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ISOLATION AND CHARACTERISATION OF FUNGAL SPECIES ASSOCIATED WITH SPOILAGE OF *MORINGA OLEIFERA* LAM. IN STORAGE

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This study isolated and characterised the fungal contaminants associated with deteriorated *Moringa oleifera* Lam. stem barks upon storage*. Moringa oleifera* stem barks freshly harvested from Oluyole Local Government Area in Oyo State Nigeria were stored at room temperature. Within 7 days storage, they showed signs of deterioration and were examined for the presence of fungal pathogen causing deterioration. Two species of *Aspergillus: Aspergillus niger and Aspergillus flavus* were identified, samples were placed on sabouraud dextrose agar (SDA) at room temperature. The presence of these microorganisms causes bio-deterioration of *Moringa oleifera* during storage. The quality of herbal drugs should be thoroughly monitored before consumption to prevent health hazards when consumed as herbal preparations by humans.

KEYWORDS; Isolation, characterization, fungi, deterioration, *Moringa oleifera* stem barks.

INTRODUCTION

Herbal drugs are becoming increasingly popular both in developed and developing countries and their use has always been part of human culture as some plants possess important therapeutic properties which can be used to cure human and animal diseases (Einsberg *et al*., 1998). Herbal preparations have been regarded as safe (Sofowora, 1993).

Moringa oleifera, a shrub and small deciduous tree of about 2.5-10 m in height, is known as drumstick tree, horseradish tree or kelor tree (Anwar and Bhanger, 2003). In Pakistan *Moringa oleifera* is locally known as sohanjna and is cultivated all over the country (Anwar *et al*., 2005). It is a well adopted tree in the tropical world and is indigenous to Northwest India and is cultivated mainly for its edible fruits (Tahiliani and Kar, 2000). It is found wild and is cultivated throughout the plains especially in hedges and house yards and it grows best under tropical climate and is abundant near the sandy beds of rivers and streams (Anwar *et al*., 2007).

According to Chaudhary and Chopra (1996), all parts of the plants are known to possess medicinal properties and are used to treat ailments such as rheumatism, venomous bites and for enhancing cardiac functions, also the leaves are used to treat scurvy and cold infections (Anwar *et al*., 2007).

The leaf extract have also been found to regulate thyroid status and cholesterol levels (Tahiliani and Kar, 2000; Ghasi *et al*., 2000). Traditionally, the seeds of *Moringa oleifera* are used in the rural areas of Sudan and Malawi for the clarification and softening of water (Anwar *et al*., 2007). According to Sharma *et al*. (2006) *Moringa oleifera* can be used as a bio-absorbent in the removal of cadmium from aqueous media.

Moringa oleifera is an important source of food nutrition in the tropics and its leaves, fruits and flowers are used as nutritive vegetable in many countries particularly in India, Pakistan, Hawaii and many parts of Africa (D' souza and Kulkarm, 1993; Anwar and Bhanger, 2003; Anwar *et al*., 2005). The leaves are good sources of vitamin C, calcium and potassium and good sources of natural antioxidants which enhance the length of storage of fatty foods these antioxidants include ascorbic acids, carotenoids, and phenolics (Dillard and German, 2000). In the Philippines, *Moringa oleifera* is known as *Mother's best friend*

because it is used to increase a woman's milk production and it is sometimes prescribed for anaemia in pregnant women (Estrella *et al*., 2000). The seeds of *Moringa oleifera* can be consumed fresh as peas or pounded, roasted, or pressed into sweet oil which is commercially known as "Ben oil" and is of high quality. The pressed cake of *Moringa oleifera* seed contain polypeptide which serve as a coagulant for water treatment (Ndabigengesere and Narasiah, 1998).

Unfortunately, the various uses of a multipurpose plant as *Moringa oleifera* is reduced by high rate of fungal attack during storage. This study is therefore aimed at isolating and identifying the fungi associated with the bio-deterioration of *Moringa oleifera* stem barks.

MATERIALS AND METHODS

Sample Collection and Pre-treatment

Moringa oleifera stem barks were collected from Olowa village via Onigambari, Oluyole Local Government Area, Oyo State. The samples were transported to the laboratory in a polythene bag for analysis. The stem barks were surface sterilized with sodium hypochlorite, rinsed with distilled water and then stored.

Isolation and Characterisation of Fungi

Within 7 days of storage, as soon as fungal growth was noticed isolation of fungi was carried out by cutting segments (1-2cm) of the stem barks affected using a pair of sterile scissors and placed on freshly prepared sabouraud dextrose agar (SDA) plates. The agar plates were then incubated at 25℃ for 48-72 hours. To obtain pure culture, each different fungus appearing was sub-cultured onto freshly prepared SDA plates and was incubated for 5 days at 25℃. Smears of each fungus were prepared on microscopic slide and stained using lactophenol cotton blue and were examined microscopically. Cultures were identified to species level using the method described into Raper and Fennel (1977).

RESULT

Two species of *Aspergillus* namely; *Aspergillus niger* and *Aspergillus flavus* were isolated from bio-deteriorated *M. oleifera* stem barks. The macroscopic features of the fungi isolated are presented in Table 1, Plates 1 and 2. *Aspergillus niger* appeared black in colour and *Aspergillus flavus* appeared yellowish green (Plates 1 and 2). Figs 1 and 2 revealed some microscopic characteristics observed for the two species. *Aspergillus niger* has a large conidial head which splits into loose columns later. *Aspergillus flavus* mostly has a radiate conidial head with round conidia which are globose to sub-globose and they have smooth to finely roughened walls and rough chains of conidia borne on the vesicle.

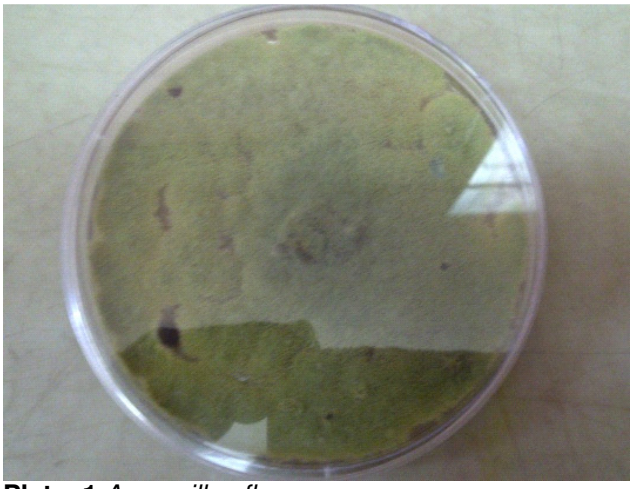


Plate: **1**:*Aspergillus flavus*

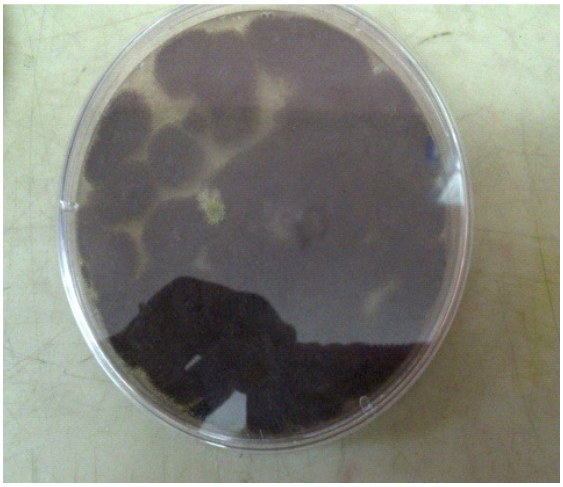
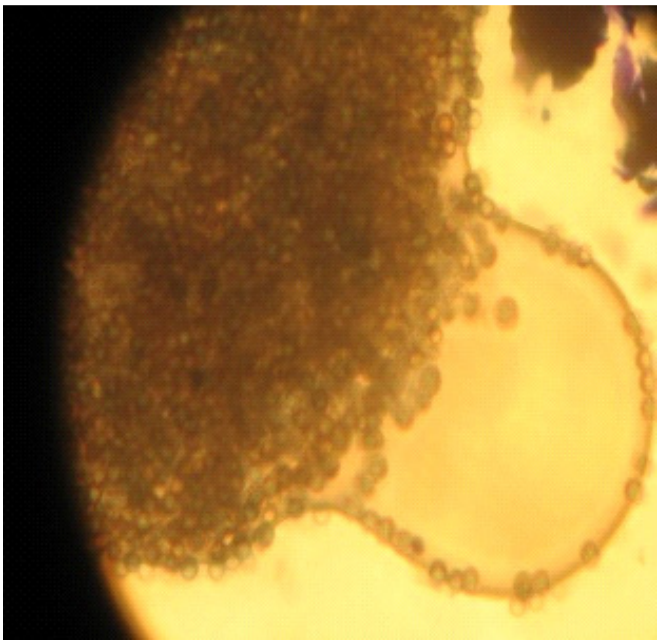
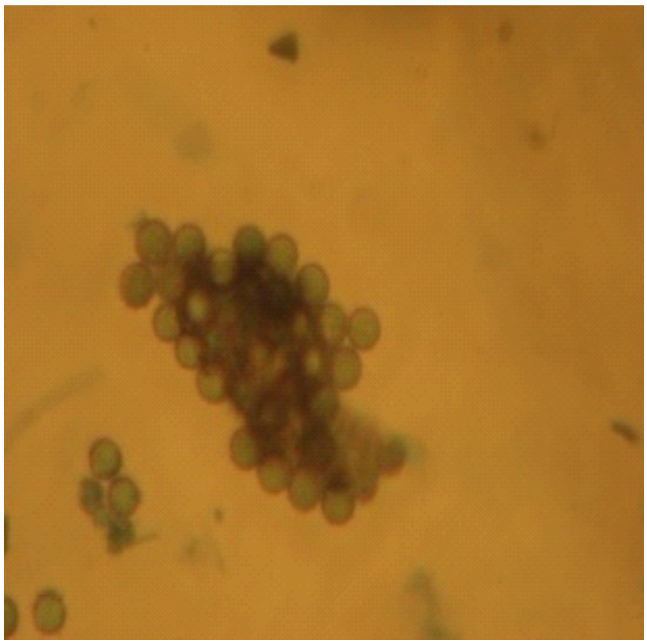


Plate: **2:** *Aspergillus niger*

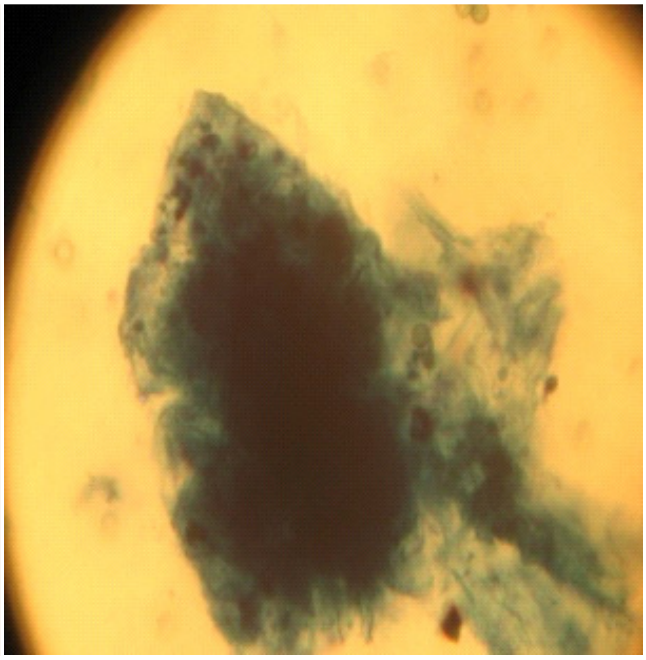


Dark brown coloured conidia



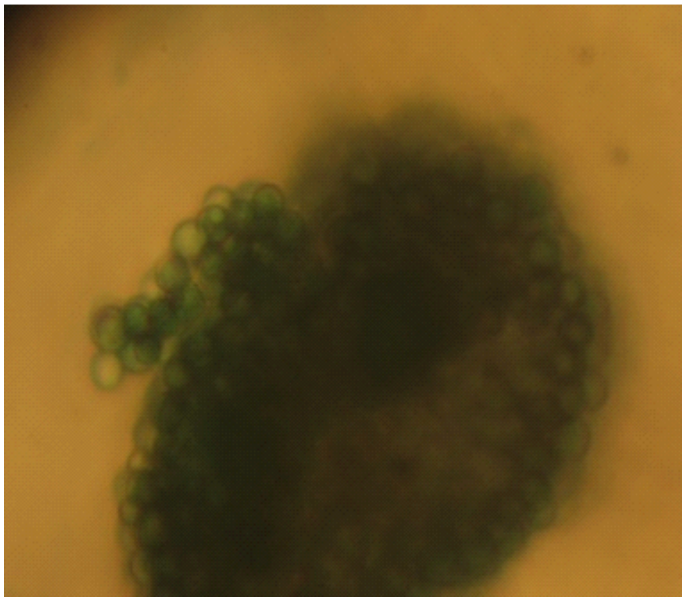
Splitted loose conidia

Conidia borne on the stipe

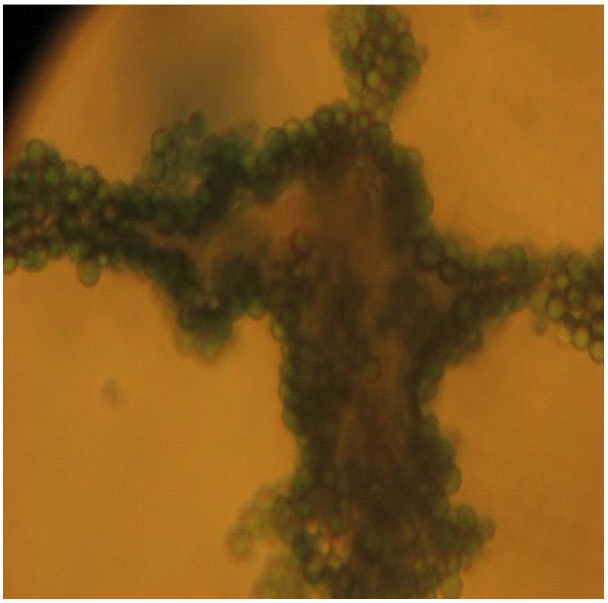


Large conidia head

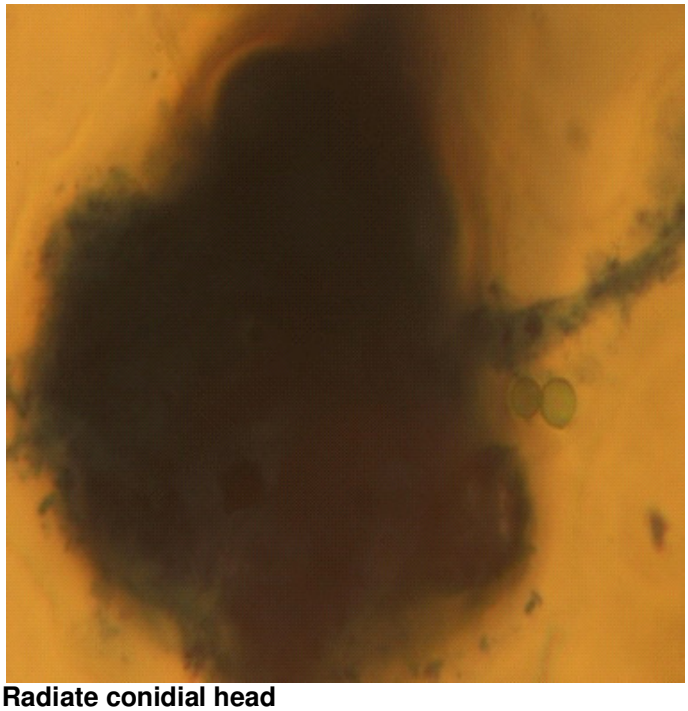
Fig1: Microphotography of *Aspergillus niger*



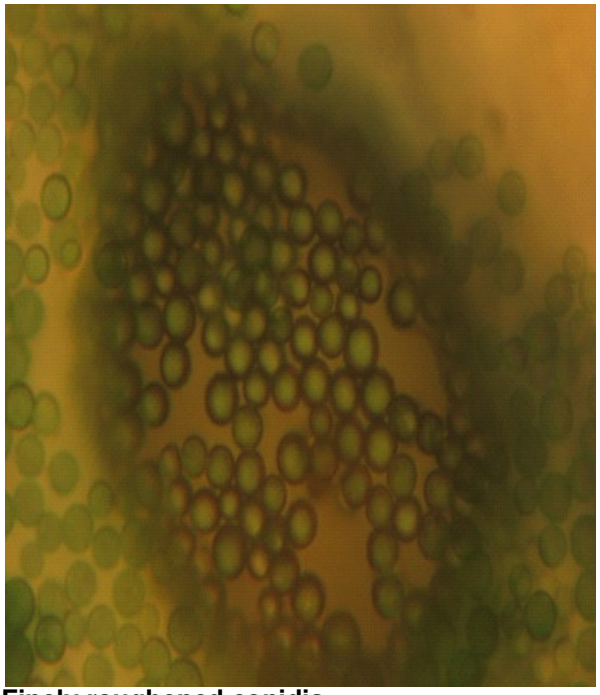
Splitted conidia mass



Rough conidia borne on the vesicle



Radiate conidial head



Finely roughened conidia

Fig 2: Microphotography of *Aspergillus flavus.*

Table 1: Identification of fungi isolates from deteriorating Moringa oleifera stem bark.

ORGANISM	MACROSCOPY	MICROSCOPY
Aspergillus niger	Surface colony colour is initially white and becomes black with white edges and later black colonies grow all over the plate with time. Growth is rapid and texture of colonies vary from downy to powdery	Conidial heads are large and become radial later. Conidial heads split into several loosed columns. Conidia are dark-brown and round in shape and are rough walled and in chains with a diameter of 4-5µm. conidiophores are smooth-walled and hyaline.
Aspergillus flavus	Growth rate is rapid and textures of colonies vary from woolly to cotton to granular. Surface colony colour is yellow-green.	Conidial heads are mostly radiate and conidial masses split into blocky column. Conidia are round, globose to sub globose and pale green. Diameters of 3-4µm. conidiophores are hyaline and finely roughened.

DISCUSSION

The result of this study showed that two species of *Aspergillus*, *A.niger* and *A. flavus* were predominant spoilage organisms on *Moringa oleifera* stem barks. In a related study in India, Moorthy *et al*., (2010) examined the mould profile of 48 herbs and spices collected from different locations. A total of 1210 fungal isolates representing 393 species were isolated and identified by them. The authors reported the occurrence of *Aspergillus* species along with other fungi such as *Curvularia* spp*, Geotricum* spp*, Penicillium* spp *and trichodema* spp. Idu *et al*. (2008) also reported the presence of *Penicillium sp, A. niger* and some bacterial species on medicinal plants in Edo State in Nigeria. The occurrence of different types of fungi in the present study and those of Moorthy *et al.,* (2010); Idu *et al.,* (2008) may be due to the fact that multiple samples were investigated and these samples were collected from various locations while in the present study, only one sample was screened and collection was limited to one location. Dubey *et al*., (2008) reported that traditional methods of collection, storage and transportation are still employed in India till date and hence make herbal raw materials prone to fungal contamination. This was corroborated by the findings of Idu *et al.,* (2008) which reported a higher microbial load in medicinal plants sold in Bini market and kept on cold floor in stalls in very humid tropical conditions. According to Tabassum *et al*., (2010) countries in the tropics have a harsh climate which is usually dried humid and such climate favours the presence of factors that are suitable for fungal growth in food.

The prevalence of fungi as spoilage organisms in other plant materials such as fruits, vegetables, seeds have been reported and in most cases *Aspergillus* species have been isolated (Okwulehie and Alfred, 2010; Amusa *et al*., 2002; Akinmusire, 2011). The occurrence of fungi on herbs was opined to originate from the soil through adherence to the leaves, stems, flowers, seeds and roots of herbs (Adeleye *et al*., 2005).

The findings of this study show the need for routine check of herbal plants during storage and in market places to ensure they are suitable for human consumption. Clearly, there is a public health problem which could arise from the ingestion of contaminated medicinal herbs, especially with such fungi that are known mycotoxins producers and other related harmful metabolites. It is therefore suggested that medicinal plants such as *Moringa oleifera* stem barks should be used within 2-3 days of harvest or immediately as this will not only prevent excessive contamination by fungal pathogens but will eliminate possibilities of contamination with mycotoxins and other related metabolites. The World Health Organization (2007) reported that contamination by deterioration

compromises safety and quality of herbal plants and this is harmful to consumers.

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