MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF POST-HARVEST FUNGAL OF GINGER (*Zingiber officinale*) RHIZOMES FUNGI

**Ikechi-Nwogu, C.G., Odogwu, B.A. and Pikibo, A.**

Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria. Email: *chinyerum.nwogu@yahoo.com, blessing.odogwu@uniport.edu.ng, and elliotpikibo@gmail.com* 
Tel: *+2348032325098, +2348036772711 and +2347067984535

**ABSTRACT**

Ginger (*Zingiber officinale*) rhizome is an economically important vegetable in Nigeria. It is used for medicinal purposes and as spices in many parts of the country. Despite the importance of the crop, it has been observed that during storage, the rhizome shelf-life is reduced by several post-harvest diseases caused by fungal pathogens. A study was conducted to isolate and identify the common fungal pathogens causing post-harvest decay of the rhizomes. Ginger rhizomes were collected from the Ayama Daily market in Abua/Odual Local Government Area in Rivers State. Pathogenicity test was conducted on fresh rhizomes; fungal isolates were collected and morphologically identified. The DNA of the most common fungal isolate, GN-01 was molecularly characterized using Internal Transcribed Spacer 1 (ITS-1) molecular markers. The morphological results indicated that the GN-01 isolate was a *Fusarium* sp. The molecular weight of the DNA of the isolate was over 500 base pairs. Based on sequence similarity, the DNA sequence of the isolate was 94.48% identical to *Nectria haematococca* strain ATSTENI and 93.39% identical to *Fusarium solani* R31 and *F. solani* T-ICA06NP respectively. The fungus *N. haematococca* is commonly referred to by its asexual name *F. solani*. Therefore, these findings showed that *F. solani* is the causal fungal pathogen of post-harvest rot of Ginger. It is expected that these findings will provide information for developing effective disease management strategy for mitigating the post-harvest losses caused by *F. solani* and also provide the basis for further study of potential mycotoxin effect of consuming diseased Ginger rhizomes.

Key words: *Zingiber officinale*, *Fusarium solani*, pathogenicity, and RBCL marker
INTRODUCTION
Ginger (*Zingiber officinale*) rhizomes is an important vegetable in Nigeria that has several medicinal and health benefit (Keenan, 2012). Nigeria is the third main producer and exporter of ginger in the world (Onuba, 2019). With its annual world production surpassing 1.5 million tons, Ginger is now one of the most important trade commodities in the world. The several forms in which Ginger is traded such as aroma, pungency, high oil and aleoresin content has led to an increase in the consumption of this crop (Onuba, 2019). Despite the importance of the crop, it has been observed that during storage, the rhizome shelf-life is reduced by several post-harvest diseases caused by fungal pathogens. Several classical methods have been used to identify these pathogens; however, these identification may sometimes be cumbersome, time consuming and sometimes not accurate. Proper identification of post-harvest fungal pathogens are pertinent because diseased Ginger rhizome caused by mycotoxin-producing fungi could be consumed resulting to serious health hazards. To accurately identify these pathogens, molecular characterization technique has been employed to authenticate the identification of the fungi pathogens (Gontia-Mishra et al., 2013; Bechem and Afanga, 2017). Therefore, this study was aimed at identifying the fungal pathogens associated with the post-harvest diseases of Ginger rhizomes using macro- and micro-morphological identification, and validating the identification using molecular characterization technique.

MATERIALS AND METHODS
Source of plant material
Fresh rhizomes of ginger were obtained in January 2019 from Ayama Daily Market in Abua/Odua Local Government Area in Rivers State.

Isolation of Fungi from Ginger (*Zingiber officinale*) using Blotter Method
Fungal pathogens associated with ginger were isolated using standard blotter method recommended by ISTA, (2016). The Petri-dishes were lined with 3 layers of the sterilized filter paper, which was soaked with distilled water. The ginger rhizomes were surface sterilized in a beaker using 70% ethanol, plated and incubated for 7 days at temperature of 25 ± 2°C at the Pathology/Mycology laboratory of the Department of Plant Science, University of Port Harcourt, Rivers State, Nigeria. The most common fungal isolate was coded (GN-01).

Morphological and microscopic characterization and identification
The fungal mycelium of the isolate GN-01 was cultured on potato dextrose agar medium at room temperature for 7
days. The morphological identification of isolate GN-01 was conducted by visually observing the mycelium and comparing with the pictorial guide by Snowdon (1990). Colonies were compared for their diameters, overall colors, colors of conidia, reverse colors, texture, zonation and sporulation. The isolate was also subjected to microscopic analysis for its characterization and identification using a light binocular microscope at X40.

Molecular characterization using the **Internal Transcribed Spacer (ITS) marker and identification**

The Genomic DNA of the isolate GN-01 was extracted following the protocol of Quick-DNA™Fungal/Bacterial Mini-PrepKit (Zymo Research Group, California, USA) as described by the manufacturer, with modifications at the Regional Center for Biotechnology and Bioresources (RCBB), University of Port Harcourt, Rivers State, Nigeria. The GN-01 isolate DNA quantity and concentration were measured using the Nanodrop 2000c spectrophotometer (Thermo fisher Scientific Inc. Wilmington, Delaware, USA). The DNA purity was measured as a ratio of absorbance at 280 nanometer (nm) to that of 260 nanometer. The quality of the DNA of the isolate GN-01 was further quantified using the Agarose gel electrophoresis performed according to the modified method of Saghai-Maroof et al. (1984). The DNA sample of the GN-01 isolate was shipped to the International Institute of Tropical Agriculture (IITA) Bioscience Center, Ibadan, Nigeria for amplification and sequencing. The primers used to amplify fragments of the nuclear ribosomal DNA (rDNA) of the GN-01 isolate were the Internal Transcribed Spacer 4 (ITS4) with the sequence TCCTCGCTATATTGATATG and ITS5 with the sequence GGAAGTAAAAGTCGTAACAAGG. The amplicons were sequenced using the ABI 3500 capillary electrophoresis sequencer. The DNA sequence file was saved in the Bioedit file with extension .ab1. The sequence was analyzed using the Molecular Evolutionary Genetics Analysis (MEGA) version 7.0.26 software, and aligned using the Basic Local Alignment Search Tool for nucleotide (BLASTN) 2.8.0 version of the National Center for Biotechnology Information (NCBI) database.

**RESULTS**

**Isolation, morphological and microscopic identification of fungi associated with Ginger**

The result of the fungal isolation is presented in Plate 1a and the micrograph is presented in Plate 1b. The isolated fungus GN-01 was found to be associated with ginger. The isolate was observed to develop a whitish mass of
mycelia which turned grey with age. From the photomicrograph, the isolate was identified as a *Fusarium* sp.

**Molecular characterization using the Internal Transcribed Spacer (ITS) marker and identification**

The genomic DNA of the isolates GN-01 of ginger was successfully extracted. The NanoDrop result showed that the concentration of the DNA of the isolates was 15.3 ng/µl. While the absorption peak of the 260nm/280nm readings was 1.73 and the 260nm/230nm readings was 0.1. The result of the Amplified PCR product generated from GN-01 isolate is shown in Plate 2 below. The amplified DNA showed a band on gel when observed under UV light. From the result, the ladder used indicated that the GN-01 isolate sequence had over 500 base pairs.

The result of the GN-01 isolate sequence alignment is presented in Fig. 3. The result indicated that GN-01 isolate sequence aligned with 100 sequences deposited in the composite biological database of National Center Biotechnology Information (NCBI). The percentage identity ranged from 93.31% to 94.48%. The GN-01 isolate sequence was 94.48% identical to *N. haemato-cocca* strain ATSTENI (orange arrow), and 93.39% identical to *F. solani* T-ICA06NP and *F. Solani* R31 respectively (blue arrows). These findings showed that isolate was a *N. haemato-cocca* or *F. Solani*.

**DISCUSSION**

Fungi are significant destroyers of food-stuffs during storage, rendering them unfit for human consumption by retarding their nutritive value and producing mycotoxins (Moreira *et al.*, 2013). Some fungal species have been reported globally to cause soft rot disease in ginger and they belong to different genus including *Fusarium*, *Pythium*, *Acremonium*, *Colletotrichum*. Moreira, *et al.*, (2013) in their work, identified the following *Acremonium murorum*, *Acrostalagmus luteo-albus*, *Fusarium* sp., *F. oxysporum*, *Lasiodiplodia theobromae* and *Sclerotium rolfsii* as the fungi causing ginger rhizome rot in Brazil. In the study conducted by Rosangkima *et al.*, (2018), *F.oxysporum*, *F. solani* and *Plectosphaerella cucumerina* were also isolated and identified from soft rot diseased ginger rhizomes.

According to Stirling (2009), the major causes of post-harvest losses of ginger rhizome is decay or wet rot caused by the fungus *F.oxysporium*, dry rot caused by the fungus *F.solani* and *F.sporium* and *Pythium myriotylum*.

In this study, *Fusarium* sp. was identified as a causal pathogen associated with post-harvest spoilage of Ginger rhizome,
and characterized by a small, brownish, irregular, water-soaked patches of *Fusarium* rot over the rhizomes and white mycelium is observed over the infected areas. This is similar to the finding by Belay *et al.* (2012), in which *Fusarium* spp. was associated with post-harvest deterioration of ginger rhizome samples collected from Hadaro-Tunto and Boloso-Bombae, Southern Ethiopia. This may be associated with the fact that *Fusarium* spp. is a soil-borne fungus and infection by *Fusarium* spp. is typically associated with wounds or insect and nematode damaged tissue (Adams and Moss, 2000; NARI, 2004). Therefore care should be taken during harvesting and drying of ginger rhizomes to minimize injuries and damages to discourage the infestation of *Fusarium* spp. Apart from temperature, rainfall, and humidity, the fungal contaminated air in open air markets where Ginger rhizomes are sold, may be more or less responsible for fungal infection in storage condition. A study of bio-aerosol in a market environment showed that most of the fungi found on vegetables and fruits originated in the field or developed during transportation and storage (Belay *et al.*, 2012). The spoiled materials, dumping plant material and debris present in the market places are likely to act as reservoirs of plant fungal pathogens. These fungi takes part in biodegradation and deterioration of the foodstuff because of their requirement for prime sources of carbon, nitrogen and other nutrients (Belay *et al.*, 2012). Mycologists have traditionally used morphology as a sole means of identifying fungal species (Hyde *et al.*, 2010; Huzefa *et al.*, 2017). The use of morphology in fungal species identification is very important to understand the evolution of morphological characters (Huzefa *et al.*, 2017). However, morphological approaches to fungal systematics, although routinely used in fungal taxonomic studies for classification of fungi at the ordinal or familial level, (Wang *et al.*, 2016; Huzefa *et al.*, 2017) may not always perform well for lower-level (species) classifications (Huzefa *et al.*, 2017). Moreover, identifying fungi based on morphology alone can be challenging, especially when non-experts are dealing with cultures of fungi, since there are a limited number of morphological characters that can be used for identification. As a consequence, DNA sequence-based methods have emerged for identifying species within the mega diverse fungi (Huzefa *et al.*, 2017). In this study, the DNA sequence alignment result (Fig. 3), indicated that the GN-01 isolate sequence was 94.48% identical to *N. haematococca* strain ATSTENI and
93.39% and identical to \textit{F. solani} R31 and \textit{F. solani} T-ICA06NP respectively. \textit{N. haematococca} is commonly referred to by its asexual name \textit{Fusarium solani}. It is a member of an evolutionary group called the \textit{F. solani} species complex (Summerbell, 2003). Therefore, the GN-01 isolate is a \textit{F. solani} fungus causing post-harvest losses of Ginger rhizomes. \textit{Fusarium solani} fungus is a mycotoxin-producing fungus (Llorens et al., 2006; Eltariki et al., 2018). Therefore, this study will enhance the knowledge of the identification of fungal species associated with post-harvest losses of important food and income security crops, and also enhance storage disease management strategy.

**CONCLUSION**

\textit{Fusarium} spp. have been found to be associated with post-harvest spoilage of Ginger rhizomes. However, molecular characterization through sequencing of the Internal Transcribed Spacer (ITS) regions was effective in the identification of the specific \textit{Fusarium} spp., which is \textit{F. solani} fungus causing post-harvest losses of ginger rhizomes.

Plate 1a: Pure Culture of Fungus Isolated from Ginger on Potatoes Dextrose Agar. 1b: micro-morphological presentation of isolate GN-01
Plate 2: Amplified PCR product generated from GN-01 isolate

REFERENCE


