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Evaluation of Post-Harvest Fungi Pathogens Associated with Fluted Pumpkin (Telfairia occidentalis Hook F) Seeds Sold Within Port Harcourt Metropolis in Rivers State, Nigeria

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Abstract

The study on the Evaluation of Post-Harvest Fungi Pathogens Associated with Fluted Pumpkin (Telfairia occidentalis Hook F) Seeds was carried out to investigate the fungal organisms associated with post-harvest rot in fluted pumpkin seeds. The samples of the plant seeds were collected from mile 3 market, Port Harcourt, Rivers State and isolation was carried out using standard microbial methods in the Plant Science and Biotechnology Laboratory, Rivers State University. The seeds were subjected to series of culturing, and sub-culturing under aseptic conditions, after which 4 pure cultures were obtained, of which three were identified as Geotrichum candidum with a 75% occurrence and one identified as Curvularia sp. with a 25% occurrence. Both microscopic and morphological characteristics were used to identify these associated organisms and pathogenicity tests confirmed their involvement in post-harvest rot of fluted pumpkin seeds.

Keywords: *Fluted pumpkin seeds, post-harvedt rot, fungi*

INTRODUCTION

Telfairia occidentalis (Fluted pumpkin) is a green leafy vegetable, it is one of the most common leafy vegetables grown in Nigeria, especially in the Eastern and Southern parts of the country. The plant consists of roots, stem, leaves, fruit and seeds. *Telfairia occidentalis* is a perennial herb widely cultivated as garden and farm vegetable (Chuku, 2015). Its common name is fluted pumpkin, it is also known as fluted gourd. Fluted pumpkin is native to West Africa and it grows in humid tropical climate in well drained fertile soil (Perseglove, 1999). Fluted pumpkin belongs to the Cucurbitaceae family, which is commonly known as gourds or cucurbits. Other members of the Cucurbitaceae family includes; watermelons, cucumbers and melons.

The seeds are sensitive to desiccation and germination, they are dark red and up to 5cm in length. The fruit length measures up to 105cm and 9cm in diameter. When not ripe, the fruits appear light green, and when ripe, it colours yellow. It has been reported that the seeds of *Telfairia occidentalis* which germinate to female plants are larger in size than those that germinate into male plants. There is also speculation among indigenous farmers that seeds extracted from the head and tail portions of the fruit pod develop into male plants, while those extracted from the middle portion of the fruit pod develop into female plants (Ajibade *et al.,* 2006; Akoroda 1990; Akoroda and Adejoro 1990; Nwangwa *et al*., 2007).

MATERIALS AND METHODS

Collection and Identification of Sample

The plant species used for this study was fresh seeds of *Telfairia occidentalis* (fluted pumpkin). The fluted pumpkin was obtained from farmer's zone mile 3 market, Port Harcourt, Rivers State, Nigeria. Sample was obtained within morning hours as farmers arrived with their freshly harvested farm produce. Identification was done by a Plant Taxonomist and Biosystematist in the Department of Plant Science and Biotechnology, Rivers State University.

Preparation of Sample

The fluted pumpkin fruit pod was cut open at both ends using a sharp sterile knife, then, the center of the pod was cut open. Seeds were extracted by hand, and pulp removed. The extracted seeds were dried in the Rivers State University, Department of Plant Science and Biotechnology Screen house and kept at room temperature under humid conditions for disease to develop for 7 days.

Preparation of Culture Media

All materials (conical flask, slides, petri dish, etc.) to be used for the study was first sterilized. Glasswares after washing properly with soap under running water were sterilized at 120^0C in the oven for one-hour, other equipment sterilized using 70% ethanol in order to reduce microbial contamination (Okogbule *et al*., 2020). Inoculating loop and forceps were dipped in ethanol and further flamed over burnson burner before use.

Sabouruad Dextrose Agar (SDA) was the nutrient media used during this study for culturing. Using a weighing balance, 19.5g of powdered SDA was obtained and dissolved in 400ml of distilled water in an Erlenmeyer flask, the opening was covered with foil and shaken to get a homogenous mixture. Then, it was autoclaved 121^0C for 15 minutes, and allowed to cool for about 15 minutes, after which the flask was removed from the autoclave and allowed to settle again for about 10 minutes to bring the temperature to 45^0C. Antibiotics (one capsule of tetracycline) was introduced into the media (this was done to inhibit the growth of bacteria) thereafter, about 15mls of the molten ager was poured plates and content oven dried at 65℃ to obtain a solid culture media.

Isolation of Fungal Pathogens from Infected Fluted Pumpkin Seeds

Using direct plating method, about 5cm were cut from each seed showing visible sign of spoilage between healthy portions and portions of establishment of spoilage. Using a well sterilized scalpel, the sections from each diseased seed were inoculated on to the afore prepared Petri dish containing sterilized Sabauraud Dextrose Agar (SDA) and incubated at 28 ± 3^0C for 3 days and later examined for the growth of the organisms. Pure culture of isolates was obtained after series of sub-culturing and isolations (Okogbule *et al*., 2022).

Subculturing and Purification Test Fungi Isolates

After the establishment of fungal growth, subcultures were prepared using inoculums from the different organisms in the mixed cultures to obtain a pure culture. This was done by transferring hyphal tips from the colony edge of the mixed cultures on to fresh plates of SDA using flame sterilized inoculating needle. After sub-culturing, the plates were properly sealed with masking tape and incubated at 28±2°C until pure cultures were obtained. The resulting pure cultures were used for characterization and subsequent identification of the fungal isolates, using identification guides (Barnett and Hunter, 1998; Hanlin, 1998; Giraldo and Crous, 2019). Stock cultures were maintained on agar slants in McCartney bottles and stored at 4°C in the refrigerator.

Identification of Test Fungal Isolates (Using Wet Mount Preparation)
Materials used were; microscope, microscope slide, coverslips, stain (Lactophenol blue), burnson burner, forceps, ethanol and cotton wool.

The work area was first sterilized using ethanol and cotton wool. The inoculating forceps sterilized by flaming over a burson burner. Thereafter, a colony was carefully picked from each culture plate and placed on a microscope slide upon which a drop of lactophenol blue had been added. A smear was made to obtain a homogenous mixture of the stain and cultured growth, a coverslip was then carefully placed over the smeared slide and gently tapped to eliminate any trace air bubbles. The covered slide was placed under X4 low per magnification, viewed and later magnified to X10 and X40 to get a clearer view of the organism and to identify the fungi based on their colonial morphology, mycelia structure and other associated feature and characteristics compared using an established key. The fungi identification done using the procedure as described by Ainswoth and Bisby, (2022). The identification was based on microscopic and macroscopic features using growth patterns, colour of mycelia, texture and shape.

Pathogenicity Test

Pathogenicity test was carried out on freshly harvested fluted pumpkin seeds using methods described by Shirin and Golam, (2012).

In this process, healthy freshly harvested fluted pumpkin fruit pod were again obtained from farmer's zone, mile 3 market Port Harcourt, Rivers State, Nigeria. The fruit pod cut open and seeds extracted from pulp. The extracted seeds were properly washed under a running tap in the laboratory (Department of Plant Science and Biotechnology, River State University). Under

aseptic conditions, a sterile knife was used to create a small opening of about 1cm into the fresh seed's endocarp and about 5mm mycelia disk from a six-day old culture of *Geotrichum candidum* and *Curvularia* sp were inserted into the small opening of the fluted pumpkin seeds. The inoculated seeds were wrapped with transparent polyethene bags and kept in the laboratory shelf at a temperature of about 28℃ for disease development. After one week of incubation, appearance of spoilage symptoms developed. The pathogen was re-isolated, identified and confirmed using specific morphological characteristics as well as microscopy.

RESULTS AND DISCUSSION

Isolation and Identification of Post-harvest Fungal Organisms Associated with *Telfairia occidentalis* Seeds.

The result from the table shows the cultural characteristics of fungal organisms associated with post-harvest rot of Telfairia occidentalis seeds. From the result, two fungal organisms were obtained. The organisms were: *Geotrichum candadidum* and *Curvularia* sp.

Table1:1 Morphological and Microscopic Characteristics of Fungal Organisms Associated with Post–harvest Rot of *Telfairia occidentalis* Seeds

Fungal isolates	Macroscopic Examination	Microscopic Examination	Fungus	% Incidence
Isolates TP1, TP2, andTP3	Colony colour ranges from white to cream with no reverse pigmentation.	Septate and branched hyphae with hyaline spore colouration, spore shape is subgloboseto cylindrical anthroconidia.	*Geotrichum candidum*	75
Isolate TP4	Colony colour range from brown to velvety-black with fluffy growth.	Septate hyphae with brown colouration, sporesarepalebrown in colour with multicelled, slightly curved shape.	*Curvularia* sp.	25

branch-like hyphae. The colonies are thin and soft (Union *et al*., 1981). *Geotrichum candidum* has several biotechnological importance. It has been used in several biotechnological processes such

as; brewing, enzyme production, production of pharmaceuticals (Eleni *et al.*, 2023). The European Food and Feed Cultures Association (EFFCA) and the International Diary Federation (IDF) has listed *Geotrichum candidum* among the micro-organisms with safe administration in fermented foods (Bourdichon *et al*., 2012).

The colour of *Curvularia* ranges from brown to black, the colonies are usually wooly or fluffy, hyphae are septate and brown and their conidiophores are simple of branched bending to its point of origin. Ekhuemelo and Otor (2021) identified *Curvularia* sp as one of the fungi associated with leaf spot disease of fluted pumpkin.

CONCLUSION

Apart from the deliciousness of fluted pumpkin, used for various dishes especially in Eastern and Southern parts of Nigeria, the plant has so much potential. Fluted pumpkin seeds are prone to attack by fungi which causes post-harvest loss thereby reducing its quality for planting and use. In this study, the fungi pathogens associated with fluted pumpkin seeds were; *Geotrichum candidum* and *Curvularia* sp.

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