999).

Phenol: One ml(1ml) of the extract was added to 1ml of FeCl_3 and mix together. The presence of blue black precipitate confirmed

the presence of phenols as described Evans and Trease (1999).

Glycoside: Approximately 2ml of glacial acetic acid were added to 5ml of the extract. Followed by one (1) drop of FeCl_2 and concentrated H_2SO_4 . Brown ring precipitate indicated the presence of glycoside.

Result

Phytochemical screening

The results of the phytochemical analysis in each of the extracts (Figure 1) showed the presence of steroids, saponins and glycoside in both methanolic and chloroform of leaf extracts in *M. indica*. However, both extracts do not contain alkaloids nor flavonoids, but the chloroform extract contains an additional phytochemical, phenol (Table 1). Similarly, the stem bark extracts have the same composition of secondary metabolites as the leaves (Table 2). Phytochemical analysis of *F. platyphylla* extracts of chloroform and methanol from leaves showed the presence of steroid, flavonoid and phenols. The leaves on the other hand have additional phytochemical (glycoside) (Table 3). The same trend of phytochemical composition was also obtained in the stem bark extract of the solvents (Table 4) (Figure 2-5).

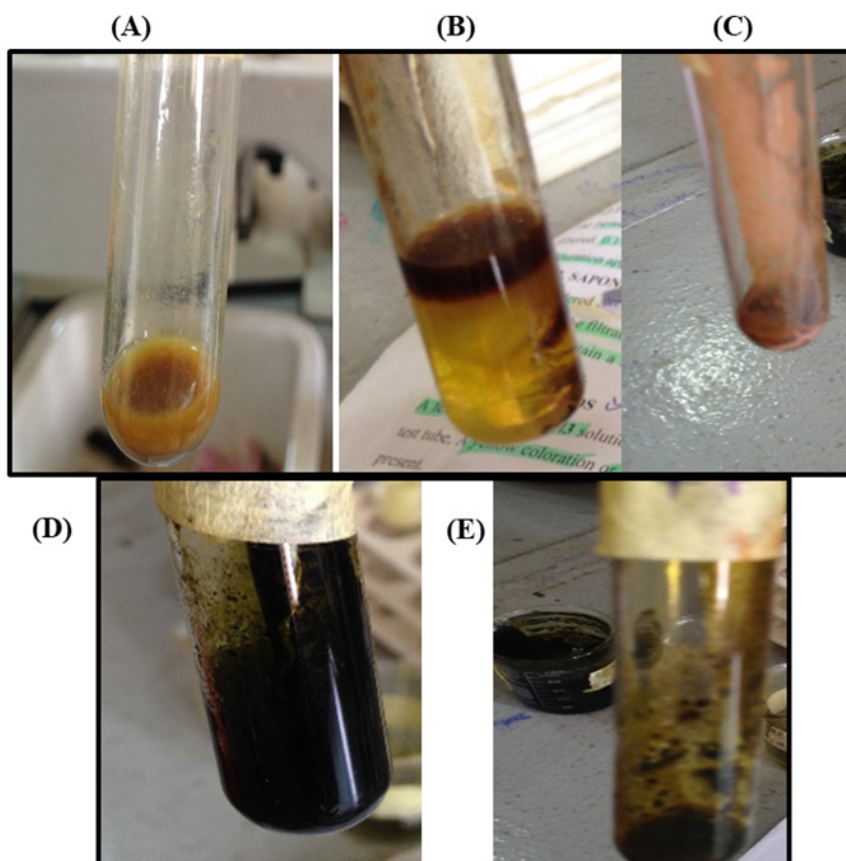


Figure 1: Pictorial representation of phytochemical test for secondary metabolites:

- | | |
|----|---------------------------------------|
| A. | Alkaloids (creamy coloration) |
| B. | Glycoside (dark brown ring formation) |
| C. | Flavonoid (cloudy formation) |
| D. | Phenols |
| E. | Tannin. |

Table 1: Phytochemical analysis of leaf extract of *M. indica*.

Phytochemical	Methanol	Chloroform
Alkaloid	-	-
Steroid	+	+
Flavonoid	-	-
Saponin	+	+
Phenol	-	+
Glycoside	+	+

Table 2: Phytochemical analysis of stem bark extract of *M. indica*.

Phytochemical	Methanol	Chloroform
Alkaloid	-	-
Steroid	+	+
Flavonoid	-	-
Saponin	+	+
Phenol	-	+
Glycoside	+	+

Table 3: phytochemical analysis of leaves extract of *F. platyphylla*.

Phytochemical	Methanol	Chloroform
Alkaloid	-	-
Steroid	+	+
Flavonoid	+	+
Saponin	-	-
Phenol	+	+
Glycoside	-	+

Table 4: phytochemical analysis of leaves extract of *F. platyphylla*.

Phytochemical	Methanol	Chloroform
Alkaloid	-	-
Steroid	+	+
Flavonoid	+	+
Saponin	-	-
Phenol	+	+
Glycoside	-	+

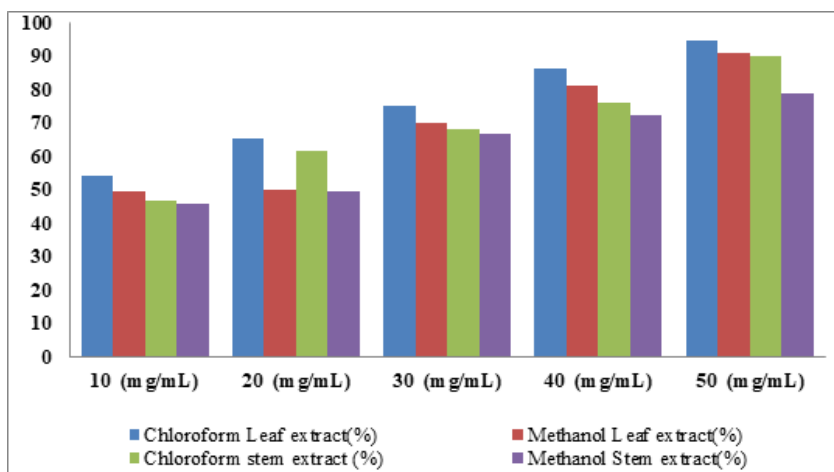


Figure 2: Percentage inhibition (%) of *M. indica* LEAF and stem back extract (chloroform and methanol) on *A. niger*.

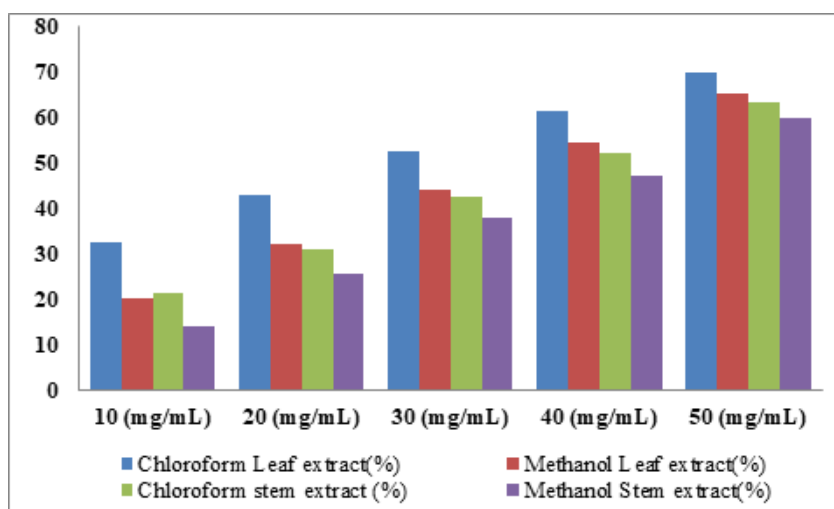


Figure 3: percentage inhibition (%) of *M. indica* leaf and stem back extracts (chloroform and methanol) on *A. flavus*.

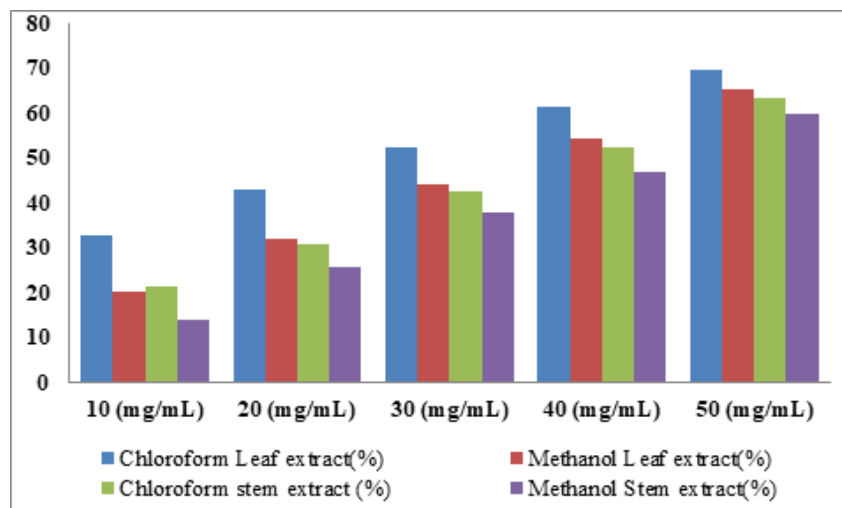


Figure 4: percentage inhibition (%) of *F. platyphylla* leaf and stem back extracts (chloroform and methanol) on *A. niger*.

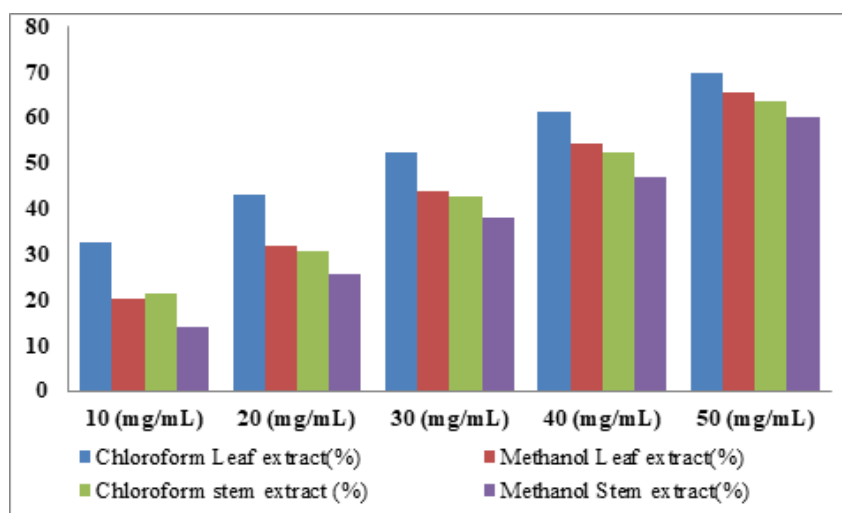


Figure 5: Percentage inhibition (%) of *F. platyphylla* leaf and stem bark extracts (chloroform and methanol) on *A. niger*.

Isolation and Identification

During the survey of fungi associated with rot disease of onions in the site, two fungi were isolated and identified as *Aspergillus niger* and *Aspergillus flavus* from three samples as presented in Table 1. This survey showed that samples 1, 2 and 3 all have higher number of *Aspergillus niger* species. However, fewer number of *Aspergillus flavus* species were found to be present in samples 1 and 3 while sample 2 indicated significant presence of the fungus. Additionally, it was found that all the samples had negative presence of *Penicillium spp.* and *Rhizopus spp.*

In vitro efficacy of *M. indica* on the growth of onion bulb rot fungi

Effect of *M. indica* leaf and stem bark extracts on the growth of *A. niger*: The percentage inhibition on the growth of *A. niger* due to application of different concentrations of *M. indica* leaf and stem bark extracts were presented in Table 5. At 50mg/ml of chloroform

leaf extracts, 94.54% inhibition was recorded compared to the methanolic extract with 91.00%. Similarly, the percentage inhibition at other concentrations of chloroform leaf extracts 10 (54.15%), 20 (65.33%), 30 (74.94%) and 40mg/ml (86.08%) also indicated increasing inhibition compared to the methanolic extracts with 10 (49.31%), 20 (50.06%), 30 (69.75%), and 40mg/ml (80.92%). The percentage inhibition of chloroform stem extracts, 46.91, 61.65, 68.03, 76.09 and 89.79% for 10, 20, 30, 40 and 50mg/ml concentrations respectively also indicates stronger inhibition against *A. niger* than the corresponding inhibition with methanol extracts with 45.60, 49.60, 66.56, 72.14 and 78.64%. This result showed that leaf extracts from both methanol and chloroform revealed higher inhibition compared to the extracts from stem back at all concentrations (10, 20, 30, 40 and 50mg/ml). However, the chloroform extracts from the leaf and stem indicate slightly higher percentage of inhibition than the methanolic extracts from these parts of the plant.

Table 5: The Results of the Organisms Isolated.

(++) = High; (+) = Moderate (–) = Absence

Isolate	Sample 1	Sample 2	Sample 3
<i>Penicillium spp</i>	–	–	–
<i>A. niger</i>	++	++	++
<i>A. flavus</i>	+	++	+
<i>Rhizopus spp</i>	–	–	–

Effect of *M. indica* leaf extract on the growth of *A. flavus*: The percentage inhibition on the growth of *A. flavus* due to application of different concentrations of *M. indica* leaf and stem bark extracts were presented in Table 6. The result showed high inhibition at 50mg/ml concentration of the extracts with 93.63 and 90.68% in chloroform and methanol extract of leaf respectively but a slightly lower percent inhibition of 89.41% and 79.15% were recorded in chloroform and methanol extracts of stem bark. At 40mg/ml concentration of chloroform and methanol leaf extracts, 85.45

and 82.65% inhibitions were recorded. However, the stem extract showed lower percentage of inhibition with 82.05 and 72.79 for both chloroform and methanolic extracts respectively. This lower trend continued as the concentrations of the extracts were decreased. These include 77.00% (30mg/ml), 70.75% (20mg/ml) and 62.06% (10mg/ml) for chloroform leaf extract and 76.20%, 68.11%, 59.53% for 30, 20 and 10mg/ml concentrations of the leaf extracts from methanol respectively. The percentage inhibition of the stem extracts from the solvents indicates also that at a concentration of 50mg/ml, maximum inhibition of 93.63% and 90.68% against *A. flavus* were recorded for leaf extracts from chloroform and methanol respectively. Similarly, 89.41%, and 79.15% inhibitions were obtained with 50mg/ml of the stem extracts of chloroform and methanol respectively. In addition, 82.05%, 76.59%, 69.34% and 65.04% inhibition and 72.79%, 67.83%, 59.73% and 51.49% inhibition against *A. flavus* were recorded for 30, 20, and 10mg/ml concentrations of stem extracts from chloroform and methanol respectively.

Table 6: Percentage (%) Inhibition of *A. niger* by extracts from *M. indica* leaf and stem bark.

Conc. (mg/ml)	LEAF		STEM BARK	
	Chloroform extract (%)	Methanol extract (%)	Chloroform extract (%)	Methanol extract (%)
10	54.15	49.31	46.91	45.6
20	65.33	50.06	61.65	49.6
30	74.94	69.75	68.03	66.56
40	86.08	80.92	76.09	72.14
50	94.54	91	89.79	78.64

Effect of *F. platyphylla* leaf and stem bark extracts on the growth of *A. niger*: The percentage inhibition on the growth of *A. niger* due to application of different concentration of *F. platyphylla* leaf and stem bark were presented in Table 7. The result showed high inhibition (77.28%) at 50mg/ml concentration of chloroform extract from leaf and 71.97% for stem bark. Moderate inhibitions were recorded at concentration of 40mg/ml with 58.34% and 54.36% in methanolic extract of leaf and stem bark respectively. This decrease in percentage inhibition continues as the concentration of the extracts in the solvents decrease which is evident for 30mg/

ml concentration with 58.78% and 53.78% in chloroform and methanol leaf extracts respectively. Similarly, 54.25% inhibition against *A. niger* was recorded for chloroform extract and 49.77% inhibition obtained for methanolic extract of the stem. Additionally, when 20mg/ml and 10mg/ml concentrations of the leaf extracts was tested against the fungus, 56.42% and 44.20% inhibitions; 41.30% and 30.95% were shown in chloroform and methanol leaf extracts respectively. The stem extracts from both solvents also revealed a decline in percentage.

Table 7: Percentage (%) Inhibition of *A. flavus* by *M. indica* Leaf and stem bark Extracts.

Conc. (mg/ml)	LEAF		STEM BARK	
	Chloroform extract(%)	Methanol extract(%)	Chloroform extract(%)	Methanol extract(%)
10	62.06	59.53	65.04	51.49
20	70.75	68.11	69.34	59.73
30	77	76.2	76.59	67.83
40	85.45	82.65	82.05	72.79
50	93.63	90.68	89.41	79.15

Effect of *F. platyphylla* leaf and stem bark extract on the growth of *A. flavus*: The percentage inhibition on the growth of *A. flavus* due to application of different concentration of *F. platyphylla* leaf and stem bark were presented in Table 8. The result showed moderate inhibition (69.71% and 65.31% in chloroform and methanol extract of leaf; 63.41% and 59.92% chloroform

and methanol extract of stem bark) at 50mg/ml concentration of the extracts. At 40mg/ml concentrations 61.38% and 54.39% were recorded in chloroform and methanolic leaf extracts; 52.18% and 46.95% recorded in chloroform extract of stem bark. 52.41% and 43.95% inhibition were recorded in chloroform and methanol leaf extracts while 42.54% and 37.92% inhibition were

shown for chloroform and methanol stem extracts at 30mg/ml. Lower inhibitory activities were recorded at 10 and 20mg/ml of the extracts as shown in Table 9. The isolates were aseptically inoculated into healthy susceptible onion, the characteristic of the organism originally observed were also noticed again, all the

fungus were confirmed as the causative agent of onion rot. The result of pathogenicity tests carried out show that, the organisms were pathogenic and were the actual agent of the spoilage onion bulbs and can also infect others plants like fruits and also many other vegetables.

Table 8: Percentage (%) Inhibition of *A. niger* by *F. platyphylla* Leaf and stem bark Extracts.

Conc. (mg/ml)	LEAF		STEM BARK	
	Chloroform extract(%)	Methanol extract(%)	Chloroform extract(%)	Methanol extract(%)
10	41.3	30.95	49.2	37.15
20	56.42	44.2	45.73	42.37
30	58.78	53.78	54.25	49.77
40	68.95	58.34	64.06	54.36
50	77.28	69.51	71.97	62.75

Table 9: Percentage (%) Inhibition of *A. flavus* by *F. platyphylla* Leaf and stem bark Extracts.

Conc. (mg/ml)	LEAF		STEM BARK	
	Chloroform extract(%)	Methanol extract(%)	Chloroform extract(%)	Methanol extract(%)
10	32.59	20.34	21.28	13.86
20	42.95	31.95	30.77	25.7
30	52.41	43.95	42.54	37.92
40	61.38	54.39	52.18	46.95
50	69.71	65.31	63.41	59.92

Discussion

Onion is a plant of economic importance and widely cultivated in the world [14,15]. It is an important vegetable crop [16,17] whose distinctive flavor is appreciated by people throughout the world and therefore ranks fourth in world production of vegetable with a volume of 64.101 metric tons annually [13]. Onions suffer from many diseases from pre harvest to post harvest period. The survey conducted at the international level revealed that about 35-40% of onions are lost due to damage caused by different diseases [18]. A number of microorganisms are responsible for bulb rotting of onion, but among them, fungi are the main causal agent responsible for pre- and post-harvest period losses in the onion, Currah and proctor [19]. Various species of *Aspergillus* pathogens are reported to cause blue mold on onion bulbs during storage. The blue molds are frequently isolated from stored diseased bulbs of local cultivars of onion, Hussain et al. [20]. *Aspergillus niger* is able to produce mycotoxin which reduces the quality and quantity of food products and feedstuff which is a potent hepatic- carcinogen in humans and animals, Paster et al. [21].

The result of this study shows that *Aspergillus niger* and *Aspergillus flavus* isolated from rotten onion bulb and were associated with onion bulb rot. The result also reveals that *A. niger* was responsible for black coloration on onion bulb, as described previously by Adongo [2] that *A. niger* also causes the black mold diseases causing significant number of postharvest losses in onion all over the world. The present study is in conformity with that of Shehu [22] and KO et al. [23] that also isolated this fungus from rotten onion bulbs and that the storage under ambient conditions in the tropics. The fungi isolated from were confirmed to be pathogenic

on onion bulbs and *A. niger* was highly pathogenic and caused the highest amount of rot on onions bulb in 4-5 days followed by *A. flavus*. However, Ibrahim [24] and Joon et al. [25] indicated that *Aspergillus* and *Botrytis* species were most frequently encountered during isolations in many parts of the tropical and humid regions. The pathogenicity test establishes infection of the onion bulbs through mechanical injuries and openings from animals etc. The effect of plant extracts on fungal growth and disease development may vary depending on the solvent used for extraction and also the concentration of the extracts [26]. Percentage inhibition of *M. indica* and *F. platyphylla* against the fungi revealed that chloroform extracts of leaf are more sensitive and effective on the fungi *In vitro* as it significantly reduces fungal growth by more than 90%. Additionally, all extracts at the highest concentration (50mg/ml) reduces disease incidence associated with the two fungi (*A. niger* and *A. flavus*). This is similar for other concentrations even though with reduced percentage inhibition. This by implication means that *M. indica* and *F. platyphylla* leaf and stem bark extracts possesses antifungal potential against *A. niger* and *A. flavus*, in a similar result reported by William [27], [28-42].

Conclusion

The findings from this study revealed that most of the spoilage of onions in Kano state are caused by these two isolated fungi, *A. niger* and *A. flavus*. These fungi were found to be highly pathogenic on onion bulbs. The study further indicated that the plant extracts had fungicidal properties with *M. indica* extracts having the more inhibitory properties than *F. platyphylla* extracts with a directly proportional increase in the concentration of the extracts compared with the control. Plant materials are cheaper to obtain and not

harmful to lives than the pesticides which are harmful and often costly. Further research is encouraged to confirm the effectiveness of these extracts on the growth of fungi *in-vivo*.

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