

Incidence of Seed-Borne Fungi on Seeds of *C. Citrullus* (*Colocynthis Citrullus* L.) from Five States of South Eastern, Nigeria

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ABSTRACT

Seed samples from Egusi melon (*Colocynthis citrullus* L.) were obtained from farmers in each of the three local government areas of Ohafia, IsialaNgwa South and Ikwuano in Abia State; Aguata, Aniocha and Orumba in Anambra State; Ishiagu, Ezza and Afikpo South in Ebonyi State, Nkanu, Nsukka and Udeniu in Enugu State; Orlu, Ideato and Mbaitolu in Imo State. The seeds were subjected to seed health test using the blotter paper method. Fungi isolated from the samples were *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus granulosis*, *Botrytoidia theobromae*, *Fusarium solani*, and *Penicillium citrinum*. *Aspergillus* species had the highest incidence in the three local government areas of the five south Eastern States surveyed. The percentage disease incidence these fungal isolates such as *F. solani* and *B. theobromae* were assessed in a decreasing order of ranking in which *P. citrinum* had the lowest disease incidence in the four states assessed. However, no fungus was isolated from seed lots collected from the three local government areas of Imo State.

Keywords: Egusi-melon, isolates, pathogens, percentage disease incidence.

INTRODUCTION

Colocynthis citrullus (L.) is a member of the Cucurbitaceae family which consists of more than 825 species which include fluted pumpkin, water melon and cucumber (Van der Vossen *et al.*, 2004, Obute and Ndukwu 2005; Oduaka and Shippers, 2004). *C. citrullus* has also been referred to in some texts as *Citrullus lanatus* (Oguremi, 1978; Okoli 1984.) *Citrullus vulgaris* (Philip, 1977; Makinde, *et al.*, 2007). Oyolo (1977) proposed that the vernacular name “egusi” be attached to *Colocynthis citrullus* to prevent confusion due to several names given to the crop and its relatives.

C. citrullus seeds are high in protein (34.86%), oil (42.29%) minerals such as Sodium (162.76ppm), Potassium (8.28%), Calcium (1.49%), from (39.71pph) Copper (3.37ppm) and Zinc (13.46ppm) (Abiodun and Adeleke, 2010). Thus *C. citrillus* is a major source of protein in the tropical regions of Africa and Asia where staples are very low in protein. Due to its high protein content *C. citrullus* is used as food. The seeds are roasted and ground into a coarse, nutritional meal which is a local staple in Kalahari regions of Africa. Also in Sudan and Egypt *C. citrullus* seeds are roasted and the pulp is eaten as a dish called ‘tasali’ (Van dervossen *et al.*, 2004).

In Nigeria, *Colocynthis citrullus* seeds are fermented and eaten as “ogiri” (Abiodun *et al.*, 2010). The seeds can also be roasted, pounded, fried and then boiled to prepare a sweater known as ‘Igbalo’ (Van der vossen *et al.*, 2004). In Abia State, ground *C. citrullus* seeds are eaten together with a mushroom *Pleurotus tuber-regium*). This is used as a substitute for meat. (Nwokolo and Sim, 1987). Also, chaff from oil extraction is used to make fried cakes called ‘robo’ which is eaten as a snack. The milled seed is used as thickener in sauces, soups and stews.

C. citrullus is an ethno-botanical crop which occupies a very prominent position in the culture and tradition of the Igbo people of South Eastern Nigeria. In the Abiriba community in Ohafia local government area, Abia State for example, large steamed balls of *C. citrullus* locally called ‘ahu’ are used as gift items during the traditional retirement ceremony known as ‘imeuche’. Despite the importance of *C. citrullus* it is infected by fungal pathogens while growing in the field and during storage. Some of the fungal pathogens of *C. citrullus* include *Sclerotium rolfsii*, *B. theobromae*, *Cercospora citrulina*, *Alternaria cucumerina*, *Collectotrichum lagenarum*, *Fusarium oxysporum* and

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Aspergillus species. Some of the *Aspergillus* spp produce mycotoxins especially aflatoxins. Bankole *et al.*, (2005) reported the presence of Aflatoxins in *C. citrullus* grown in Nigeria.

There are few published research studies on the pathology of *C. citrullus* particularly in the South-Eastern region of Nigeria, hence this work was aimed at isolating and identifying seed-borne fungi associated with *C. citrullus* seeds from the five South-Eastern States of Nigeria.

MATERIALS AND METHOD

The *C. citrullus* seed cultivar used in the experiment was ‘serewe’ which is popular in the South-Eastern part of Nigeria. The *C. citrullus* samples were collected from farmers in three local government areas each of five South-Eastern States namely, Isiala Ngwa South, Ohafia and Ikwuano in Abia State; Aguata, Onicha North and Aniocha in Anambra State; Ishiagu, Afikpo South and Ezza in Ebonyi State; Mbaitolu, Ideato and Orlu in Imo State.

A total of 400 seeds were randomly selected from each sample for seed health test. These seeds were surface sterilized with 2% sodium hypochlorite solution for 2 minutes after which they were raised in three changes of distilled water. The sterilized seeds were plated in 9cm Petri dishes (10 seeds/dish) using standard blotter method (ISTA, 2007). The plates were laid out in Completely Randomized Design (CRD) and incubated at room temperature for seven days. Fungi growing from the seeds were identified using fungi identification guides by Barnett and Hunter (1999) and Alexopoulos *et al.* (2002).

Data collected from the experiment were subjected to analysis of variance (ANOVA) while Least Significant Difference (LSD) was used to test significant difference between means.

RESULTS

A total of seven fungal species namely *A. niger*, *A. flavus*, *A. fumigatus*, *A. fumigatus*, *A. granulosis*, *B. theobromae*, *F. solani* and *P. citrinum* were isolated from the seed lots obtained from each of the three local government areas in Abia, Anambra, Ebonyi and Enugu states except in seed lots from Imo State which had no *P. citrinum*.

Aspergillus species were mostly isolated from all the five South-Eastern States. Seed-lots from Ohafia Local Government Area had the highest percentage occurrence of *Aspergillus* spp such as *A. niger*, *A. flavus*, *A. fumigatus* and *A. granulosis* with 11.85%, 7.50% and 7.18% while *B. theobromae* and *P. citrinum* each had 1.75% occurrence (Fig 1).

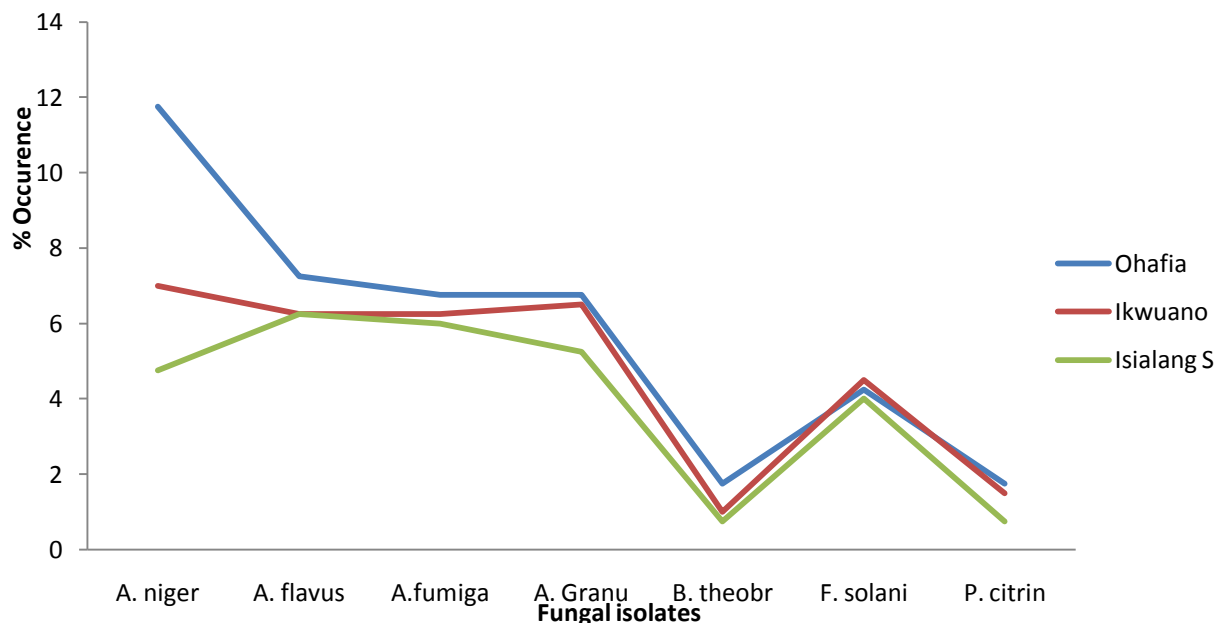


Fig1: Percentage occurrence of fungi isolated from *C. citrullus* seeds obtained from three Local Government Areas of Abia State.

This was followed by seed lot from Ikwuano local government area while the seed lot from IsialaNgwa South had the least percentage of fungi occurrences. The percentage occurrence of *A. niger* in seed-lots from Ohafia, Ikwuano and Isiala Ngwa South local government areas differed significantly from each other. However, there were no significant differences in the percentage occurrence of other fungal isolates from the three local government areas tested.

Fig 2 shows that the percentage occurrence of *A. niger* from the seed-lots obtained from Aguata local government area was 11.75%, while *A. flavus*, *A. fumigatus*, *A. granulosis*, *B. theobromae*, *F. solani* and *P. citrinum* were 6.75%, 6.75%, 6.50%, 1.75%, 4.70% and 2.00% respectively.

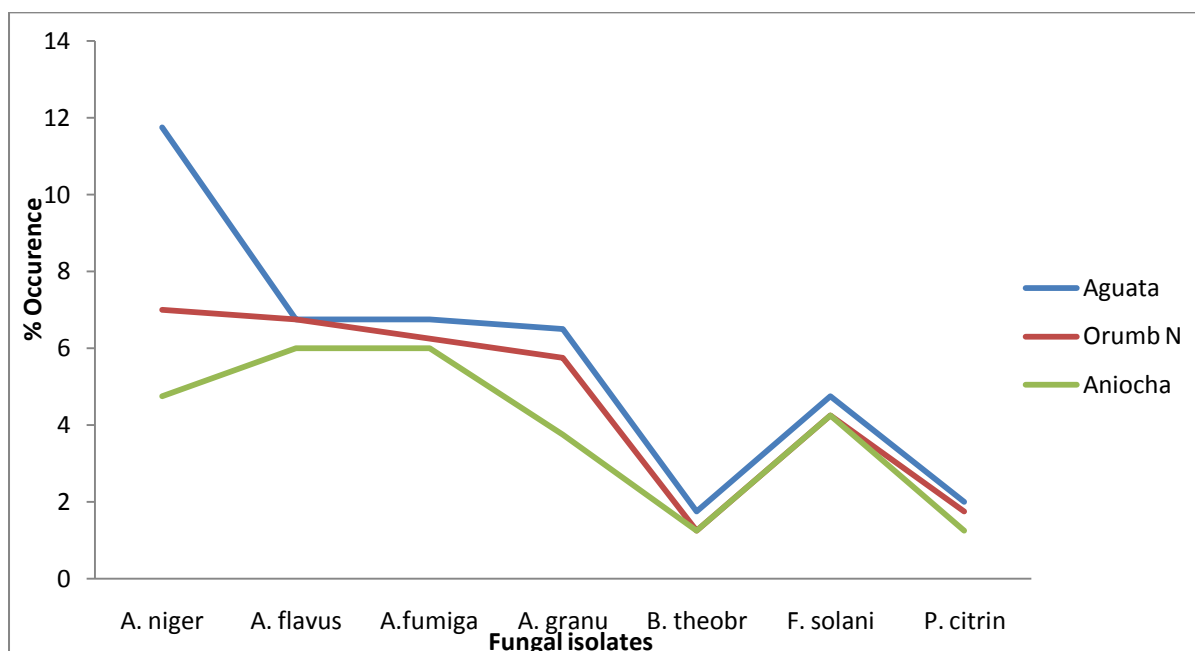


Fig 2: Percentage occurrence of fungi isolated from *C. citrullus* seeds obtained from three Local Government Areas of Anambra State.

But the percentage occurrence of fungi in seed-lots from Orumba local government area were reduced to 7.00%, 5.75%, 4.25%, 1.75% and 1.25% for *A. niger*, *A. granulosis*, *F. solani*, *P. citrinum* and *B. theobromae* respectively. The result also shows reduction in the percentage occurrence of *A. niger* (4.75%), *A. flavus* (6.00%), *A. fumigatus* (6.00%), *A. granulosis* (3.75) and *P. citrinum* (1.25%). Percentage occurrence of *A. niger* in seed lot from each of the local government areas tested in Anambra State differed significantly ($P=0.05$). The percentage occurrence of *A. granulosis* in seed lot obtained from Aguata local government area also differed significantly from seed-lot obtained from Aniocha local government area.

Fig 3 shows the percentage occurrence of all fungal isolates (*A. niger*, *A. flavus*, *A. Fumgatus*, *A. granulosis*, *B. theobromae*, *F. solani* and *P. citrinum*) of seed-lots collected from Ishiagu local government areas were higher than the fungal isolates from Ezza local government area while seed-lots collected from Afikpo South local government area gave the lowest percentage occurrence of *A. niger* (4.75%), *A. flavus* (6.00%), *A. fumigatus* (6.00%), *A. granulosis* (3.70%) and *P. citrinum* (1.25%).

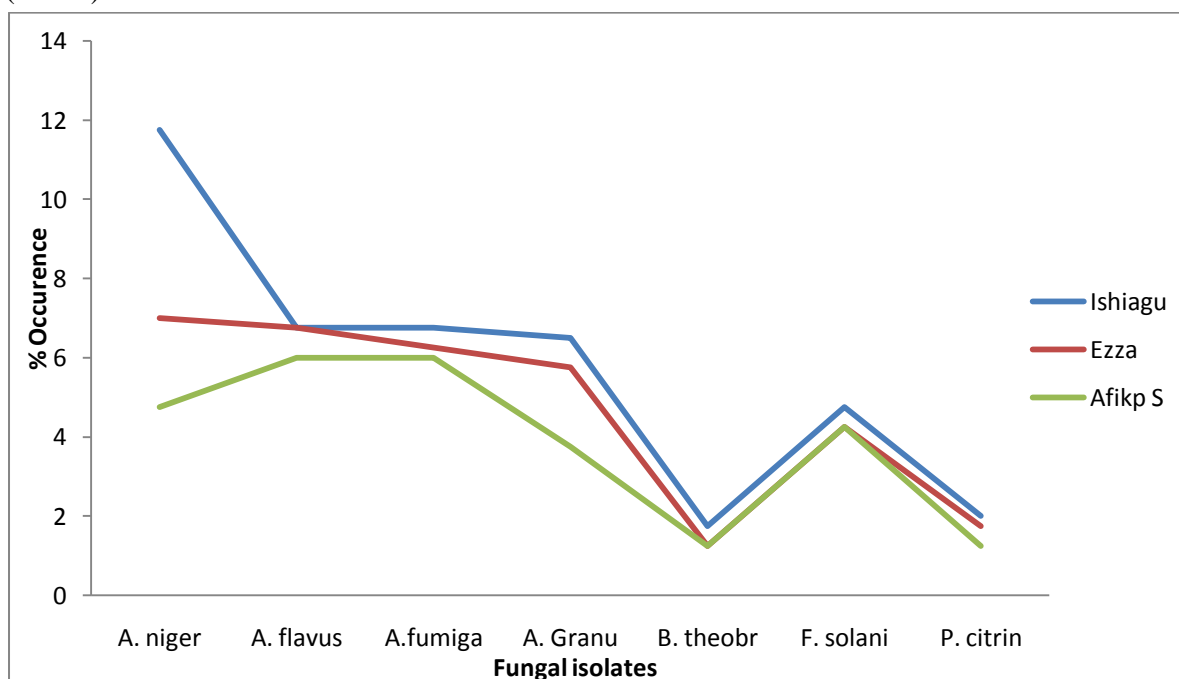


Fig3: Percentage occurrence of fungi isolated from *C. citrullus* seeds obtained from three Local Government Areas of Ebonyi State.

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The result also shows that the percentage occurrence of *A. niger* differed significantly in seed-lots from the three local government areas tested in this experiment. The percentage occurrence of *A. granulosis* in seed-lots from Ishiagu was 6.50% which differed significantly from the seed-lot obtained from Afikpo South local government area. Significant difference ($P=0.05$) in the frequency of occurrences of *A. granulosis* in the seed lots from Ezza and Afikpo south local government areas were also recorded (Fig 3)

Fig 4 shows the result of the percentage occurrence of fungi isolated from Nkanu local government area as follows: *A. niger* (5.75%), *A. flavus* (6.00%), *A. fumigatus* (4.25%), *A. granulosis* (4.00%), *F. solani* (2.50%), *B. theobromae* (1.00%) and *P. citrinum* (0.75%) which were higher than the fungal isolates from Udenu local government area.

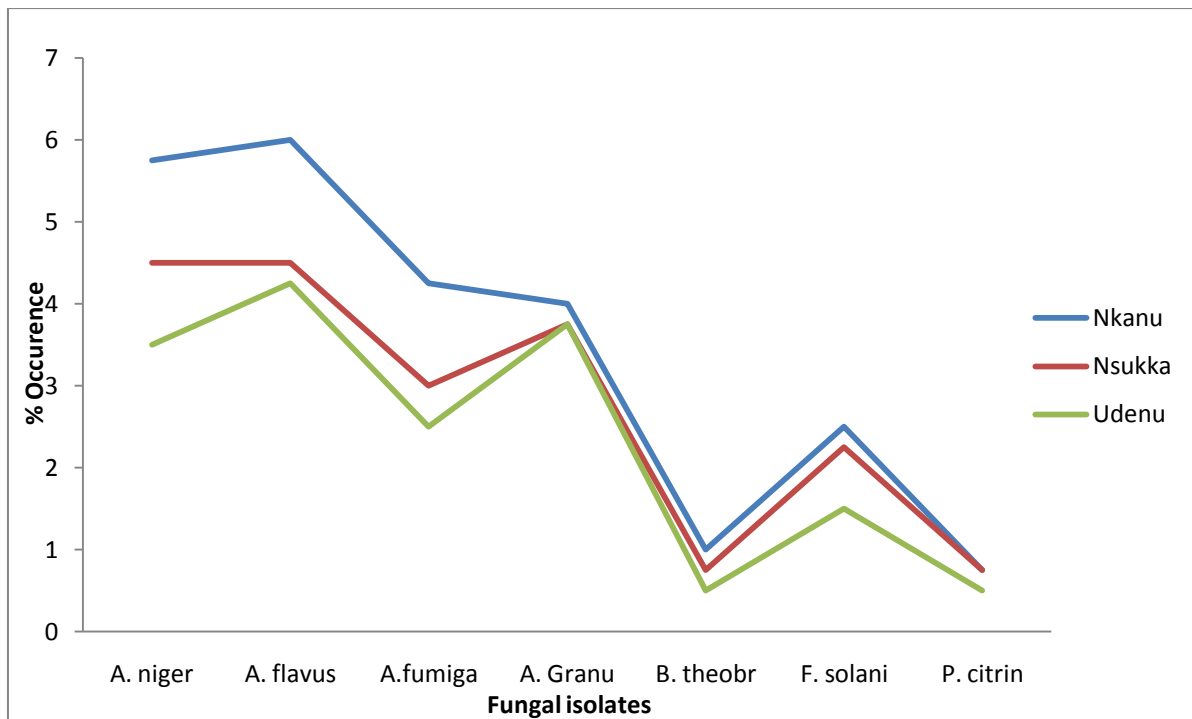


Fig 4: Percentage occurrence of fungi isolated from *C. citrullus* seeds obtained from three Local Government Areas of Enugu State.

The result also shows that except *A. granulosis*, *B. theobromae*, and *P. citrinum* all other fungal isolates of *C. citrullus* seeds from Nkanu local government area differed significantly ($P=0.05$) in their percentage occurrence when compared with seed-lots from Udenu local government area. However there were no significant differences in the percentage occurrence of the fungi in the seed-lots from Nkanu and Nsukka local government areas.

Fig 5 indicates that seed-lot from Mbaitolu local government area had fungal occurrence as follows: *A. niger* (4.00%), *A. flavus* 4.50%, *A. fumigatus* (3.00%), *A. granulosis* (3.25%) and *F. solani* (1.80%). The result also shows *A. niger* (3.75%) from Orlu seed lots and (3.75%) and *F. Solani* (1.50%) from seed-lots collected from Ideato local government *A. flavus* area. The seed-lot from Orlu local government area gave the lowest fungal incidence of *A. flavus* (3.00%) *A. granulosis* (2.50%) and *F.solani*(1.00%).

However there was no record of incidence of *P. citrinum* from all the seed-lots from the three local government areas in Imo State. The result of the analysis shows no significant difference in the percentage occurrence of the fungal isolates from the seed-lots used in this study.

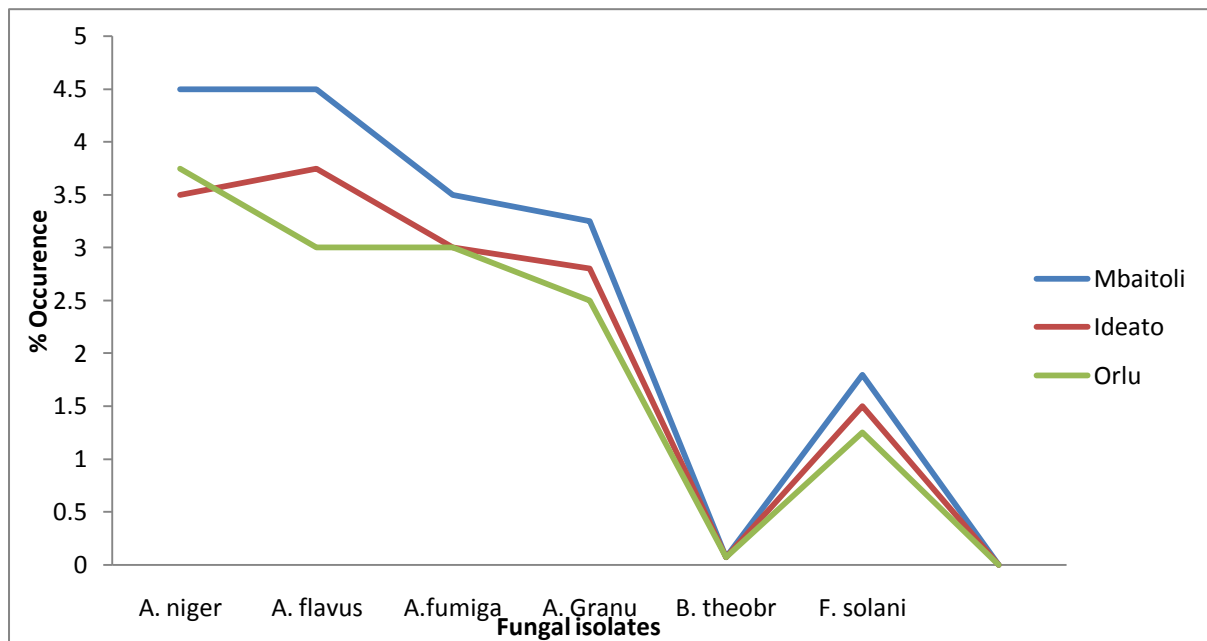


Fig5: Percentage occurrence of fungi isolated from *C. citrullus* seeds obtained from three Local Government Areas of Imo state.

DISCUSSION

The results of this study have shown that *C. citrullus* ('serewe' cultivar) collected from the five South Eastern States of Nigeria were infected by *A. niger*, *A. flavus*, *A. fumigatus*, *A. granulosis*, *F. solani*, *B. theobromae* and *P. citrinum*. Many researchers have reported similar fungal pathogens as isolates of *C. citrullus* grown in Nigeria (Bankole and Joda, 2004; Chiejina, 2006; Kehinde, 2008; Kehinde, 2011)

The study also showed that *Aspergillus* species were mostly isolated from the seed-lots collected from all the local government areas tested in this investigation. Other fungi were isolated in order of decreasing incidence of *F. solani*, *B. theobromae* and *P. citrinum*. This is in agreement with the report of Bankole *et al* (2005), that stored *C. citrullus* harbor many species of *Aspergillus* and a few of *Penicillium*. The reason for this may be due to post harvest handling practices such as threshing and drying which may create wounds on seeds thereby facilitating easy entry of these pathogens.

Some of the fungi isolated from *C. citrullus* seeds are storage fungi which attack stored seeds under high temperature and relative humidity (Amadi and Adeniji, 2009; Aboloma and Ogunbusola, 2012). Moreover, the five South-Eastern State are known for experiencing these environmental conditions that are suitable for the proliferation of these storage fungi. Furthermore, *C. citrullus* farmers also store their seeds in sacks under room temperature which also favour the growth and development of these fungi pathogens. To discourage the attack of these fungi on *C. citrullus* seeds and to preserve healthy seedlots for consumption and cultivation, farmers should be careful during harvesting, threshing and drying practices to avoid inflicting injuries on the seeds which expose them to high risk of fungal infection. They should also dry the seed to reduce their moisture content and store them in air tight containers as high seed moisture content plays a very important role in fungal infection of *C. citrullus* seed.

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