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RESEARCH ARTICLE



# Identification and characterization of fungi pathogen causing fruit rot disease of watermelon (*Citrullus lanatus*)

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**ABSTRACT**

Postharvest rot caused by *Fusarium* species is considered one of the most devastating diseases limiting production and commercialization of water melon worldwide. Two economically important fungi species are generally responsible for fruit rot in watermelon. The aim of this study was to identify and characterize the fruit rot causing pathogen in watermelon in Ibadan Nigeria. Symptomatic watermelon leaves and fruits expressing severe wilt and rot were collected and cultured on solidified Potato dextrose agar. Pure culture of the isolated pathogen was identified based on morphological and molecular characterization as a new species of *Fusarium* viz. *Fusarium chlamydosporium*. The pathogenicity of *F. chlamydosporum* confirmed on watermelon and golden melon fruits revealed its capability to cause infection on healthy fruits. Golden melon fruits artificially inoculated with *F. chlamydosporum* isolate had higher rot diameter (81.7 mm) than watermelon (40.0 mm), disease incidence and severity also was significantly higher in golden melon with 88.3% and 100.0% respectively. To our knowledge this is the first report of *F. chlamydosporum* as a causal pathogen of watermelon wilt and fruit rot disease in Nigeria.

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**KEYWORDS**
*F. chlamydosporum*; infection; inoculated; isolate; symptomatic; wilt

## Introduction

Watermelon (*Citrullus lanatus*) is a tropical plant of the cucurbitaceae family (Amadi et al. 2014) which is of great economic importance due to its vital role in human nutrition, poverty reduction and enhancement of socio-economic status of the farmers through income generation (Kim 2008). The fruit is highly nutritious and a good source of potassium that helps in controlling blood pressure and to prevent stroke. Watermelon fruit contains vitamins C and A in the form of beta-carotene (IITA 2013).

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The watermelon fruit is high in amino acids such as citrulline, the body uses citrulline to make another amino acid, arginine which is used in the urea cycle to remove ammoniacal from the body Figueroa et al. (2017). Watermelon, a dessert fruit and a delectable thirst quencher in the very dry parts of Africa is relished by both man and animals as a source of water as the seeds are source of food particularly protein and oil (Hassan et al. 2008). Dehulled watermelon seeds were reported to contain about 50% fat and 35% protein and dietary fibre (Biswas et al. 2017).

Over the years, production of watermelon is widely and mostly from the northern part of Nigeria due to suitable weather condition but the trend now is changing as other regions of the country now grow the crop largely due to its high nutritional value and the demand across the country making its production financially profitable (Adeoye et al. 2009).

Diseases play an important role in reducing the quality and quantity of fruits and vegetables during production and storage. Watermelon like other plants in the Cucurbitaceae family is affected by several diseases caused by fungi, some of which are Alternaria leaf spot/blight (*Alternaria cucumerin*), anthracnose (stem, leaf and fruit) (*Colletotrichum orbiculare*), belly rot (*Rhizoctonia solani*), black root rot (*Theilaviopsis basicola*) and Fusarium fruit rot caused by *Fusarium equiseti* (Roberts and Kucharek 2006).

Postharvest rot caused by *Fusarium* species is considered one of the most devastating diseases limiting production and commercialization of water melon worldwide (Mahdikhani and Davoodi 2016). The fungal genus *Fusarium* has a cosmopolitan distribution and includes a vast number of species. These species are commonly recovered from a variety of substrates including soil, air, water and decaying plant materials (Leslie and Summerell 2006). They have diverse ecosystem functions in soils and are also able to colonize living tissues of plants and animals, including humans, acting as endophytes (microbial organisms existing inside plant tissues), secondary invaders or becoming devastating plant pathogens (Nelson et al. 1994). In addition to their ability to colonize a multiplicity of habitats, *Fusarium* species are present in almost any ecosystem in the world (Leslie and Summerell 2006).

Several species of *Fusarium* have been identified as causal pathogen(s) of watermelon rot. In Thailand, species of the *Fusarium incarnatum-equiseti* have been reported as the causal pathogen of melon rot (Wonglom and Sunpapao 2020), same species have also being reported in China to cause melon rot (Li et al. 2019). In another report, *Fusarium sulawesiense* have been linked with melon rot in Brazil (Lima et al. 2021). Based on the information on postharvest rot in melons, the possibilities that post

harvest rot of water melon may be caused by diversity of *Fusarium* species not previously reported may not be ruled out hence the objectives of the study. The study was carried out with the aim of identifying and characterizing *Fusarium* species associated with rot of melon fruits in Ibadan, Oyo State, Nigeria and to establish the pathogenicity of the identified isolates.

## Materials and methods

### *Collection and isolation of pathogens from diseases tissue*

Watermelon fruit showing rot symptoms were collected from the Vegetable Research field of National Horticultural Research Institute (NIHORT), Ibadan South western Nigeria. The infected portions were cut into smaller pieces using sterile scalpel and surface sterilized for 1 min in 1.0% sodium hypochlorite solution. The sterilized samples were rinsed in three changes of sterile distilled water (SDW) and blotted dry using sterile filter papers. The dried tissues were placed in 9 cm petri-dishes containing solidified chloramphenicol modified potato dextrose agar (PDA) to suppress bacterial contamination. The inoculated plates were incubated at room temperatures 30 ± 2 °C for 5 days after which sub-culturing was done to obtain pure culture of the isolate.

### *Pathogenicity*

The Pathogenicity of *Fusarium chlamydosporum* was tested on two melon types (Watermelon and Golden melon). Healthy watermelon and Golden melon fruits were collected from Vegetable Research unit of NIHORT, Ibadan. Six fruits each were surface sterilized using 70% ethanol, washed severally under running tap water and blotted dried using sterile cotton wool. A hole was bored on each fruit using 5 mm sterile cork borer and mycelia plug (3 mm diameter) taken from 7-day culture of the isolate was introduced into the hole bored on the fruits. The hole afterwards was sealed with Vaseline petroleum jelly, control samples were inoculated with a sterile PDA without mycelia growth. The inoculated fruits were carefully arranged in separate clean bowls moistened with wet absorbent cotton wool to create micro humidity environment. Diameter of rot was assessed from the point of inoculation and converted to rot indices on a scoring scale of 1–5 using formula adopted from Colle et al. (2014) with some modifications where 1= no symptom, 2 = 1–20 mm rot diameter, 3 = 21–40mm, 4 = 41–60 mm, 5 = above 60 mm. Percentage rot incidence was calculated according to the formula,

$$WI = n/N \times 100.$$

where WI, % incidence; *n*, number of fruits showing rot symptoms; *N*, number of fruits per treatment.

To fulfil Koch postulate, *Fusarium chlamydosporum* was re-isolated from infected fruit and compared with the initial original isolate.

## Isolates identification

### Morphological identification

A mycelial plug (diameter 3 mm) was inoculated in the middle of PDA medium and incubated at room temperature (30 ± 2 °C). The appearance of the colonies, the occurrence of sectors, and the growth of the pathogen was described after 7 days of incubation. The isolated pathogen was identified based on their colony and spore morphology under a microscope according to Boerema et al. (2004).

## Molecular characterization

Fungi DNA extraction was done using a modified protocol described by Dellaporta et al. (1983). In brief, one hundred milligrams (100 mg) of fungal mycelia were taken into a 1.5 ml Eppendorf tube, 1 ml of DNA extraction buffer containing proteinase K (0.05 mg/ml) was added and the mycelia was macerated with sterile pestle. Fifty microliter (50 µl) of 20% sodium dodecyl sulphate (SDS) was added and the mixture was incubated in a water bath at 65 °C for 30 min. The tubes were allowed to cool at room temperature ±28 °C then, 100 µl of 7.5 M potassium acetate was added, mixed briefly and centrifuge at 13,000 rpm for 10 min. The supernatant was transferred into new fresh autoclaved tubes. To the supernatant, 2/3 volumes of cold Isopropanol was added, tubes were inverted 3–5 times gently and incubated at −20 °C for 1 h. The supernatant was discarded after centrifuging at 13,000 rpm for 10 min, 500 µl of 70% ethanol was added and centrifuge for another 5 min at 13,000 rpm. Supernatant was carefully discarded with the DNA pellet intact and traces of ethanol were removed by drying the DNA pellets at room temperature. DNA pellets were re-suspended in 50 µl of Tris-EDTA (TE) buffer and stored at −20 °C for further laboratory analysis.

The isolated DNA was used as templates in polymerase chain reaction (PCR) using degenerate primers pairs ITS1/ITS4 (ITS 1: 5′ TCC GTA GGT GAA CCT GCG G 3′/ITS 4: 5′ TCC TCC GCT TAT TGA TAT GC 3′). A 12.5 µl of reaction cocktail consisting of 5x GoTaq reaction buffer (10 mM Tris–HCl (pH 8.8), 50 mM KCl, 1.5 mM MgCl2, 0.01% Triton-X 100), 0.25 mM of each dNTPs, 25 mM MgCl2, 10 pmol each of primers and 0.3 units of Taq DNA polymerase was dispensed into the tubes containing 2 µl of DNA template. Amplifications were carried

out using the following thermocyclic conditions; 94 °C for 5 min for initial denaturation, followed by 35 cycles of denaturation at 94 °C for 1 min, an annealing step at 55 °C for 1.5 min, an extension at 72 °C for 1 min, and a final extension at 72 °C for 7 min ended the PCR reaction. The PCR products were analysed on 1.5% agarose gel electrophoreses and observed under a UV- illuminator. The amplified fragments were purified with ethanol in order to remove the PCR reagents. Briefly, 7.6 µl of 3 M sodium acetate and 240 µl of 95% ethanol were added to 40 µl PCR amplified product in a new sterile 1.5 µl Eppendorf tube, the mixture was mixed thoroughly and kept at −20 °C for 30 min. Centrifugation was done for 10 min at 13,000×*g* and thereafter, supernatant was removed. Pellets were washed by adding 150 µl of 70% ethanol and mixed before centrifuge for 15 min at 7500×*g*. Supernatants were removed and the pellet allowed to dry in the fume hood at room temperature for 10–15 min. Re-suspension was done with 20 µl of sterile distilled water and kept in −20 °C prior to sequencing.

The sequences were initially compared to known fungal sequences using the BLAST program available at the National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/BLAST/). Sequence identities was calculated from the "Sequence Identity Matrix" option in MEGA 7 windows software, Phylogenetic trees was constructed from the Muscle-aligned sequences using MEGA version 7 (Tamura et al. 2013) and a neighbour-joining method was adopted. Evolutionary divergences were estimated using MEGA version 7 (Tamura et al. 2013).

Nucleotide sequences of the CP coding region of the isolates was aligned with the consensus region using Muscle programme and compared to related sequences available from Gen-Bank. Selection of related sequences was done using a basic local alignment search tool for Nucleotide Sequences (BLASTN). Alignment of ITS DNA sequences was done using Muscle program. 1000 bootstrap replicates were performed and high bootstrap replication percentages were given on the tree's internal nodes.

## Result and discussion

The cultured isolates were subjected to PCR assay resulting in amplification of the ~600 bp DNA fragment of ITS gene (Figure 1). The primer set used in this study have been designed to amplify in a region of about 500–600 bp from DNA of a majority of *Fusarium* fungi (Singh and Kumar 2001). These results confirmed the isolates as fungal species according to Singh and Kumar (2001). Several pair primers have been reported for detection of fungal species however both primer sets (ITS1/

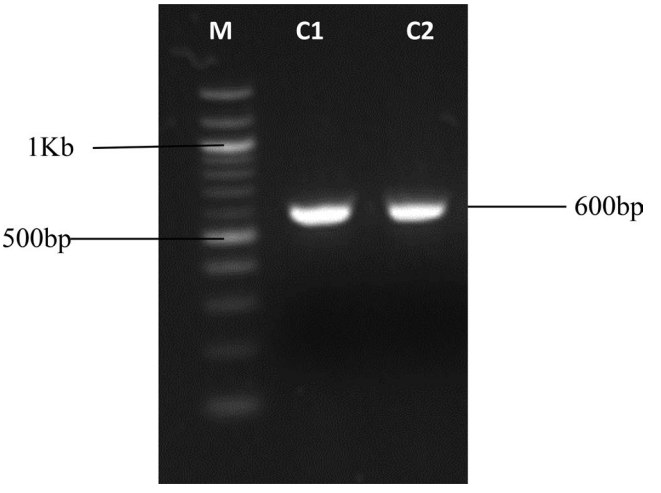


**Figure 1.** Gel electrophoresis pattern of PCR products from cultured mycelia using degenerate primer pair (ITS1 and ITS4). M, 100 bpGeneRuler™ DNA ladder (Thermo Scientific); C1 and C1, sample.

ITS4) produced amplicons of the expected size from all the isolates obtained in this study (Figure 1) Molecular characterization of the isolates was performed using ITS sequencing. The two nucleotide sequences generated in this study were similar with 99% identity and a consensus of 510 nucleotides was assigned GenBank accession number MT408911. The generated nucleotide sequence of the isolates was compared to those available in GenBank which identified the isolate as *F. chlamydosporum* and showed high level of nucleotide identity of 99.8% to an Iranian isolate and 99.6% to an isolate from Hungary.

The phylogenetic analysis of nucleotide sequence generated from this study and other fungal species used for comparison formed three clades on the phylogenetic tree (Figure 2). Clade I subdivided into three subclades and *F. chlamydosporum* isolate obtained in this study was placed in subclade 1 with other *F. chlamydosporum* isolates from different geographical locations and various sources. Clade II comprise of isolate of *F. oxysporum* isolated from Ireland while clade III included *F. oxysporum* Isolated from South Africa and Italy. It is however important to assess the distribution, rate of spread, and extent of damage caused by *F. chlamydosporum* on melon and other hosts in Nigeria to determine its current and potential economic significance as diseases play an important role in reducing the quality and quantity of fruits and vegetable during production and storage. Pathogen isolated in this study was identified culturally and based on morphological features as *Fusarium chlamydosporum*. Microscopic view of the pathogen showed the presence of micro and macro conidia. Macro conidia were formed straight while micro conidia were abundant and occur singly having oval shape. Chlamydospores were

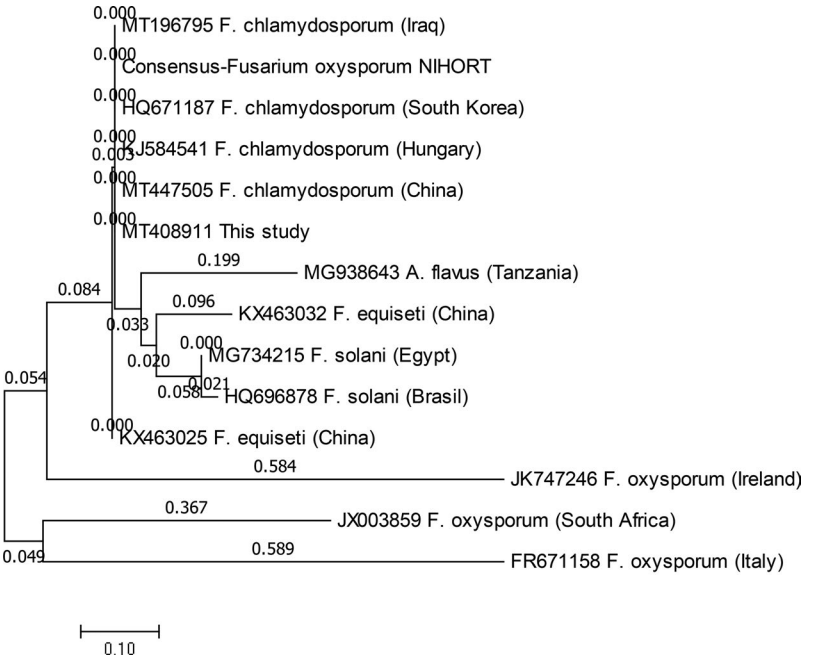


**Figure 2.** A maximum likelihood tree showing the phylogenetic relationships among the isolate of *F. chlamydosporum* collected in this study and the related species found in GenBank based on the ITS sequences. Country of isolation of the respective isolates/strains is shown in the parenthesis.

**Plate1.** Growth of the *F. chlamydosporum* on PDA plate.

abundant and formed rapidly in single or cluster which is typical of *F. chlamydosporum*. Colony culture of *F. chlamydosporum* produces white, raised, smaller concentric ring on PDA (Plate 1). *Fusarium chlamydosporum* have been described as one of the pathogens with a world-wide

occurrence (Jacobs et al. 2018). They are associated with various plant hosts as well as soils and can also produce mycotoxins in food commodities or be associated with diseases of animals or humans (Leslie and Summerell 2006). *Fusarium chlamydosporum* was found to be responsible for asparagus root and crown disease (Elmer 2015). It has also been regarded as an endophyte on many plants Chaturvedi et al. (2014). Endophytic *F. chlamydosporum* have been isolated from leaf and stem of a medicinal plant, *Tylophora indica* and also from the root of *Dendrobium crumenatum* (Siddiquee et al. 2010). Result from the pathogenicity test revealed that *F. chlamydosporum* isolated from diseased watermelon fruits was capable of causing disease in healthy water and golden melon fruits. Seven (7) days post inoculation, whitish mycelium was produced from the point of inoculation on both fruits (Plate 2(A,C)) while no infection was observed in un-inoculated controls (Plate 2(B,D)). Soft rot was observed around the point of inoculation in golden melon. *F. chlamydosporum* induced significantly higher diameter of rot (81.7 mm), likewise significantly higher disease incidence and severity of 83.3 and 100.0% respectively in golden melon (Table 1). The differences in virulence of

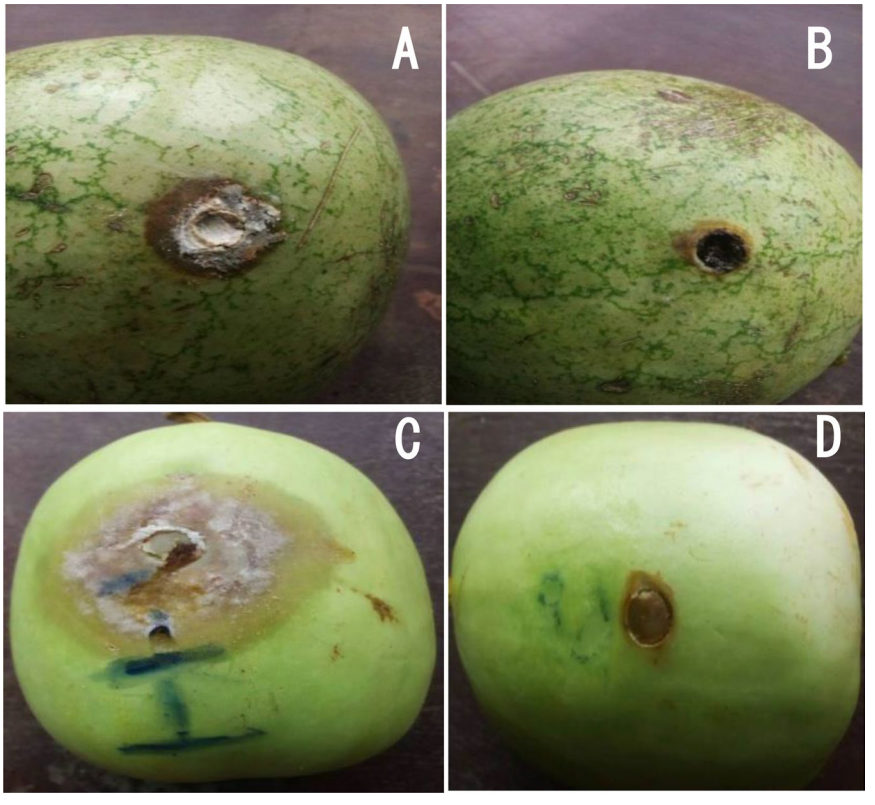


**Plate 2.** Symptoms induced by *F. chlamydosporum* on water and golden melon fruits (A and C) compared with uninoculated fruits (B and D).

**Table 1.** Pathogenicity of *F. chlamydosporum* on two melon varieties under artificial inoculation.

| Plant type | Rot diameter (mm) | Incidence (%) | Severity (%) |
|---|---|---|---|
| Golden melon | 81.7 | 88.3 | 100.0 |
| Water melon | 40.0 | 46.7 | 60.0 |
| Control | 3.0 | 0.0 | 0.0 |
| $LSD_{(0.05)}$ | 8.86 | 14.63 | 4.72 |

*F. chlamydosporum* on the varieties could be related to genetic factors (Narayanasamy 2011). Different levels of susceptibility experienced between the two melon varieties in this study could also be an indication of differences in the ability of the *Fusarium* isolates to colonize the host and establish the disease. *Fusarium chlamydosporum* is regarded by some as non-important plant pathogen but it has been linked with several diseases on several crops such as fruit rot of chili (Krishna et al. 2012), wilt of guava (Gupta and Misra 2012) and damping-off of Aleppo pine (Lazreg et al. 2013). In addition, results from this study have revealed the capability of *F. chlamydosporum* to induce rot in healthy water and golden melon fruits. Golden melon was found to be more susceptible to rot induced *by F. chlamydosporum* compared to the water melon. The results of the present study also provide useful information on the occurrence and pathogenic variations of *F. chlamydosporum* in association with Water and Golden melon fruit rot.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

## Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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