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Abnormal Symptoms of Fungi-Induced Morphological Changes in Infected Melon (*Colocynthis Citrullus* Linn.) Seeds During Storage

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Abstract: *Bruising, morphological distortion, decaying, discolouration and mycotoxin production in stored melon seeds ensued partly as a result of several microbial activities within the storage environment. Shelled and unshelled melon seeds were collected from store houses in local markets within Lagos, Nigeria. Disease diagnosis, characterization and Laboratory analysis were conducted on samples collected. Absidia corymbifera and Penicillium spp caused decay and mummification of unshelled melon seeds, Cladosporium spp and Aspergillus fumigatus degraded the unshelled seed hilum, while A. flavus, Curvularia spp and Cladosporium spp caused tanning of the shell of stored melon seeds. Also, A. niger, A. flavus, A. fumigatus, and Penicillium spp caused internal ramification and morphological distortion of unshelled melon seeds. The ability of these storage fungi to degrade melon seeds were mostly restricted by their capacity to survive and thrive within the storage environment.*

Keywords*: Mycotoxin; Stored melon seeds; Microbial activities; Disease diagnosis; Fungi.*

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I. Introduction

The plant family "Cucurbitaceae" comprises of Summer squashes, Marrows, Pumpkins and Melon (Bankole *et al*., 2005). Several species of melon exist worldwide, they include the Winter melon [*Benincasa hispida*], Watermelon [*Citrullus lanatus*] (Zohary *et al*., 2012), Horned melon [*Cucumis metuliferus*] (Njorogo and Van Luijk, 2004), Muskmelon (*Cucumis melo*), The European Cantaloupe (Chiffolo and Hesse, 2006), The Persian melon (Vos, 2010), Casabas, Honeydew, Argos, Canary melon, Hami melon, Sugar melon (Constant, 1986), Tiger melon (Bastyra and Canning, 1990), and Melon [*Cucumeropsis mannii* Naud-Holl (Bankole and Joda, 2004), *Lagenaria siceraria* (Achigan-Dako *et al*., 2008) and *Colocynthis citrullus* Linn.(Obani *et al*., 2019)] popularly known as "Egusi" in Nigeria (Nwokocha and Opara, 2016).

The seeds of *Colocynthis citrullus* Linn. (Egusi) are fermented and eaten as "ogiri" by the Yoruba people of Southwestern Nigeria (Abiodun *et al.,* 2010). It can also be roasted, pounded, fried and then boiled to prepare "Igbalo" (Van der vossen *et al.,* 2004). In Southeastern Nigeria, pulverized melon seeds are eaten with *Pleurotus tuber-regium* (An edible mushroom) which was used as substitute for meat; also, the milled seeds were used as thickener in sauces, soups and stews (Nwokocha and Opara, 2016). Many groups of fungi are known to contaminate "Egusi" seeds during storage causing seed rot, formation of sclerotic layers on seeds, and seed discolouration resulting in deterioration of food reserves in the cotyledon of the seed, reduction of shelf-life, quality/viability of stored seeds, nutrient composition, production and accumulation of toxic metabolites like Mycotoxins in the seeds (Obani *et al*., 2019).

The development of appropriate crop protection measures depend on the knowledge of the diseases symptoms exhibited by the stored melon seeds linked with the implicated causal pathogen(s) (Fagbohun *et al*., 2011). The traditional method of intercropping melon with other crops has helped peasant farmers to evolve crop protection techniques that are readily adaptable (Kehinde, 2013), however, a major constraint to the adoption of this methodology is the difficulty in appropriate identification of disease symptoms before it escalates. Similarly, causal agents of diseases and their peculiar symptoms are major points of reference when control strategies are being developed. For melon, there is paucity of information in the characteristic symptoms associated with each causal pathogen in Southwestern Nigeria (Kehinde, 2013). This issue need to be addressed urgently in order to give the farmer an added advantage in terms of a more unified and targeted control effort to manage the challenges posed by melon diseases in farms and store houses (Kehinde, 2013).

II. Methodology

Sample Collection

Shelled and unshelled melon seeds were collected from store houses in local markets within Lagos, Nigeria. The collected melon seed samples were aseptically packaged, labelled accordingly with date and time of collection, and stored in ice bags while on transit to the Botany Laboratory, Lagos State University, Ọjọ, Lagos, Nigeria for characterization and other Laboratory analysis.

Sorting

Bruised, morphologically distorted, decayed and tarnished melon seeds were selected from the collected samples using standard diagnostic procedure. The excised diseased melon seeds were grouped based on market location. Surface sterilization was done 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 and transportation. The purified samples were rinsed in three (3) successive changes of sterile distilled water to remove deposits or residues of Ethanol in the seeds.

Categorization of melon seeds by their disease symptoms

The second stage of disease diagnosis involved the categorization of the infected melon seeds based on morphological appearance of the selected melon seed samples. The following morphological taxa were defined from the market samples analysed:

- Unshelled melon seeds with decayed hilum
- Unshelled melon seeds with discoloured shell
- Unshelled melon seeds with black dots
- Mummified unshelled melon seeds
- Morphologically distorted and internally ramified unshelled melon seeds
- Shelled melon seeds with transparent base
- Shelled melon seeds with bruised and degraded apex
- Shelled melon seeds with physically damaged base showing signs of deterioration

Isolation of fungal pathogens of melon seed

The isolation of fungal strains from the sorted, purified and categorized melon seed samples was carried out using standard laboratory techniques (As described by Atehnkeng *et al*., 2008). The infected melon seed samples were inoculated on freshly prepared 39g/L of PDA (full strength) reinvigorated with 100 drops of lactic acid using Pasteur pipette to suppress bacterial growth. Inoculation was conducted in batches of ten (10) infected melon seed samples per culture plate with five (5) replicates from each market location. The cultured samples were incubated at 25 ± 2^{o}C, for a period of seven (7) days with constant observation for fungal growth, after which pure cultures were obtained from continuous screening of the incubated samples and a series of sub-culturing of the initial isolates.

Identification of fungal isolates

The isolated fungi were identified using standard mycological techniques and laboratory procedures. Slides were prepared for each isolates using lactophenol in cotton blue stain and examine under a digital trinocular microscope (Olympus CX31 HD Digital microscope). Fungal isolates were carefully identified based on their mycelia morphology and orientation on culture plates, production of metabolites, and the presence of various fruiting bodies like the sporodochia (macrospores), phiallides, microspores, conidia and sporangia.

Classification of fungal isolates based on disease symptoms

The identified fungal pathogens were classified according to the observable disease symptoms produced on the infected melon seed samples and the point of isolation of the pathogen from the diseased melon seeds. Some pathogens were isolated from multiple infection points.

III. Results

The fungal isolates were grouped based on the disease symptoms produced in stored melon seeds (Table 1). *Absidia corymbifera* and *Penicillium* spp caused decay and mummification of unshelled melon seeds, *Cladosporium* spp and *Aspergillus fumigatus* degraded the hilum, while *A. flavus*, *Curvularia* spp and *Cladosporium* spp caused total discolouration of the shell of stored melon seeds. Dotted shells were instigated by the metabolic activities of *A. corymbifera*, *Penicillium* spp and *Curvularia* spp, while *A. niger*, *A. flavus*, *A. fumigatus*, and *Penicillium* spp caused internal ramification and morphological distortion of unshelled melon seeds. *Cladosporium* spp degraded the base of shelled melon seeds leaving behind the transparent waxy and

water repellent cuticle. Also, bruising leading to decay of the naked hilum was influence by *Curvularia* spp and *Cladosporium* spp. Finally, *Rhizopus oryzae*, *Curvularia* spp, *Penicillium* spp, *Mucor* spp, *A. niger* and *A. flavus* each caused bruising and decay of the basal end of shelled melon seeds in store houses (Table 1).

Table 1: Classification of fungi isolates based on disease symptoms

S/N	Disease Symptoms	Fungal Isolates
1	Unshelled melon seeds with decayed hilum	▪ *Cladosporium* spp ▪ *Aspergillus fumigatus*
2	Unshelled melon seeds with discoloured shell	▪ *Aspergillus flavus* ▪ *Curvularia* spp ▪ *Cladosporium* spp
3	Unshelled melon seeds with black dots	▪ *Absidia corymbifera* ▪ *Penicillium* spp ▪ *Curvularia* spp
4	Mummified unshelled melon seeds	▪ *Absidia corymbifera* ▪ *Penicillium* spp
5	Morphologically distorted and internallyramified unshelled melon seeds	▪ *Aspergillus niger* ▪ *A. fumigatus* ▪ *A. flavus* ▪ *Penicillium* spp
6	Shelled melon seeds with transparent base	▪ *Cladosporium* spp
7	Shelled melon seeds with bruised and degraded apex	▪ *Cladosporium* spp ▪ *Curvularia* spp
8	Shelled melon seeds with physically damagedbase showing signs of deterioration	▪ *Rhizopus oryzae* ▪ *Curvularia* spp ▪ *Penicillium* spp ▪ *Mucor* spp ▪ *Aspergillus niger* ▪ *A. flavus*

The fungal isolates with the most entry points of infection in stored melon seeds were *Penicillium* spp, *Cladosporium* spp and *Curvularia* spp (Table 2), each of the isolates had four (4) different points of penetrating stored melon seeds. *Rhizopus oryzae* and *Mucor* spp had the least entry point for infecting stored melon seeds with only one route each (Table 2).

Table 2: The number of entry points of the identified fungal species

S/N	Fungal Isolates	No. of Infection points on melon seeds
1	*Aspergillus niger*	2
2	*Aspergillus flavus*	3
3	*Aspergillus fumigatus*	2
4	*Penicillium* spp	4
5	*Rhizopus oryzae*	1
6	*Mucor* spp	1
7	*Cladosporium* spp	4
8	*Curvularia* spp	4
9	*Absidia corymbifera*	2

Aspergillus niger, *A. flavus*, *Penicillium* spp, *Cladosporium* spp and *Curvularia* spp each had the tendency to infect both shelled and unshelled melon seeds while in storage (Table 3). *Rhizopus oryzae* and *Mucor* spp only had affinity for infection of shelled melon seeds in store houses, while *A. fumigatus* and *Absidia corymbifera* were mostly domiciled in unshelled melon seeds during storage (Table 3).

Table 3: The type of Melon seeds infected during storage

		Seed Infection mode	
S/N	Fungal Isolates	Shelled Melon Seeds	Unshelled Melon Seeds
1	*Aspergillus niger*	+	+
2	*Aspergillus flavus*	+	+
3	*Aspergillus fumigatus*	-	+
4	*Penicillium* spp	+	+
5	*Rhizopus oryzae*	+	-
6	*Mucor* spp	+	-
7	*Cladosporium* spp	+	+
8	*Curvularia* spp	+	+
9	*Absidia corymbifera*	-	+

Key
+ Present
- Absent

The percentage occurrence of each fungal isolates in stored melon seeds was described in Table 4. *Penicillium* spp, *Cladosporium* spp and *Curvularia* spp each had 50% chances of occurrence in stored melon seeds; *Aspergillus flavus* had 37.5% chances of occurrence, while *A. fumigatus*, *A. niger*, and *Absidia corymbifera* each had 25% chances of occurrence in stored melon seeds. *Rhizopus oryzae* and *Mucor* spp had the least chances of occurrence with just 12.5% chances each (Table 4).

Table 4: Percentage occurrence of fungal species on stored melon seeds

S/N	Fungal Isolates	% Occurrence on stored melon seeds
1	*Aspergillus niger*	25.0
2	*Aspergillus flavus*	37.5
3	*Aspergillus fumigatus*	25.0
4	*Penicillium* spp	50.0
5	*Rhizopus oryzae*	12.5
6	*Mucor* spp	12.5
7	*Cladosporium* spp	50.0
8	*Curvularia* spp	50.0
9	*Absidia corymbifera*	25.0

IV. Discussion

This research showed that fungal pathogens were specific in their mode of attack on stored melon seeds i.e. they had preference for causing infection at a peculiar spot on each melon seeds. This observation has not yet been noted by other researchers in the field as at the time of filing this report, and as such, more research interest should be channelled towards this direction to help farmers improve on the disease management strategies developed mainly for stored produce as the pathogens involved are sometimes peculiar to the storage environment. Several species of fungi isolated from stored melon seeds were able to cause infection from multiple entry points on both shelled and unshelled variety of *Colocynthis citrullus* Linn. This observation is a pioneer report in the field of postharvest deterioration of melon seeds and as such there are very few reports (if available) to back up this finding.

It was noticed that some fungal pathogens had preference for attacking shelled or unshelled melon seeds only, while few species of storage fungi were able to cause damage in both forms (shelled and unshelled melon seeds) during storage. This was earlier noted by Chiejina (2006), who stated that more fungi were associated with the unshelled seeds in all the cultivars of melon investigated than with the shelled seeds. The percentage occurrence of fungal species on stored melon seeds varied differentially with their ability to survive and thrive within the storage environment. This was in agreement with the findings of Chiejina (2006) who stated that it is surprising that the percentage incidence of infection by some storage fungi was higher with the shelled melon seeds than with the unshelled samples in all the three melon cultivars and this could be attributed to the handling procedure during the shelling and the ventilation in the storage places.

V. Conclusion

The fungal pathogens responsible for spoilage of melon seeds while in storage are largely dependent on their ability to infect the melon seeds from different entry points and mostly restricted by their ability to survive and thrive within the storage environment.

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