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Fungi associated with *Irvingia gabonensis* (Ogbono) and *Colocynthis citrullus*(Egusi) seeds sold in markets in Ado-Ekiti , Ekiti State Nigeria

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ABSTRACT

Irvingia gabonensis are seeds of a small mango-like fruit found in West Africa. The seeds when ground into paste are highly mucilaginous and are used in thickening soup while Colocynthis citrullus (Egusi) are seeds of a creeping annual and an intercropping plant made use of in traditional farming practices. When the seeds are coarsely ground up, the also assist in thickening soups. Fifteen samples each of Irvingia gabonensis (Ogbono) and Colocynthis citrullus (Egusi) seeds were randomly purchased from 5 retailers in each of the three (3) major markets in Ado-Ekiti Ekiti state, Nigeria. The seeds were cultured on potato dextrose agar in the laboratory and incubated at 25° C for 5-7days. The fungi isolated from Irvingia gabonensis were Rhizopus nigricans, Mucor mucedo, Trichoderma viride and Aspergillus flavus. The most frequently occurring fungi was Mucor mucedo, then Trichoderma viride while Aspergillus flavus had the least frequency of occurrence. fungi isolated from Colocynthis citrullus were Aspergillus niger, Mucor mucedo, Rhizopus nigricans, Trichoderma viride and Penicillium italicum of these the most frequently occurring fungi was Rhizopus nigricans followed closely by Mucor mucedo. Trichoderma viride and Aspergillus niger had the least frequency of occurrence. It was concluded that the fungi associated with these seeds were of the group Micromycetes. The significance of these results was discussed.

Keywords: *Irvingia gabonensis*, *Colocynthis citrullus*, Micromycetes**,** Ado-Ekiti.

INTRODUCTION

Irvingia gabonensis is a genus of African and South-East Asian trees in the family Irvingiaceae. The small mango like fruits sometimes called bush or wild mango are eaten raw. The seeds or kernels popularly called ogbono are oily. When used in soup thickening, the seeds are ground, heated to melt the fat, pepper and other spices or seasonings are added.

The soup is then eaten with pounded yam, eba, semovita, agidi(eko) or other carbohydrates meals which are eaten as bolus.

Colocynthis citrullus is mostly cultivated in West Africa, especially in Nigeria. It thrives in tropical, subtropical, desert and temperate regions especially on rich light soil of South-western and Eastern Nigeria [1]. In Nigeria, melon seeds are used in many ways as major soup ingredient and a common component of daily meals. When seeds are coarsely ground up, they used in thickening soups. Some are soaked, boiled fermented and wrapped in leaves to form a favourite food seasoning called Ogiri [2]. The kernels are milled and used as vegetable or for the producing vegetable oil for domestic use because melons contain over 50% oil

Irvingia and *Colocynthis* fruits constitute very important soup condiments in Nigeria. They have high moisture content so need to be properly dried before storage. They are stored because they are seasonal crops. Drying therefore ensures they are available during the off season. Improper drying leads to mould growth on the seeds. The seeds of *Irvingia* contain high moisture as a result they are easily covered with moulds if not properly dried. Moisture and relative humidity led to greater fungal growth and thus low storability of the seeds of *Irvingia gabonensis*[3]. The same trend was noticed in *Colocynthis citrullus*[4].These two seeds were used in this study because of their importance in the daily meal of Nigerians since they are used in preparing two major soups. In any restaurant one is either served egusi or ogbono soup so the importance of these two seeds cannot be over emphasised.

MATERIALS AND METHODS

Collection of Materials

Seeds of *Irvingia gabonensis* and *Colocynthis citrullus* were purchased from 15 retailers randomly selected from the 3 major markets in Ado-Ekiti , Ekiti state, Nigeria.

Isolation of Fungi from seeds

Seeds were surface sterilized immersing in 1% Sodium hypochlorite for 2mins and rinsed in several changes of sterile distilled water, The surface sterilized seeds were plated on Malt extract agar, Potato dextrose agar, Sabouraud dextrose and Yeast-Malt extract agar. To each of the plates 50mg/l chloramphenicol was added [5]. All plates were incubated at 25^{o}C for 5-7 day. Subcultures were on sterile media plates and incubated appropriately.

Identification of fungi isolates

The identification of the isolated fungi was done both macroscopically and microscopically. The gross morphology of the fungal growth on plates was studied including their colors. Later small portions of the fungal pure culture were teased and mounted in lactophenol in cotton blue dye on a clean slide, covered with a clean cover slip and observed under the microscope [6]. The identity of the fungi were certified using cultural characteristics and pathogenicity tests as well as comparing them with confirmed representatives identified by means of key.

Determination of % occurrence of the fungal isolates

This was done to determine the incidence of occurrence of the different fungal isolates. The total number of each isolate in all samples was obtained against the total number of all the isolates in all the samples screened. Frequency of occurrence was therefore determined using method described by [7].

$$\text{Percentage of frequency} = \frac{\text{No. of observations in which a species appeared}}{\text{Total no. of observations}} \times 100$$

RESULTS

The results obtained showed that *Trichoderma viride* and *Mucor mucedo* (30%) had the highest frequency of occurrence followed by *Rhizopus nigricans*(25%) while *Aspergillus flavus* had the least frequency of occurrence (15%) in *Irvingia gabonensis*

In *Colocynthis citrullus*, *Rhizopus nigricans* had the highest frequency of occurrence (28.5%) followed by *Mucor mucedo* (25.5%) *Penicillium italicum* (17.1%). *Trichoderma viride* and *Aspergillus niger* had the least frequency of occurrence (14.3%)

DISCUSSION

The fungi isolated from *Irvingia gabonensis* are similar to those isolated by [8] in which seed rot fungal pathogens of *Irvingia gabonensis* was investigated

It had been previously observed that *Aspergillus flavus*, *Rhizopus spp* were amongst the fungi that reduced the viscosity and storability of *Irvingia gabonensis* [3]. *Aspergillius*, *Rhizopus* and *Penicillium* amongst other organisms have been isolated from shelled melon seeds [9] purchased from markets in some randomly selected towns in some

states in Nigeria. Fungi of the group *Aspergillus*, *Rhizopus*, *Penicillium* amongst others are seed pathogens of some Nigerian melons[10].

Most of these organisms are storage fungi that have been variously implicated in the spoilage of fruits and vegetables. Members of the fungal group micromycetes which include all the fungi isolated from the present work have implicated as being responsible for the spoilage of stored fruits of the family Cucurbitaceae to which *Colocynthis citrullus* belongs [4]. also report have shown the isolation of some micromycetes like *Mucor spp*, *Aspergillus* as causing spoilage of melon seeds in storage [11]

Some of the fungal isolates e.g *Penicillium* and *Aspergillus* are known to be producers of mycotoxins which are secondary metabolites that are known to cause a lot of deleterious effects when consumed in food by man.

Aflatoxin B1 has been found in some melon seeds that were seen to have moulds of the genera *Aspergillus*, *Penicillium*, *Rhizopus* etc[12]. The detection of Aflatoxin in *Irvingia* spp sold in Uyo, Nigeria was reported [13].

The occurrence of *Aspergillus* agrees with the report of [14], where *Aspergillus* was reported as one of the most frequent organisms associated with seed rot of melon. (9) Studies on melon seeds also found that *Aspergillus* was the most frequently occurring mould in market sold melon [9]. It has also been reported that *Aspergillus* was found to be the most frequent fungi contaminating products of plant origin [5], [15] and [16].

The seeds are purchased, ground in the market and the ground seed taken home to make soup. The seeds are not sorted to remove mouldy seeds so moulds will definitely be ground along with the seeds. These moulds if mycotoxigenic, can lead to diseases in due course.

If the moulds are mycotoxigenic, the mycotoxins will be swallowed along with food since they cannot be destroyed by heat or processing.

Most of the moulds may be field to store organisms whose spores may have attached to the seeds during processing of the fruits to extract the seeds and subsequent drying before sending to the market in this process mycotoxins may be elaborated on the seeds by the infecting fungi. Mycotoxin accumulation in fruits and vegetables may occur in the field, and during harvest, postharvest and storage and factors affecting mycotoxin production include the fruit or vegetable type and cultivar, geographical location, climate, pre-harvest treatments, method of harvest, postharvest treatments and storage conditions[17］.

CONCLUSION

Seeds of *Irvingia gabonensis* and *Colocynthis citrullus* sold in Ado-Ekiti markets in Ekiti state, Nigeria are contaminated with fungi of the group micromycetes of which some could be producers of mycotoxin. Farmers and traders alike are hereby advised to use improved methods of storage to discourage proliferation of these organisms on the seeds. Storage facilities should be cleaned out before storing new consignment into them. Buyers of these seeds from the market should examine them closely and remove mouldy seeds before grinding the apparently healthy seeds for use.

Table 1: Fungi isolated from *Irvingia gabonensis* and frequency of occurrence

Fungi isolated from *Irvingia gabonensis* seeds	**Frequency of Occurence of fungal isolates**	**% frequency of Occurrence of fungal isolates**
Rhizopus nigricans	10	25
Mucor mucedo	12	30
Trichoderma viride	12	30
Aspergillus flavus	06	15

Table 2: Fungi isolated from *Colocynthis citrullus* and frequency of occurrence

Fungi isolated from *Colocynthis citrullus* seeds	**Frequency of Occurence of fungal isolates**	**% frequency of Occurrence of fungal isolates**
Rhizopus nigricans	10	28.6
Mucor mucedo	09	25.7
Trichoderma viride	05	14.3
Aspergillus niger	05	14.3
Penicillium italicum	06	17.1

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