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Identification of Fungi Associated with *Irvingia gabonensis* (Ogbono) Seeds Spoilage in Benin City, Nigeria

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ABSTRACT: *Irvingia gabonensis* (Ogbono) is one of the forest tree species of great domestic importance and its postharvest shelve life is affected by various species of fungi. This study is to investigate the effect of various species of fungi that attack *Irvingia gabonensis* seeds in post-harvest. Ready to use cotyledons of Ogbono were obtained from six (6) markets in Benin City (New Benin market, Aduwawa market, Uselu market, Oba market, Ikpoba Hill market, Santana market) Edo State, in sterile transparent polyethylene bags and transported to the laboratory for analyses. The proximate composition of the Ogbono seeds was determined using standard protocols. The pour plate method was used for isolation using a potato Dextrose Agar which was supplemented with streptomycin. The fungi were isolated based on their cultural and microscopic characteristics. The result of the highest and lowest fungal counts were too numerous to count and 3.3×10^3 cfu/g respectively. A total of seven species of fungi were isolated and identified in this study which included *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Mucor* sp., *Neurospora* sp., *Penicillium* sp., *and Rhizopus* sp. *Aspergillus flavus* was the most prevalent fungi with prevalence rate of 48 (90.6%), while *Neurospora* sp. was the least prevalent fungi with prevalent rate of 04 (18.2%). The presence of these fungi especially *Aspergillus* species portrays a serious public health implication as regard food poisoning, mycotocosis and food security. Proper handling methods of Ogbono seeds, coupled with good orientation are necessary to ensure the safety of ogbono seeds.

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Irvinga gabonensis has a variety of important uses; both the fruit and kernel of the seed are edible and thus play important roles in nutritional supplements and food security in West and Central Africa, especially among rural dwellers and its fruits constitute a very important soup condiment in Nigeria Onyekwelu and Bernd (2006). It is rich in vitamin C and is widely consumed as a dessert fruit or snack throughout Western and Central Africa Leakey and Newton (1994). *I. gabonensis* is in high demand due to its nutritional, medicinal, economical worth and agricultural potentials Ndoye *et al.*, (1997); Van, 2010. *I. gabonensis* is especially valued for their fat and protein rich seed a kernel which serves as a sauce thickening agent and oil Matos *et al*., (2009). A major setback in the sales and consumption of *Irvingia* seed kernels is their susceptibility to postharvest spoilage fungi and its intended health risks. Several studies have shown that *Irvingia* seed kernels displayed on shelves for sales in Nigerian markets are often contaminated with spoilage fungi like *Aspergillus flavus* and *A. parasiticus* and in turn the contaminated seed kernels possess aflatoxin, which are harmful to the consumers (Adebayo-Tayo *et al*., (2006); Iyayi *et al*., (2010); Wu and Khlangwiset, (2010). Consumption of high levels of aflatoxin in food has been reported to have caused illness among several hundreds of Kenyans in 2004, and leaving 125 people dead and it is estimated that more than 5 billion people in developing countries worldwide are at risk of chronic exposure to aflatoxins through contaminated foods Lewis *et al*., (2005); Strosnider *et al*., (2006). It becomes imperative therefore to handle postharvest *Irvingia* seeds in ways that would minimize postharvest fungal contamination. Hence the aim of this study was to isolate and identify the post-harvest fungal species that infest *Irvingia gabonensis* cotyledons sold in some markets within Benin City.

MATERIALS AND METHODS

Samples and Materials Preparation: Ready to be used cotyledons of *Irvingia gabonensis* (Ogbono) were obtained from different markets in Benin City (Oba market, Santana market, New Benin market, Ikpoba hill market, Uselu market and Aduwawa market) Edo state, Nigeria. The samples were transported to the LABORATORY for analyses.

Mycological Studies: Enumeration of total culturable fungi (THF): The medium of choice was the potato dextrose agar (PDA) with 10% tartaric acid using the spread plate method. The medium was prepared according to the manufacturer's (oxoid, Basingstoke, Hants) instructions and sterilized at 121℃, 15psi for 15 minutes before dispensing into sterile disposable petri plates. A 0.1ml aliquot of appropriate dilutions of sample was inoculated unto the media. The plates were incubated for 5-7 days at room temperature and

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colonies formed were counted and expressed as cfu/gram.

Identification of fungi isolated: Moulds that are utilizing petroleum hydrocarbon were identified based on preliminary and conventional methods. Pigment production was noted, and wet mount carried out by picking fungal colony onto grease free slide containing two drops of Lactophenol Cotton blue. This wet preparation was covered with cover slip and viewed under ×4 and ×40 objectives lenses. The methods have been previously reported by Chukwurah *et al*., (2007). Preliminary identification of Hydrocarbon utilizing fungi (moulds) was based on the keys and details on Smith's Introduction to Industrial Mycology Onions *et al*., (1981).

RESULTS AND DISCUSSION

The fungal counts obtained from *Irvingia gabonensis* cotyledons in this work are shown in Table 1. The fungal count ranged from 3.3 X 10^3 to a large number of colonies that were too numerous to count (TNTC). This is contrary to the findings of Iyayi *et al*., (2010) whose findings had fungal count ranged between 2.7 X 10^3 and 4.5 X 10^5 with the same dilution factor. This may be due to various environmental factors, climate, pre-harvest treatments and method of harvest.

Table 1: Total population of mould in the samples of *Irvingia gabonensis* collected from various markets in Benin City.

Source of sample	Volume used	Dilution factor	Number of colony	Fungal count cfu/g
Oba market	1 ml	10^2	33	3.3×10^3
Oba market	1 ml	10^4	20	2.0×10^5
Santana market	1 ml	10^2	90	9.0×10^3
Santana market	1 ml	10^4	64	6.4×10^5
New Benin market	1 ml	10^2	88	8.8×10^3
New Benin market	1 ml	10^4	56	5.6×10^5
Ikpoba hill market	1 ml	10^2	50	5.0×10^3
Ikpoba hill market	1 ml	10^4	TNTC	TNTC
Uselu market	1 ml	10^2	80	8.0×10^3
Uselu market	1 ml	10^4	56	5.6×10^5
Aduwawa market	1 ml	10^2	50	5.0×10^3
Aduwawa market	1 ml	10^4	22	2.2×10^5

***Key**: TNTC = Too numerous to count*

The fungal isolates found in the *Irvingia gabonensis* cotyledons samples analyzed at the different market locations are shown in Table 2-7. These isolates includes: *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Mucor* sp., *Neurospora* sp., *Penicillium* sp., *and Rhizopus* sp. Among these, *Aspergillus niger, Rhizopus sp., Aspergillus flavus, Penicillium* sp. *and Mucor* sp. This is in agreement with studies by Agrios, (1978) where these fungi had earlier been isolated from *Irvingia* cotyledons in storage and are recognized to be among the most common group of fungi that infect grains after harvest, and grow on them during storage Agrios, (1978). Researches by Adebayo-Tayo *et al*., (2006), Iyayi *et al*., (2010) and Aboloma and Ogunbusola (2012) also isolated similar fungal species from *Irvingia* species cotyledons/seeds displayed for sale to consumers in some Nigerian markets. *Aspergillus fumigatus* and *Neurospora spp* were isolated in this study; this is contrary to other studies by Etebu and Bawo (2012) where *Candida tropicalis, Phytophthora* sp. and *Fusarium oxysporum* were isolated from the *Irvingia gabonensis* cotyledon. This may be due to differences in geographical location as both works were not conducted on the same location.

Table 2: Distribution of fungal isolates from samples in Oba market

Fungal isolates	No. of isolates	% frequency
Aspergillus flavus	05	25
Aspergillus niger	15	75
Total	20	100

Table 3: Distribution of fungal isolates from samples in Santana market

Fungal isolates	No. of isolates	% Frequency
Penicillium sp.	04	6.3
Aspergillus flavus	48	90.6
Rhizopus sp.	02	3.1
Total	54	100

Table 4: Distribution of fungal isolates from samples in New Benin market

Fungal isolates	No. of isolates	% frequency
Aspergillus flavus	36	56.3
Aspergillus fumigatus	03	4.7
Rhizopus spp	05	7.8
Aspergillus niger	09	14.1
Mucor spp	03	4.7
Total	56	100

Table 5: Distribution of fungal isolates from samples in Ikpoba hill market

Fungal isolates	No. of isolates	% frequency
Aspergillus flavus	40	80
Aspergillus fumigatus	5	10
Penicillium spp	5	10
Total	50	100

Table 6: Distribution of fungal isolates from samples in Uselu market

Fungal isolates	No. of isolates	% Frequency
Aspergillus fumigatus	02	6.3
Penicillium spp	03	9.4
Aspergillus flavus	23	71.8
Aspergillus niger	04	12.5
Total	32	100

Table 7: Distribution of fungal isolates from samples in Aduwawa market

Fungal isolates	No. of isolates	% Frequency
Aspergillus fumigatus	09	40.9
Aspergillus flavus	04	18.2
Aspergillus niger	05	22.7
Neurospora species	04	18.2
Total	22	100

Two fungi species were identified and isolated from the samples collected from Oba market namely *Aspergillus flavus* and *Aspergillus niger* as shown in Table 2. Of the two isolates *Aspergillus niger* had the highest prevalent rate of 15(75%) while *Aspergillus flavus* 5(25%).

Table 3-6 shows the prevalence of fungal isolates in the samples obtained from Santana market, New Benin market, Ikpoba hill market and Uselu market, where *Aspergillus flavus* 48 (90.6%), 36(56.3%), 40(80%), 23(41.1%) respectively had the highest prevalent rate. Table 7 shows the prevalence of Fungal isolates from the samples obtained from Aduwawa market, where *Aspergillus fumigatus* 9(40.9%) had the highest prevalent rate. The dominance of *Aspergillus sp*. here is in agreement with Visconti *et al*., (2001) who reported that *Aspergillus sp.* are the pioneer fungal colonizers in seed-borne infection of Sorghum before other species of fungi arrive. One of the fungi isolated from this study of *Irvingia* cotyledons have the tendency to habour aflatoxin, which if ingested by man or animals, could lead to serious health challenges, and this fungi was *Aspergillus flavus.* Aflatoxins are produced primarily by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* Wu and Khlangwiset (2010). Certain post-harvest conditions have been implicated as pre-disposing factors that lead to fungal attack and aflatoxin production in contaminated food, and some of these post-harvest conditions include storage conditions, duration, transportation and processing methods Wu and Khlangwiset (2010).

Conclusion: The ultimate result obtained from this study is while different fungi are present and responsible for Ogbono spoilage. The presence of these fungi especially *Aspergillus* species is most abundant and responsible for most food spoilage in Benin City and it portrays serious public health implication as regard food poisoning, mycotocosis and food security.

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