

Stereotyping Fungi Affecting Stored Melon Seeds within Local Markets in Lagos, Nigeria

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Abstract

Introduction: Despite the dietary importance and medicinal potentials of melon as domestic remedy for mild infections in humans, melon seeds are vigorously affected by fungal pathogens while in storage. Conventional methods have been used to determine the identity of the pathogens responsible for spoilage of melon seeds but not much have been done in local markets within Lagos, Nigeria.

Objective: This research was aimed at stereotyping postharvest spoilage fungi of melon seeds within local markets in Lagos, Nigeria.

Methodology: Visibly distorted, mouldy and discoloured seeds were selected from healthy and viable melon samples using empirical laboratory procedure. Isolation, characterization and identification of the pathogens were carried out using direct plating technique, morphological diagnosis and international manuals of mycology.

Result: Nine (9) pathogenic fungi were isolated from the diseased melon seeds and five (5) of the fungal strains (*Aspergillus niger*, *A. flavus*, *Penicillium chrysogenum*, *Rhizopus oryzae* and *Cladosporium* spp) tested had severe spoilage effects on the inoculated disease free melon seeds.

Conclusion: This research was able to affirm that *Aspergillus*, *Penicillium*, and *Rhizopus* species were serious threats to the viability of melon seeds in local markets within Lagos, Nigeria.

Keywords: Conventional methods; viability of melon seeds; spoilage fungi; empirical laboratory procedure; Local markets within Lagos, Nigeria.

Introduction

Colocynthis citrullus Linn. (Melon) is a beneficial food crop that transcends cultural, ethnic and religious barriers in Nigeria, mostly valued by the Igbo communities in South-South and South-Eastern, Nigeria [1]. Melon (Family: Cucurbitaceae) is a drought resistant leguminous plant with optimum growth in regions of the world with warm temperate and tropical climate [2]. It is produced in abundance in different parts of Nigeria [3]. The oil extracted from melon seeds are widely used for cooking, production of margarine and salad oil, and as a preservative sauce for canned fish [4]. Melon also contains medicinal properties which are widely used as domestic remedies for urinary tract infection, hepatic congestion, intestinal disorders, catarrh, worm expellant, abnormal blood pressure [2].

Despite the importance of *C. citrullus* as dietary supplement and domestic remedy for mild infections in humans, melon seeds are adversely affected by fungal pathogens in the field and in store houses [1]. Species of *Aspergillus* and *Penicillium* have been predominantly associated with stored melon [5]. Fungal infections in the field occur

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before harvest and can be detected by routine inspection. The effects of field infection decrease while in storage since the melon seeds are kept at optimum storage conditions [6].

Storage fungi are usually not always present before harvest, although, in most cases small quantities of spores or infectious propagules of the pathogens may be present on the melon seeds prior to storage through seed spillage during packaging, exposure to soil in order to facilitate decomposition during processing of the melon fruit, improper handling of the seeds and direct contact with infected storage equipment or structures [7]. This small amount of inoculum can increase rapidly leading to significant seed infection under improper storage conditions [7]. The most common storage fungi are species of *Aspergillus*, *Rhizopus*, *Penicillium* etc. amidst other pathogens that have been listed as seed-borne pathogens of stored melon in Nigeria [8,9].

The effects of these storage fungi on melon seeds include deterioration and spoilage [10,11], reduction in nutrient composition and market value [12] and production of chemical substances that are toxic to human health [13]. Therefore, this research was intended to determine the type of storage fungi found in stored melon seeds within local markets in Lagos State, Nigeria in order to create consumer awareness on the dangers associated with the consumption of infected melon.

Materials and Methods

Sample locations

The satellite imagery of the local markets in Lagos State, Nigeria was shown in figure 1. The markets were divided into stations for ease of identity as stated below:

- **Station A:** Iyana-Iba market, situated along Lagos-Badagry expressway, close to Lagos State University main campus, Lagos State, Nigeria. Latitude 6.458195°N and Longitude 3.204234°E.
- **Station B:** Mammy market, Ojo military cantonment,

Lagos State, Nigeria. Latitude 6.461971°N and Longitude 3.224063°E.

- **Station C:** Ojo central market, Ojo local government area, Lagos State, Nigeria. 6.455097°N and Longitude 3.203976°E.

Sample collection

A total of 500g of shelled and unshelled melon seeds (*Colocynthis citrullus* Linn.) were randomly collected weekly from each of the markets from October to November 2006. The melon seeds were aseptically packaged, labelled accordingly and stored in ice bags while on transit to the Botany Laboratory, Lagos State University, Ojo, Lagos, Nigeria for further analysis.

Sorting and characterization

Visibly distorted, mouldy and discoloured seeds were secluded from the healthy and viable melon seeds using empirical laboratory diagnostic procedure. The isolated diseased melon seeds were characterized according to their point of collection and surface sterilized using 70% Ethanol to eliminate airborne spores and opportunistic microorganisms deposited on the seeds by wind, dust or rain-splash in the course of handling. The sterilized diseased melon seeds were rinsed in three (3) successive changes of sterile distilled water to remove traces of Ethanol in the seeds. The sterilized melon seeds were placed in sterile blotter papers in the lamina air flow chamber to air-dry prior to inoculation and incubation.

Isolation of the causal organism(s)

Standard laboratory procedure for isolation of fungal pathogens from the diseased melon seeds was used [14]. Infected melon seeds initially surface sterilized in 70% ethanol and rinsed in three (3) separate changes of sterile

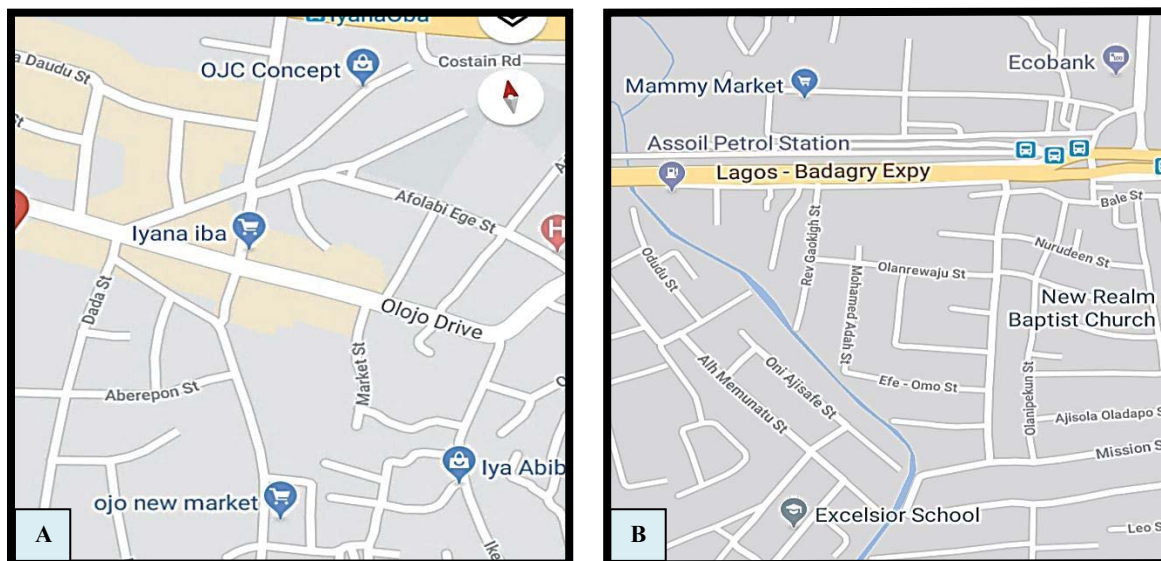


Figure 1: The satellite view of the markets (a) Iyana-Iba and Ojo new market (b) Mammy Market.
Source: Google Satellite Map.

distilled water, were inoculated in batches of ten (10) seed sample per plate using direct plating technique into replicates of freshly prepared 39g per litre of potato dextrose agar (PDA) at full strength. The replicated cultured samples were incubated at standard room temperature ($25 \pm 2^\circ\text{C}$), for a period of seven (7) days and observed daily for fungal growth, after which pure cultures were obtained from direct screening and a series of sub-culturing of the isolates.

Microbial load determination

Pure culture of each isolate were grown on a full strength (39g/L) potato dextrose agar (PDA) slants in 14ml McCartney bottles and used as stock cultures. A 7day old pure culture of the isolates was used to conduct this experiment. The culture medium was flooded with sterile distilled water, swirled and decanted into a sterile container; to extract the spores. 1ml of the spore suspension was calibrated using a spore counter and the number of spores per ml was recorded. The resulting spore values obtained was multiplied by the corresponding dilution factor so as to give a true representative of the actual spore count.

Identification of the causal agent(s)

The identification of the pathogens was done using standard laboratory techniques and international manuals of mycology. Slides were prepared for each isolates and the visibility of the fungi was enhanced by the application of lactophenol in cotton blue dye. The prepared specimens

were examined under a digital trinocular microscope (Olympus CX31 HD Digital microscope). Each isolates was carefully identified based on its mycelia morphology and orientation on culture plates; production of metabolites; and the presence of various fruiting bodies like the sporodochia (macrospores), phialides, microspores, conidia, sporangia and sporangiospores.

Pathogenicity test

In order to establish the fact that the isolates obtained from the diseased samples were actually the causal agents of the disease and the symptoms/signs manifested by the infected stored melon seed samples, a re-introduction of the isolates into healthy melon seeds was carried out using Koch's postulate [15]. Each isolate was inoculated into healthy melon seed samples and incubated in a General Lab model incubator for seven (7) days. At the end of the experiment, the isolates were further re-isolated from the induced disease situation and the new symptoms produced on the inoculated melon seeds were compared with the original symptoms from melon seed samples collected from the markets. The level of deterioration of the melon seeds by the isolates was also measured using a modified method of [16].

Results

The fungal pathogens isolated from the diseased melon seeds were identified thus:

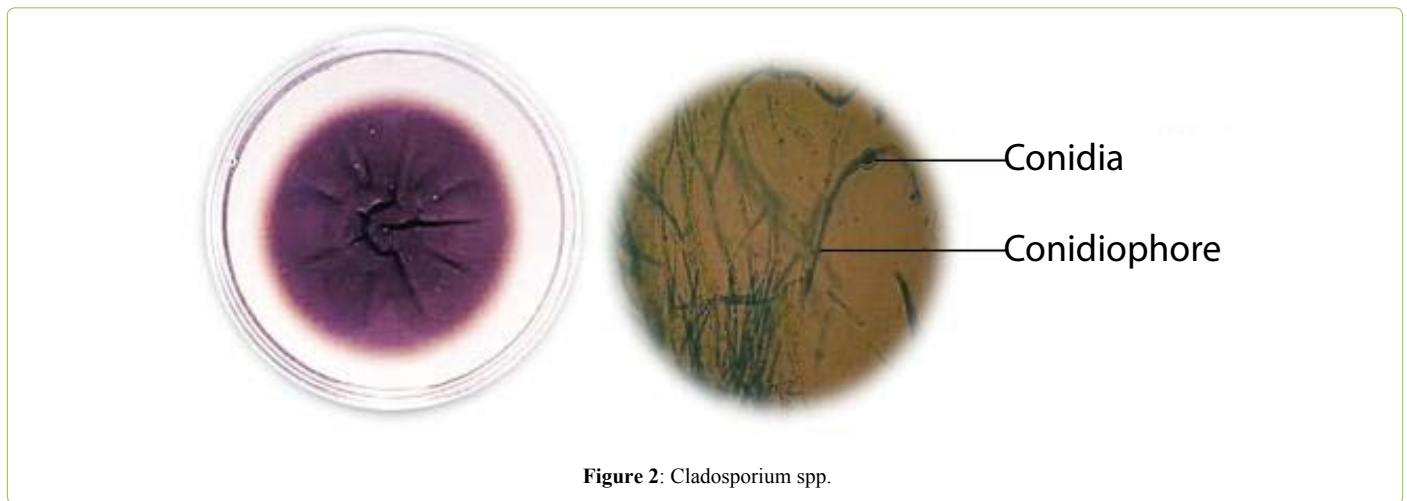


Figure 2: Cladosporium spp.

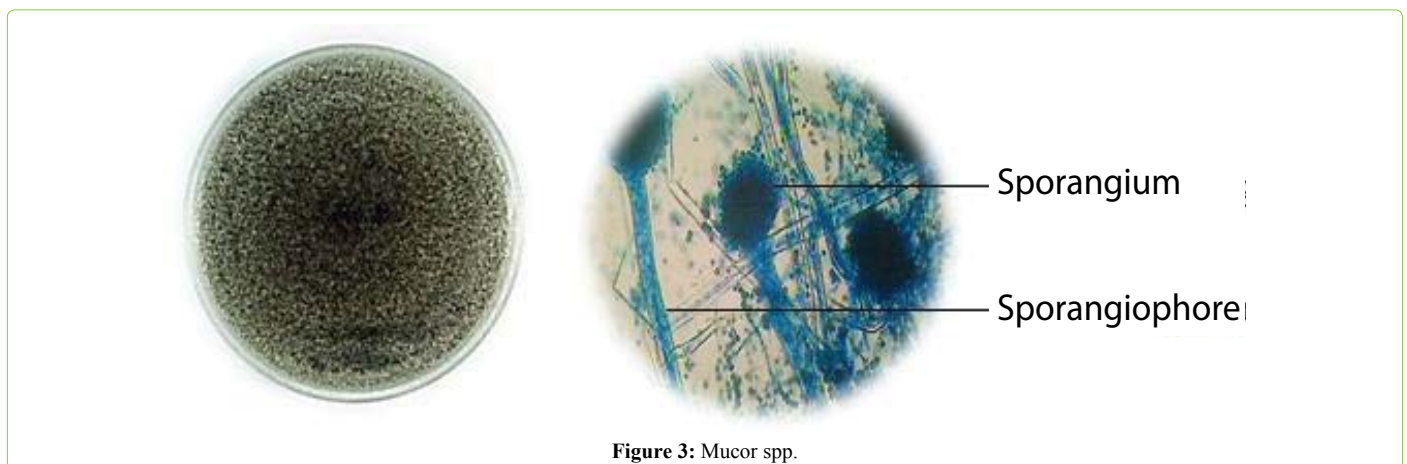


Figure 3: Mucor spp.

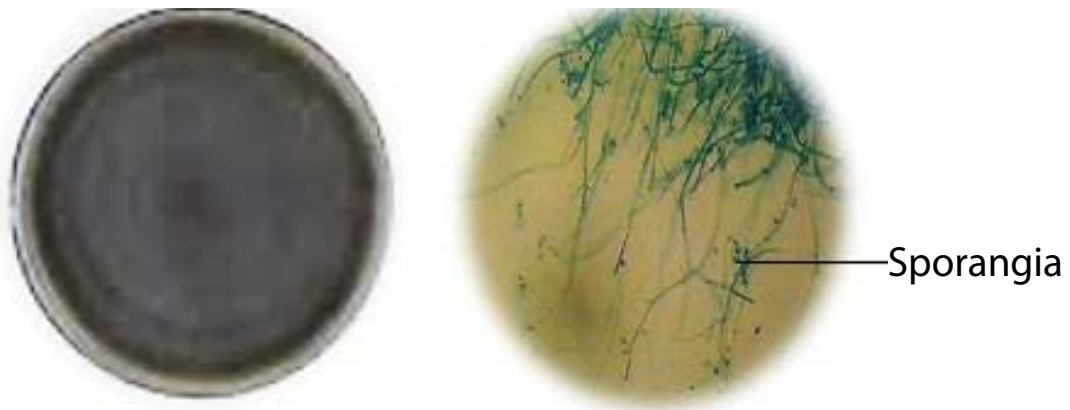


Figure 4: Curvularia spp.

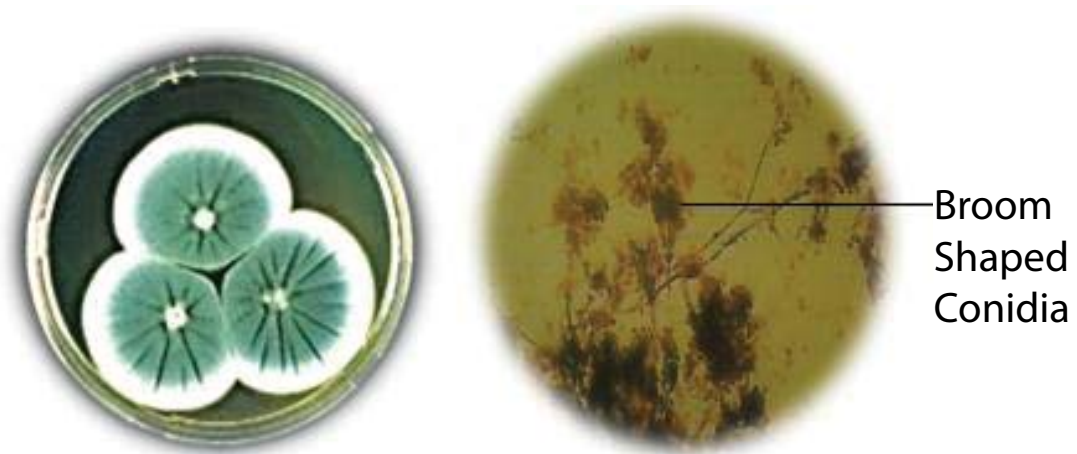


Figure 5: Penicillium chrysogenum.

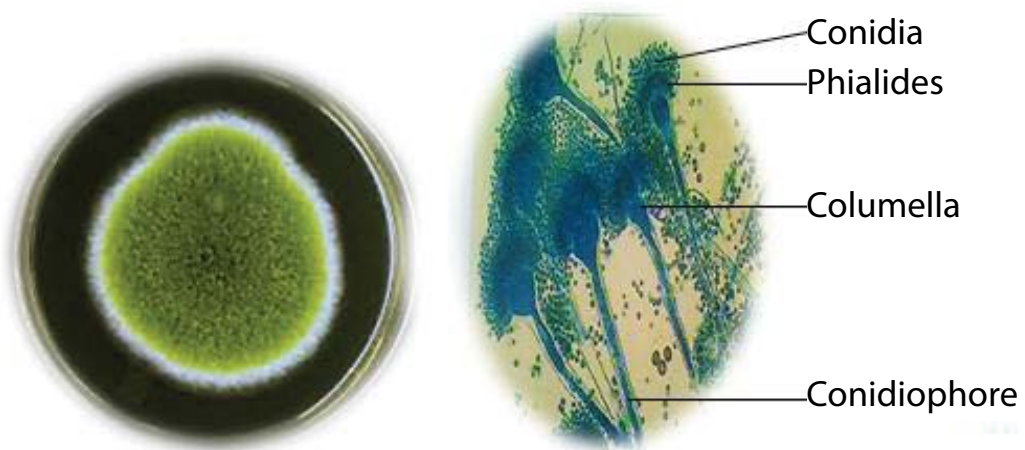


Figure 6: Aspergillus flavus.

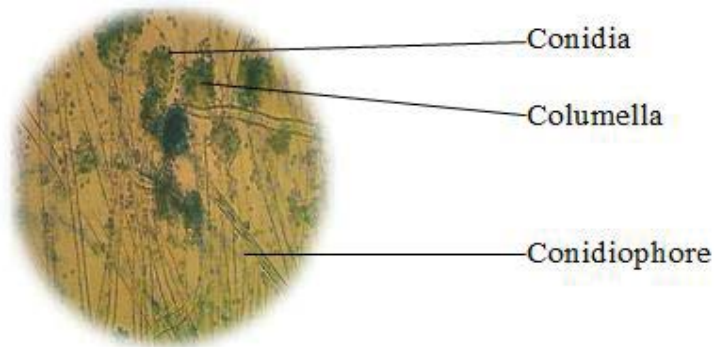


Figure 7: *Aspergillus fumigatus*.

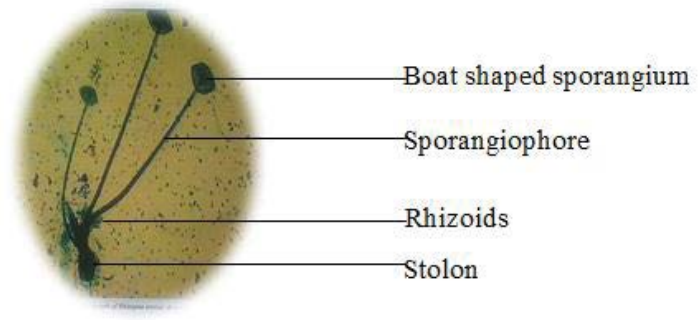


Figure 8: *Rhizopus oryzae*.

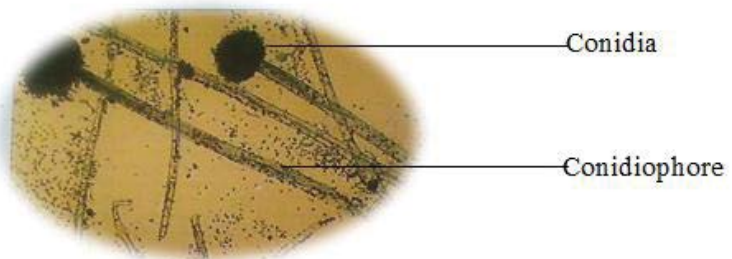


Figure 9: *Aspergillus niger*.

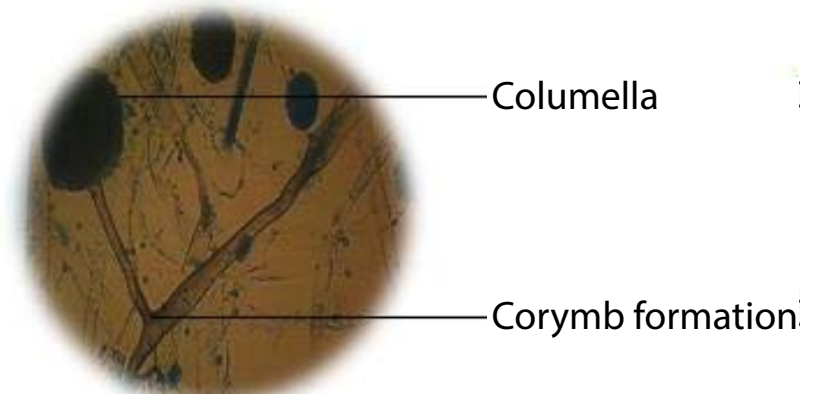


Figure 10: *Absidia corymbifera*.

Aspergillus flavus, *Cladosporium* spp, *A. niger*, *Penicillium chrysogenum* and *Rhizopus oryzae* had severe spoilage effects on the inoculated healthy melon seeds (Table 1), whereas, *Absidia corymbifera*, *Mucor* spp, *Curvularia* spp and *Aspergillus fumigatus* each had a mild deteriorative effects on the inoculated healthy melon seeds during Pathogenicity test (Table 1). Standard regulation for mycotoxin occurrence in stored products was shown in table 2.

Table 1: The effects of the isolates on healthy melon seeds (Pathogenicity test)

S/N	Pathogen	Level of deterioration of Melon seeds
1	<i>Absidia corymbifera</i>	+
2	<i>Aspergillus flavus</i>	+++
3	<i>Aspergillus fumigatus</i>	+
4	<i>Aspergillus niger</i>	+++
5	<i>Cladosporium</i> spp	++
6	<i>Curvularia</i> spp	+
7	<i>Mucor</i> spp	+
8	<i>Penicillium chrysogenum</i>	+++
9	<i>Rhizopus oryzae</i>	+++

Key
 - No spoilage of melon seeds
 + Mild spoilage of melon seeds
 ++ Medium spoilage of melon seeds
 +++ Severe spoilage of melon seeds

Table 2: Standard regulation for mycotoxin occurrence in stored products.

Product	Toxin Level (mg/kg)	Portion
Brassica vegetables	0.1	Head cabbages and kohlrabi: whole commodity as marketed, after removal of obviously decomposed or withered leaves. Cauliflower and broccoli: flower heads (immature inflorescence only). Brussels sprouts: "buttons" only.
Bulb vegetables	0.1	Bulb/dry onions and garlic: whole commodity after removal of roots and adhering soil and whatever parchment skin is easily detached.
Fruiting vegetables	0.05	Whole commodity after removal of stems. Sweet corn and fresh corn: kernels plus cob without husk.
Leafy vegetables	0.3	Whole commodity as usually marketed, after removal of obviously decomposed or withered leaves.
Legume vegetables	0.1	Whole commodity as consumed. The succulent forms may be consumed as whole pods or as the shelled product.
Pulses	0.2	Whole commodity
Root and tuber vegetables	0.1	Whole commodity after removing tops. Remove adhering soil (e.g. by rinsing in running water or by gentle brushing of the dry commodity). Potato: peeled potato.

Note: There are no standard regulations for pathogen(s) occurrence in stored products only standard or safe levels of some mycotoxins.

Discussion

Nine (9) pathogenic fungi were isolated from the diseased melon seeds. This was similarly reported by Kehinde, Nwokocha and Opara, Obani *et al.* (2019) [1,4,7]. They confirmed the fact that fungal pathogens have a high pedigree for postharvest deterioration of melon seeds in the field and in store houses. The pathogenicity test conducted using healthy melon seed samples as surrogate hosts showed that five (5) of the fungal strains (*Aspergillus niger*, *A. flavus*,

Penicillium chrysogenum, *Rhizopus oryzae* and *Cladosporium* spp) tested had severe spoilage effects on the inoculated disease free melon seeds. This was in line the researches carried out by Chiejina, Somorin and Bankole, Fagbohun *et al.* who affirmed the fact that *Aspergillus*, *Rhizopus*, *Penicillium* species are the most common and devastating storage fungi of melon seeds found in Nigeria [5,8,9]. The complexity in microbial pool could be related to the genetic diversity of microbial species present within the storage environment.

Conclusion

This research was able to affirm the fact that *Aspergillus*, *Penicillium*, and *Rhizopus* species were serious threats to the prolong shelf life of melon seeds. The continuous occurrence of these pathogens in stored melon seeds have been proven to cause serious health hazards to humans and as such drastic measures should be taken to limit the prevalence of these organisms in our food so as to minimize the risks of contacting acute diseases from the consumption of infected melon seeds and safe guard millions of lives.

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