Journal of Advances in Biology & Biotechnology

24(4): 24-38, 2021; Article no.JABB.68075
ISSN: 2394-1081

Fungi Diversity on Some Fruits and Biological Control using Two Plants Extracts

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Authors' contributions

This work was carried out in collaboration between both authors. Author TRK conceived and designed the research proposals and contributed to the review and revision guidance of the paper author PNB performed the experiments and analyzed the data, and the writing of the original draft. All authors have read and agreed to the published version of the manuscript.

Article Information

DOI: 10.9734/JABB/2021/v24i430209
Editor(s):
(1) Dr. Maria Serrano, University Miguel Hernández, Spain.
Reviewers:
(1) Sayed Mohammad Reza Khoshroo, Islamic Azad University, Iran.
(2) Shazia Nouren, Govt. College Women University, Pakistan.
Complete Peer review History: http://www.sdiarticle4.com/review-history/68075

Original Research Article

Received 10 March 2021
Accepted 17 May 2021
Published 25 May 2021

ABSTRACT

Fruits play an essential role in human nutrition by contributing the necessary growth factors like essential minerals and vitamins in human daily diet maintaining a good and normal health. But rot diseases caused by fungi cause severe losses of agricultural and horticultural crops every year. This work aimed to study fungi diversity on some fruits and carry out biological control using two plant extracts. A total of 17 infected fruit samples were collected from two local markets, small pieces of infected parts were inoculated on prepared plates of Potato Dextrose Agar. Incubation was done for 7 days and pure cultures were made, and pure isolated fungi were identified according to the recommended references. Ethanolic leaf extracts of *Ocimum gratissimum* and *Moringa oleifera* were evaluated for *in vitro* antifungal activities on *Aspergillus* and *Fusarium* species isolated from spoilt tomatoes and banana using the Agar Dilution Method. Eleven different fungi species comprising nine genera were isolated from the 17 fruits collected from the Nkwen and main markets of Bamenda. The fungi were identified as *Saccharomyces cerevisiae, Aspergillus niger, Penicillium digitatum, Mucor sp, Fusarium sp, Mucor racemosus, Alternaria alternata, Colletotrichum sp, Nodulisporium sp, Fusarium oxysporum* and *Aspergillus flavus.* There was some diversity in isolation frequency of the fungi from the fruits. *Aspergillus, Penicillium,* and *Fusarium* were the most

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common genera that colonized the fruits, with *Aspergillus sp.* found to be the most dominant fungi responsible for extensive damage of fruits. *Ocimum gratissimum* and *Moringa oleifera* leaf extracts had inhibitory activities on the test fungi. The diversity of the fungi identified in this study could be regarded as the most common causes of post-harvest deterioration of fruits. The findings of this study bring further evidence that *Moringa oleifera* and *Ocimum gratissimum* leaves extracts have the potential of becoming powerful and safe alternative means of fungi control on fruits instead of the harmful, expensive, environmentally unfriendly chemical fungicides.

Keywords: Biological control; diversity; fungi; fruits; post-harvest.

1. INTRODUCTION

In human nutrition, fruits play an essential role contributing the necessary growth factors such as essential minerals and vitamins in human daily diet, and maintaining normal and good health. It has been recognized that fruits are commercially and nutritionally essential food products. But rot diseases caused by fungi provoke severe losses of agricultural and horticultural crops every year [1]. Tropical fruit production knows more and more increases in reducing in crop yield and quality with significant economic losses [2]. Fruits and grains are also contaminated with poisonous fungal secondary metabolites called mycotoxins. The ingestion of such mycotoxin-contaminated fruits by human beings and animals has enormous public health importance because these toxins are capable of causing diseases in man and animals [3]. Some fungi that produce these mycotoxins are *Fusarium sp*. The relatively short shelf-life period provoked by pathogens is one of the most important limiting factors that impact the economic value of fruits. Pathogens deteriorate approximately 20-25% of the harvested fruits during post-harvest handling, even in advanced countries [4]. The post-harvest losses are often harsher in developing countries due to lack of storage and transportation facilities. Fruit infections by fungi may appear during the growth period, harvesting, handling, transportation, and post-harvest stockpile and marketing conditions, or after procuring by the consumer.

Fruits incorporate high levels of nutrients and mineral elements like iron, magnesium, and calcium. They are also a source of vitamins such as vitamin C and sugars. Fruits are also consumed for medical reasons. Their low pH values make them exceptionally desirable to fungal decay [5] Fungi are considered an essential post-harvest loss agent of different fruits, based on cultivar, season, and production area amid other factors [6-7]. Fungi are the most common and crucial pathogens and the main cause of crop diseases. They infect a wide range of fruits and vegetables during storage and transportation [8].

Surveys conducted by Hartill & Everett [9] Everett et al. [10] showed that fruit rot, anthracnose stem rot and galls were the most important fungal diseases. Up to 90% incidence of these diseases have been reported in areas with high relative humidity [11]. There is limited information is available on the fungi associated with some fruits in Cameroon. This study will be aimed at isolating, identifying, and determining the diversity of the fungi associated with fruit diseases in Bamenda and using plant extracts to control the fungi.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fungi-infected fruits were selected by looking for fruits showing rot symptoms and comparing the fruits with symptoms found in books containing fungi-infected fruits. Seventeen different fruits samples were collected from Nkwen and Bamenda main markets in Bamenda Town, as shown in Fig. 1. Samples were separately kept in clean zip-lock plastic bags, and stored inside a cooler. The samples were taken to the laboratory of the University of Buea for isolation, identification, and diversity determination of fungi. The criteria for selecting particular fruits were based on the nutritional value, the availability, the market value, and the medicinal value.

2.2 Fungi Isolation, Identification and Diversity

For isolation and identification of the fungi, the fruits were cultured using the protocol of Pitt & Hocking [12] the margin of the infected lesions of each fruit was cut with a sterilized razor blade. In order to carry out surface sterilization, these samples were then immersed in 1% sodium hypochlorite (NaClOH) for three minutes, then in

70% alcohol for one minute, and rinsed in three changes of sterile distilled water for one minute each. This procedure aimed to eliminate saprophytes and non-pathogenic microorganisms found on the fruit's tissue surface. Two pieces of the surface-sterilized lesions were separately inoculated onto the Petri dishes of prepared Potato Dextrose Agar (PDA). Bacterial contamination was inhibited by adding 0.3g/10mL of streptomycin into PDA solution prior to autoclaving and pouring into petri dishes. A pair of forceps was sterilized with 70% ethanol for one minute; it was taken out and passed over the flame on the Bunsen burner then allowed to cool. After cooling, the pieces of sterilized lesions were picked up and spread over the media in the Petri dishes. These Petri dishes were then sealed with Parafilm and incubated at 25^{o}C for 7days according to Leslie and Summerell [13]. As a control, some healthy fruits were also selected. A small portion of these healthy fruits were cut using a sterile scalpel and inoculated onto a freshly prepared PDA. The inoculated plates were then incubated for 7 days to observe for Fungi growth according to the protocol of Iniekong et al. [14].

After 7 days of incubation, wet mounts were made from the growth at the margins. An isolation loop was sterilized in 70% ethanol for one minute, then passed over the flame and allowed to cool. Mycelia were then collected using the isolation loop and placed over a sterile microscope slide (this was done beside the flame), then stained using methylene-blue and covered with the slide cover, then observed under the microscope with an objective lens of x10 and x40 magnification. This procedure was aimed at identifying the fungi. When the identity was confirmed, the fungi were sub-cultured; this was aimed at getting pure cultures. This was done by preparing media (PDA), pouring in Petri dishes, and allowed to solidify. Then an isolation loop was sterilized in 70% ethanol for one minute, passed over a flame and allowed to cool. It was then used to scoop the part of the fungal mycelia that was pure then placed at the center of the newly prepared media. The observation was done after 7 days of incubation. As a control, some healthy fruits were also selected. A small portion of these healthy fruits were cut using a sterile scalpel and inoculated onto a freshly prepared Nutrient agar. Incubation was done for 7 days for the inoculated plates to observe for Fungi growth according to the protocol of Iniekong et al. [14].

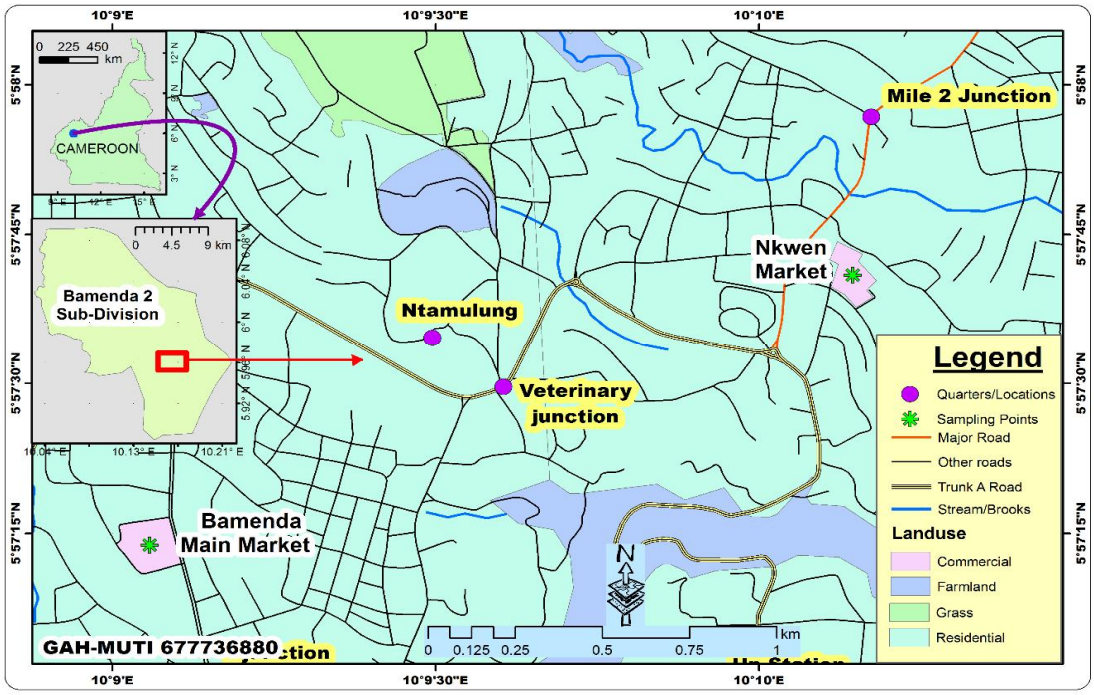

Fig. 1. Location of Nkwen market and main market Bamenda

2.3 Identification of Isolated Fungi

Isolates of the pure fungi were identified using both cultural and morphological characteristics such as surface texture (glabrous, suede-like, powdery, granular, fluffy, downy, cottony), Surface topography (flat, raised, heaped, folded, domed, radial grooved), surface pigmentation (white, cream, yellow, brown, pink, grey, black etc.), reverse pigmentation (none, yellow, brown, red, black, etc.) as well as comparing them with confirmed representatives of different species [15]. The microscopic identification was carried out by placing a drop of methylene-blue onto a clean slide. A small portion of representative fungi mycelium was removed using a flamed inoculating loop and teased onto the methylene-blue using a sterile needle. A coverslip was gently placed on the part of the slide with the stain with little pressure applied to avoid air bubbles. The slide was then mounted and viewed under the microscope with the 100 and 400 magnifications objective lens [16]. Characters such as mycelia structure and spores were observed. Pictures of the Fungi isolates were taken for further characterization and comparison with a documented book of fungi [17] and other representative pictures of fungi species.

2.4 *In Vitro* Control of Fungi on Fruits using Plant Extract

2.4.1 Harvesting of the leaves of the plants used for extraction

The extract of two plants was used; *Moringa oleifera* and *Ocimum gratissimum***.** These two plants were selected because of their availability, accessibility, affordability or low cost, environmental friendliness, their high versatile antimicrobial spectrum [18-19] and because they cause no health issues on humans when consumed. The leaves of the two plants were harvested in February 2020 from Mile 2 Limbe, South West region of Cameroon and sealed in bags and transported to the University of Dschang for extraction. The identification of the leaves was confirmed through the consultation in the Herbarium of the Department of Plant Biology, University of Dschang.

2.5 Preparation of Extraction

Fresh leaves of *Moringa oleifera* and *Ocimum gratissimum* were washed under tap water and surface sterilized with 2% sodium hypochlorite solution followed by thorough rinsing with sterile water. The plant samples were air-dried at room temperature and ground in a mortar with the use of a pestle. Thereafter, 100 g of the resulted powder were macerated in 500 ml of ethanol and mixed thoroughly. The mixture was filtered using cheese cloth followed by Whatman filter paper No. 1 after 48 hours incubation at room temperature. The extracts were transferred into labeled sterile bottles and store at 4°C [20]. Extraction was performed in the Laboratory of Microbiology and Antimicrobial Substances of the University of Dschang. Ethanol was used for the extraction instead of water because according to Parekh et al*.* [21] plant extracts from organic solvents give more consistent antimicrobial activity compared to those from water because their active ingredients will dissolve more in organic compounds than in water. Data was analyzed using descriptive statistics in Microsoft Excel version 10.

2.6 Fungi Diversity

Two plant extracts from *Moringa oleifera* and *Ocimum gratissimum* were used. *Fusarium sp. and Aspergillus sp.* isolated from tomato and banana were used. The invitro antifungal activity was assessed according to the Agar Dilution Method [22]. After preparing the media, $6cm^3$ of the media was poured into each petri dish to ensure the media was of equal volumes in each petri dish. For each fungus species and plant extract, three replicates were cµltured making it 6 replicates for each fungus. In preparing the replicates, a 0.5 micro pipette was used to measure each plant extract to 0.5µL, 1.0µL, 1.5µL and added into the PDA in three different petri dishes and swirled. This is to ensure that the PDA and the extract mix properly. The mixture was allowed to solidify. When the mixture was solidified, a mounting pin was used to remove a small portion of the fungi mycelia and placed on the PDA, closed and sealed with paraffin paper to avoid contamination. This process was carried out over a Benson flame to ensure that spores of other organisms do not cause contamination. Two Petri dishes were prepared as control for each fungus and no plant extract was introduced into them. The petri dishes were incubated at 27^0c for 7days.

3. RESULTS

3.1 Fungi Diversity

Eleven different fungi comprising nine genera were isolated from the 17 fruits collected from Bamenda. The fungi were identified as

Saccharomyces cerevisiae, Aspergillus niger, Penicillium digitatum, Mucor sp, Fusarium sp, Mucor racemosus, Alternaria alternata, Colletotrichum sp, Nodulisporium sp, Fusarium oxysporum, Aspergillus flavus. These fungi were isolated from the fruits collected.

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The biodiversity of the fungal species listed in Table 1 could be regarded as common post-harvest decay agents of the various fruits being sold in Bamenda. From the results obtained, eleven fungi species were isolated from the different fruits that were cultured. These fungi are diverse on the fruit. *Aspergillus*, yeast, *Penicillium*, *Fusarium* and *Colletotrichum* were the most common fungi that caused post-harvest decay on many of the fruits. Of the different fungi identified, *Aspergillus* was the most dominant fungus appearing as a post-harvest fungus on oranges, tomatoes, pear, pineapple, mango, and guava. Two different species of *Aspergillus* were represented, that is, *Aspergillus niger* and *Aspergillus flavus. Saccharomyces cerevisiae* was the following dominant species appearing in apples, lime, passion fruit, lemon and watermelon. Three species of *Colletotrichum* were identified, namely *C. acutatum, C. truncatum*, and *C. gloeosporioides.* They were isolated from plumes, pawpaw, banana and mango. Also dominant was *Fusarium* species which was isolated from plumes, tomatoes, pineapples and grapes. *Fusarium oxysporum* was the species identified. *Penicillium digitatum* was identified on oranges, and three other *Penicillium* species were identified on Chinese apple, passion fruit and pineapples. *Alternaria alternata* was isolated from mango and pear. *Mucor racemosus* was isolated from tomato. One species of *Rhizopus stolonifer* was also isolated from tomato. One species of *Nodulisporium* was isolated in banana.

Table 1. Fruits samples, scientific names and the fungi isolated

Fruit sample	Scientific name	Diversity of isolated fungi
Apple	*Malus domestica*	*Saccharomyces cerevisiae*
Limes	*Citrus aurrantiifolia*	*Saccharomyces cerevisiae*
Oranges	*Citrus sinensis*	*Penicillium digitatum* *Aspergillus sp.*
Passion fruit	*Passiflora edulis*	*Saccharomyces cerevisiae* *Penicillium sp.*
Plumes	*Dacryodes edulis*	*Fusarium sp.* *Colletotrichum sp.*
Tomatoes	*Lycopersicon esculentum.*	*Fusarium sp.* *Aspergillus flavus* *Mucor racemosus* *Rhizopus stolonifera*
Pear	*Persea amerricana*	*Aspergillus sp.*
Pineapple	*Ananas comosus*	*Alternaria alternata* *Aspergillus sp.* *Penicillium sp.* *Fusarium oxysporum*
Soursop	*Annona muricate*	*Saccharomyces cerevisiae*
Mango	*Mangifera indica*	*Aspergillus niger* *Alternaria sp* *Aspergillus flavus* *Colletotrichum acutatum*
Guava	*Psidium guajava* L	*Aspergillus niger*
Pawpaw	*Carica, papaya* L	*Colletotrichum trumcatum*
Chinese apple		*Penicillium sp*
Lemon	*Citrus limon*	*Saccharomyces cerevisiae*
Grapes	*Vitis vinifera*	*Fusarium oxysporum*
Banana	*Musa acuminate*	*Nodulisporrium sp* *Colletotrichum gleoesporioides.*
Water melon	*Cucumis melo L*	*Saccharomyces cerevisiae,*

The diversity of the fungi was also examined in the individual fruits; the highest diversity of fungi was found in *Lycopersicon esculentum* from where *Fusarium sp, Aspergillus flavus, Mucor racemosus* and *Rhizopus stolonifer* where isolated. This was followed by pineapple from where *Alternaria alternata, Aspergillus sp, Penicillium sp,* and *Fusarium sp* were isolated, then *Mangifera indica* from where *Aspergillus niger, Alternaria sp, Aspergillus flavus,* and *Colletotrichum sp* were isolated. The following fruits each had two fungi isolated from them; *Citrus sinensis,* had *Penicillium digitatum* and *Aspergillus sp, Passiflora edulis* had Yeast, and *Penicillium sp, Dacryodes edulis* had *Fusarium sp,* and *Colletotrichum sp* and *Musa acuminate had Nodulisporium sp* and *Colletotrichum sp.* The rest of the fruits had one fungus isolated from them: *Malus domestica, Citrus aurantiifolia, Annona muricata* and *Citrus limon* all had *Saccaromyces cerevisiae*. *Vitis vinifera* had *Fusarium oxysporium, Psidium guajava* had *Aspergillus niger, Carica papaya had Colletotrichum trumcatum*, Chinese apple had *Penicillium sp, Persea americana* had *Aspergillus sp*. The frequency of occurrence of the different fungi and fruits isolated from is shown on the Table 2.

Photos cultural morphology and microscopy of some fungi isolated and identified from fruits are shown in Figs 2, 3, 4, 5, 6, 7 and 8.

Table 2. Fungi isolated the frequency of occurrence and fruits isolated from

Fungi isolated	Frequency of occurrences	Isolated from,
Saccharomyces cerevisiae	6	Apple, limes, passion fruit, lemon and watermelon, sour soft
Aspergillus sp	7	Oranges, tomato, pear, pineapple, mango, guava, sour soft.
Penicillium sp	4	Orange, passion fruit, pineapple, Chinese apple
Mucor sp	1	Tomato
Fusarium sp	4	Plumes, tomato, pineapple
Alternaria sp	2	Pineapple, mango
Colletotrichum	4	Plumes, mango, pawpaw, banana
Nodulisporium sp	1	Banana
Rhizopus stolonifera	1	Tomato
	Total=30	

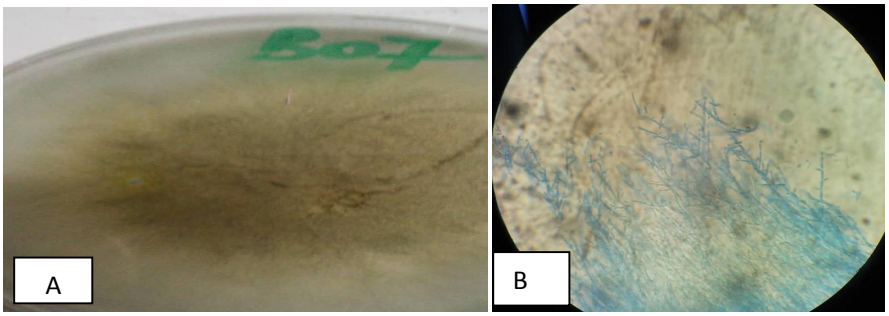

Fig. 2. Pure culture and mycelia (A) and conidia of *Aspergillus flavus* (B) age 7 days (X400)

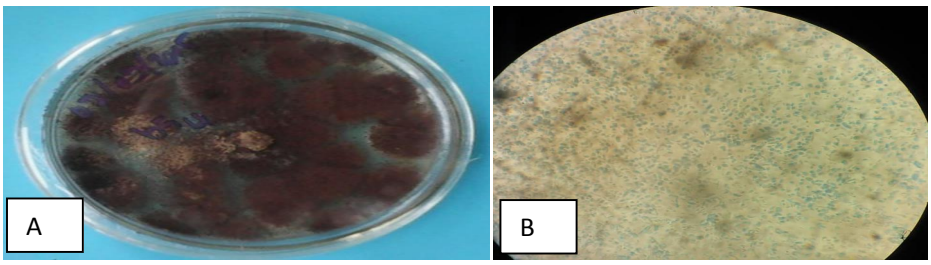

Fig. 3. Pure culture and mycelia (A) and conidia of *Aspergillus niger* (B) age 7 days at (X400)

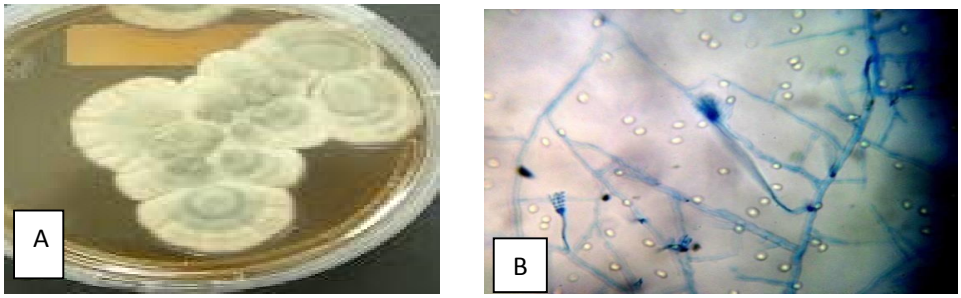

Fig. 4. Pure culture and mycelia (A) plus spores (B) *Penicillium sp age* 7 days at (X400)

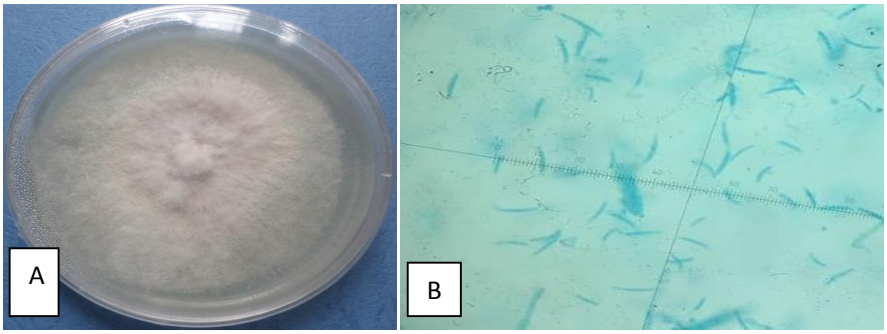

Fig. 5. Pure culture *(A) and* conidia *(B)* of *Fusarium solani* age 7 days at (X400)

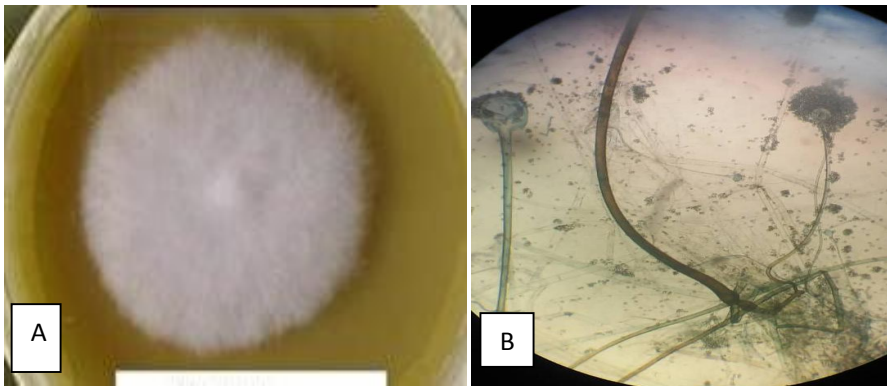

Fig. 6. Pure culture (A) and conidia (B) *of Mucor sp age* 7 days at (X400)

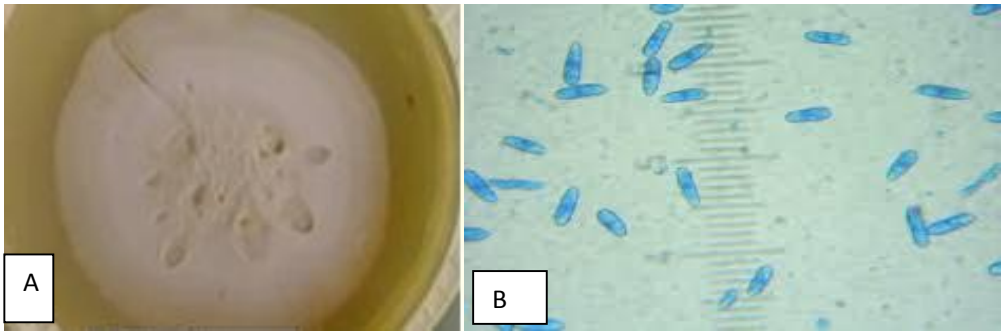

Fig. 7. Pure culture (A) and conidia (B) *of Colletotrichum gloeosporioides age* 7 days at (X400)

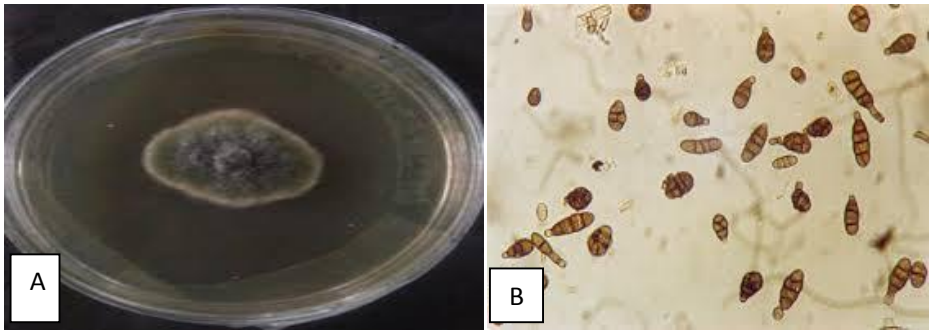

Fig. 8. Pure culture (A) and conidia (B) *of Alternaria alternata age* 7 days at (X400)

3.2 *In vitro* Antifungal Activity of *Ocimum gratissimum* on *Aspergillus sp*

Ocimum gratissimum showed antifungal activity on *Aspergillus sp* at different concentrations of the plant extract on the Petri dishes. The antifungal activity increased with an increase in the extract's concentration from 0.5µL to 1.0µL to 1.5µL. At 0.5µL, the extract's slight antifungal activity as rapid growth and spread of the fungi colony was observed on the petri dish. At 1.0µL there was a higher antifungal activity of the plant extract on *Aspergillus*. This was observed by a decrease in the colony growth of the fungi on the Petri dish compared to the colony at 0.5µL of plant extract. At 1.5µL of the plant extract, there was a very drastic increase in the antifungal activity of the plant extract as the growth of the colony was very small after 7days. This implies that the plant extract of *Ocimum gratissimum* can eliminate the growth of *Aspergillus* at higher concentrations. The control had a very rapid growth of the colony that spread throughout the Petri dish.

3.3 *Invitro* Antifungal Activity of *Ocimum gratissimum Extract* on *Fusarium oxysporum*

Ocimum gratissimum extract had antifungal activity against *Fusarium sp* at different concentrations. It was observed that, at concentration of 0.5µL of extract, there was a minimal antifungal activity of the extract on the *Fusarium sp* as the growth of the fungal colony was noticeably high. But from concentration of 1.0µL of extract, there was a great decreased in growth of the fungal colony. As the concentration of the extract increased to 1.5µL, the antifungal activity of the extract was noticeably higher as the growth of the fungal colony dropped just to a tiny colony. That was an indication that, the extract of *Ocimum gratissimum* has antifungal activity on *Fusarium sp,* and can effectively control the growth of *Fusarium in vitro*. And at higher concentration of the extract, *Fusarium sp* growth will be eliminated.

3.4 *In vitro Antifungal Activity of Moringa oleifera on Aspergillus sp*

Moringa extract showed antifungal activity against *Aspergillus sp.* The antifungal activity also increased with increased in the concentration of the extract from 0.5µL, to 1.0µL to 1.5µL. The control showed a rapid and high rate of fungal colony growth after 7days. This was observed by the way the fungal colony spread throughout the Petri dish with spores on it. At 0.5µL of plant extract, there was a slight decrease in the growth of the fungi colony. As the extract concentration increased to 1.0µL, there was a noticeable decrease in the growth of the fungal colony. Meanwhile, at 1.5µL of plant extract, there was an apparent in the growth of the fungal colony. Just a small portion of the fungus could be seen growing on the PDA. From the results observed, the extract of *Moringa oleifera* can effectively eliminate the growth of *Aspergillus* at a higher concentration of the extract.

3.5 *Invitro* Antifungal Activity of Extract of *Moringa oleifera* on *Fusarium sp*

Extract of *Moringa oleifera* had a noticeable antifungal activity against *Fusarium oxysporum* at different concentrations. Observing the three replicates of different concentrations of the *Moringa oleifera* extracts, it was observed that, at a concentration of 0.5µL of extract, there was no noticeable antifungal activity of the extract on the *Fusarium sp* as the growth of the fungal colony was high. But from the concentration of 1.0 µL of

Moringa oleifera extract, there was a significant decrease in growth of the fungal colony. As the concentration of the extract increased to 1.5 µL, the antifungal activity of the *Moringa oleifera* extract was higher as the growth of the fungal colony dropped just to a tiny colony Fig. 9. That was an indication that the extract of *Moringa oleifera* has antifungal activity on *Fusarium sp,* and can effectively control the growth of *Fusarium sp in vitro*. At higher concentrations of the *Moringa oleifera* extract, *Fusarium* growth was eliminated.

Comparing the antifungal activity of the extract of *Ocimum gratissimum* and *Moringa oleifera* against *Aspergillus sp*, extracting *Ocimum gratissimum* had higher antifungal activity on *Aspergillus sp* than *Moringa oleifera* at the same concentration. This comparison was made by looking at the rate of antifungal activity of both extracts at concentrations of 0.5µL and 1.5µL. The colony diameter of the fungi at 0.5µL and 1.5µL concentrations of *Ocimum gratissimum* extract were smaller compared to that of *Moringa oleifera* at the same concentration. This is an indication that the extract of *Ocimum gratissimum* was more effective in the in vitro control of *Aspergillus sp* than extract of *Moringa oleifera* at equal concentrations of the extracts.

Comparing the antifungal activity of extract of *Moringa oleifera* and *Ocimum gratissimum,* against *Fusarium sp*., though both extracts had noticeable antifungal activity against *Fusarium sp., Ocimum gratissimum* had higher antifungal activity against *Fusarium sp.*, than *Moringa oleifera* at the same concentration of the extracts Fig. 10. The rate of growth of the fungal colony for both extract at concentrations of 1.5µL was the observation noiced. The colony diameter was observed in the Petri dish containing the extract of *Ocimum gratissimum* to be smaller than that of *Moringa oleifera,* which indicated that *Ocimum gratissimum* has a higher antifungal activity against *Fusarium sp* than *Moringa oleifera.*

Fig. 9. Comparative antifungal effect of equal concentration of A) *Ocimum gratissimum and* B) *Moringa oleifera* on *Aspergillus sp*

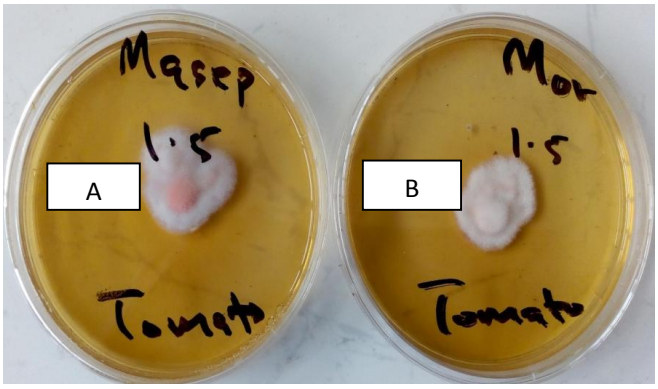

Fig. 10. Comparative antifungal effect of equal concentration of A) *Ocimum gratissimum* and B) *Moringa oleifera* on *Fusarium sp*.

4. DISCUSSION

4.1 Diversity of Fungi

A total of eleven fungal belonging to nine genera were isolated from 17 different spoilt fruit. The fungal isolates were identified as; *Aspergillus niger, Aspergillus flavus, Saccharomyces cerevisiae*, *Penicillium sp, Mucor sp, Fusarium sp, Alternaria sp, Colletotrichum acutatum, Colletotrichum gleoesporioides, Nodulisporium sp* and *Rhizopus stolonifer.* All diseased fruits were found to be infected by fungi, with tomato, pineapple and mango being the most infected fruits with the highest diversity of fruits. It indicated a number of fungal pathogens responsible for various post-harvest disease that is, storage disease pathogens in fruits. This is in accordance with the report of Singh (2001) that different genera of fungi that are pathogenic to the fruits live in the necrotic areas and cause disease in humans if consumed directly. Among the fungal isolated, *Aspergillus sp* was found to be the most dominant fungi responsible for extensive damage of fruits. Similar results on post-harvest fungal pathogens on market storage of fruits were reported by earlier works [23]. This is also in agreement with Salihu [24] who worked on the isolation and identification of pathogenic fungi associated with fresh edible fruit. Similarly, Rathod [25] reported post-harvest fungal diseases of some fruits of Marathwada regions of Maharashtra. Sani et al. [26] also isolated fungi from fruits grouping them into eight taxonomic genera namely; *Aspergillus, Rhizopus, Mucor, Alternaria, Neurospora, Penicillium, Cladosporium sp and Fusarium sp*. Fruits contain high levels of sugars and nutrients, and their low pH values make them particularly desirable to fungal infection [5]. Some of these fungi are reported by several authors to be commonly implicated in the post-harvest deterioration of many fruits and vegetables in the Tropics [27-28] Some of the fungi isolated from these fruits, like *Aspergillus sp* have been reported to produce toxins [29]. Some of these moulds have also been reported to produce secondary metabolites such as aflatoxins which have been associated with cancer of the liver, aflatoxicosis, and acute hepatitis in humans, especially in the developing world [30]. These fungi have also been reported to be pathogenic and could cause diseases [31]. *Aspergillus* sp are known to produce several toxic metabolites such as malformins, naphthopyrones, and ochratoxins, which pose a serious health hazard to human and animal health [32-33]. It is therefore pertinent that adequate storage facilities be put in place for these important fruits. Good hygiene such as proper cleaning of transportation vans, fruits, storage facilities and selling sheds must also be observed during their handling, transportation and processing. Besides, fruits with any symptoms of spoilage must be properly disposed off and not be sold and consumed by the public because of their adverse effects on health. The adverse effect of fungi in plants and fruits has resulted in the shortage of fruits for consumption [34]. Spoilage of fruits by fungi leads to a shortage of consumption and loss of profits to the farmers and industries whose raw material is fruits [34]. So appropriate majors must be taken to control fungi on fruits. The highest diversity of fungi was found in *Lycopersicon esculentum* from where *Fusarium sp Aspergillus flavus, Mucor racemosus* and *Rhizopus stolonifer* where isolated, pineapple from where *Alternaria alternata, Aspergillus sp, Penicillium sp,* and *Fusarium sp* were isolated and *Mangifera indica* from where *Aspergillus niger, Alternaria sp, Aspergillus flavus,* and *Colletotrichum sp* were isolated. The following fruits each had two fungi isolated from them; *Citrus sinensis,* had *Penicillium digitatum* and *Aspergillus sp, Passiflora edulis* had *Saccharomyces cerevisiae* and *Penicillium sp.*, *Dacryodes edulis* had *Fusarium sp,* and *Colletotrichum sp* and *Musa acuminate had Nodulisporium sp* and *Colletotrichum sp.* The rest of the fruits had one fungus isolated from them: *Malus domesca, Citrus aurantiifolia, Annona muricata* and *Citrus limon* all had *Saccaromyces cerevisiae* isolated from them. *Vitis vinifera* had *Fusarium oxysporium, Psidium guajava* had *Aspergillus niger, Carica papaya* had *Colletotrichum trumcatum*, Chinese apple had *Penicillium sp.*, and *Persea americana* had *Aspergillus sp*. The diversity of fungi isolated is related to the results obtained by Yaouba & Mpounze, [35]. The biodiversity of fungal species could be regarded as common post-harvest decay agents of various studied fruits. Through their investigation at 28 ± 2^{o}C nine fungal species attributed to six genera were isolated. *Aspergillus, Cercospora, Colletotrichum, Fusarium* and *Veticillium* were the most common genera that colonized banana, mango, and *Dacryodes edulis* fruits with different incidences. *Aspergillus* was represented by *A. niger, Cercospora* (3 species), *Fusarium* and *Verticillium* by one species. *Cercospora* contained 3 species, namely *C. capsici, C. mangiferae* and *C. musae. Fusarium* and *Verticillium* genera were represented by one species each, namely *F. oxysporum* and

Verticillium alboatrum. *Cercospora* was by far the most common genus affecting the different kinds of fruits. It appeared on 50 % each of banana, mango and *Dacryodes edulis* fruits. *Aspergillus, Colletotrichum, Fusarium* and *Verticellium* were the second most common genus affecting these fruits. *A. niger* was found on banana (8.62%), and mango (15%) and *Dacryodes edulis* (12%). *Colletotrichum gloeosporioides* appeared with variable incidences on banana (8.62%), mango (15%) and *Dacryodes edulis* (22.92%). Other species showed higher affinity towards certain fruits such as *Rhizoctonia solani* on mango and *Dacryodes edulis* fruits. The results on the diversity of the fungi identified are also related to that of Samuel et al. [36] in their study of Fungi assessment in some spoilt fruits sold in Gwagwalada market of Abuja, Nigeria. The diversity of the fungi isolated was *Aspergillus niger, Fusarium avanaceum, Penicillium digitatum, Rhizopus stolonifer, Saccharomyces species, A. flavus,* and *F. solani* with *Aspergillus niger* being prevalent with the percentage of 38% in fruits such as pineapple, watermelon, oranges, pawpaw as well as tomatoes while *Fusarium avanaceum* had the frequency of 31% in pineapple, watermelon, oranges, pawpaw, and tomatoes *Penicillium digitatum* and *Rhizopus stolonifer* was also isolated from fruits such as tomato and oranges and they have the least occurrence with the frequency of 4%. *Saccharomyces species*, *Fusarium solani* and *A. flavus* were also isolated in this study with the frequency of 10%, 8%, and 5%, respectively. Fruits and vegetables are exposed to contamination by microorganisms through direct contact with soil, dust, water and by handling at harvest during transportation, during their sale in the market, or during post-harvest processing. This makes them harbour a wide range of microorganisms, including plant and human pathogens [37].

4.2 *In vitro* Antifungal Control Using *Moringa oleifera*

The uses of plant-derived products as disease control agents have been studied since they tend to have low mammalian toxicity, less environmental effects and wide public acceptance. To develop environment-friendly alternatives to synthetic fungicides to control fungal plant diseases, the interest in essential oils and plant extracts has been increased [38-39]. In this study, we investigated the antifungal activities of extract of *Moringa oleifera* leaves against *Fusarium sp* and *Aspergillus sp in vitro*. Our results clearly show that ethanol extract of *Moringa oleifera* leaves tested at different concentrations had antifungal activity against *Fusarium sp* and *Aspergillus sp*., *in vitro*. This work also showed that the antifungal activities of the tested ethanol extract of *moringa* extracts increased by increasing the extract's concentration. The inhibition of the fungal growth was observed from the decrease growth of the fungal colony as the concentration of the extract was increased. Our studies showed that both fungi were affected by the ethanol extract of *Moringa oleifera* especially at high concentrations. Different researchers have carried out similar studies on the antifungal activity of extracts of many plants [40]. The fungicidal effect of Moringa extracts on some soil-borne fungi such as *Rhizoctonia, Pythium* and *Fusarium* were recorded by many investigators [41-43]. Indicate that *Moringa oleifera* extracts (leaves, bark and seeds) 75 % (v/v) showed significant inhibition in the mycelial growth of *Fusarium solani* and *Fusarium oxysporum*. These results are consistent with those obtained by other investigators who found an antifungal activity of *Moringa* plant extracts against several pathogens [42] [44-45]. These extracts, however, contained specific components that can inhibit the growth of certain microorganisms [42] [18]. *Moringa oleifera* provides a rich and rare combination of zeatin, quercetin, b-sitosterol, caffeoylquinic acid, and kaempferol antifungal and antibacterial activities [46-48]. That explains why the extract of Moringa was able to inhibit the growth of the two fungi tested *in vitro.* Many plant extracts have been found to be potent fungi toxic agents against many plant pathogens [49-50]. This result confirms that plant extracts can be used as natural fungicides to control pathogenic fungi of fruits and reduce the dependence on synthetic fungicides.

4.3 *In vitro* Antifungal Control Using *Ocimum gratissimum*

Two dominant fungi species that were used in this study are *Fusarium sp* and *Aspergillus sp*. The results obtained from this study indicated that ethanolic extract of *Ocimum gratissimum* can inhibit the growth of both fungi *in vitro.* The results concur with that of Nwinyi et al. [51], who showed that the leaf extracts of *O. gratissimum* possess antifungal activity. These results also agreed with earlier works on plant products' inhibitory action on the mycelia and spore germination of other pathogenic fungi [52-53].

This study corroborates the report of other workers [54] [19] that *O. gratissimum* is among important plants whose extracts can checking the spread of many fungal diseases ~~of~~ food crops. The findings of this study also showed that the inhibitory activity of the plant extract of *Ocimum gratissimum* against both fungi was greatly affected by the concentrations of the extract. The antifungal activity of the plant extract increased with increasing concentration of the extract. This result agreed with that of [55], who explained that the activity of plant extracts increases with the concentration of extract due to a higher quantity of active ingredients. This finding agrees with Mares et al. [56] that a higher concentration of antimicrobial substance showed appreciation in growth inhibition of fungi. Phytochemical studies have also shown that the antimicrobial properties of the plant extract of *Ocimum gratissimum* are due to certain active ingredients, especially the oils such as saponins tannins and flavonoids which have antifungal and antibacterial activity [57]. Several reports stated that the extracts of medicinal plants play an important role in controlling many phytopathogenic fungi [58-59]. The inhibitory effect of some medicinal plants might be due to the presence of steroids, terpenoids, alkaloids, citral, geraniol, flavonoids, eugenol, cytronolal, geranyl acetate, beta cariofiln, tannins, phenolic compounds, saponins and farnsul [57]. The antifungal activity of *Ocimum gratissimum* was greater than that of *Moringa oleifera* probably because the antifungal ingredients in *Ocimum* are more active and effective than those in *Moringa.*

5. CONCLUSION

The fungal species identified in this study could be regarded as the most common causes of post-harvest deterioration of local fruits. Results suggests the need to develop an appropriate management strategy to control post-harvest diseases caused by fungi, especially as some of the fungi have toxicological and pathologic impacts on humans and animals. The use of plant extracts in this work was to study their antifungal activity against the fungi on fruits. In this study, we used *Moringa oleifera* and *Ocimum gratissimum* extracts as bio-fungicides and as eco-friendly means to control fungal fruit diseases since the plants used extraction are readily available, environmentally safe, less risky for developing resistance in fungi, and pest resurgence, has less adverse effect on plant growth, less harmful to humans and animals and above all, less expensive. The findings of this study bring further evidence that *Moringa oleifera* and *Ocimum gratissimum* leaves extracts have the potential of becoming powerful and safe alternative means of fungi control of fruits instead of the harmful, expensive, environmentally unfriendly fungicides. The present investigation findings could be an important step towards the possibilities of using natural plant products as fungicides in the control of fruit diseases caused by fungi.

ACKNOWLEDGEMENT

The authors thank Mr. Mbaabe Felix for assistance in the laboratory work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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