



## Investigating the Morphological Characteristics, and Host Range of three Pathotypes of *Choanephora cucurbitarum* using Fifteen Commonly Grown Vegetable Crops

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### Abstract

The cultivation of vegetable crops is often challenging to farmers as a result of disease conditions caused by plant pathogens including fungi, bacteria, viruses, nematodes. *Choanephora cucurbitarum* is a notable fungus responsible for fruit rot, flower rot and leaf blight on a variety of plants. This study was conducted to morphologically characterize three pathotypes of *Choanephora cucurbitarum* and assess their host range on selected commonly cultivated vegetable crops. To obtain pathotypes of *C. cucurbitarum*, sections (3 mm<sup>2</sup>) of infected leaves of *Abelmoschus esculentus*, *Amaranthus hybridus* and *Solanum melongena* were aseptically taken, surface sterilized using 70 % ethanol, and rinsed with distilled water. Sterile sections were incubated in PDA at 27±1 °C. Established cultures were sub-cultured to obtain pure cultures. The Susceptibility of the vegetable crops to the isolated pathotypes (A1, O2 and E3) was tested by using a spore suspension (1 x 10<sup>6</sup> spores per ml of distilled water) of pathotypes pure culture to inoculate healthy seeds of test plants. Morphological characteristics of the pathotypes were studied by viewing prepared slides of *C. cucurbitarum* pure culture, using Olympus microscope under 40x and 100x magnifications. Results show that out of fifteen species of vegetables employed in the host range test, only eight species germinated, while only five out of these were established. Morphological characteristics of the three isolates of *C. cucurbitarum* revealed that the isolate from Amaranthus leaf (A1) had a monosporous Sporangiphore that was long and slender. Isolates from okra (O2) had mycelium that was hyaline, unbranched and without septations. While isolates from eggplant (E3) had a cottony colony, with scanty white mycelium. Host range test confirmed the vegetables (*Vigna unguiculata*, *Cucumis sativus*, *S. melongena*, *A. esculentus*, *A. hybridus*) were susceptible to the pathotypes of *C. cucurbitarum*. Farmers are encouraged to practice good hygiene measures, such as cleaning and disinfecting tools, equipment to prevent the spread of the pathogen.

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## 1.0 Introduction

Vegetables are grown worldwide as important source of dietary fibers, minerals, antioxidants and vitamins. Okra (*A. esculentus*), Amaranthus (*A. hybridus*), eggplant (*S. melongena*), cucumber (*C. sativus*) and cowpea (*V. unguiculata*) are all tropical and subtropical vegetable crops widely grown in Nigeria for their dietary fibers, antioxidants, vitamins, proteins and mineral elements (Bosland and Votava, 2000). They are excellent source of income for both peasant and commercial famers in Nigeria. They equally contribute to improvement of food security, thereby making them among the most economically important crops in Nigeria. However, cultivation of these crops is often challenging to farmers as a result of disease conditions caused by fungi, bacteria, viruses, nematodes and also by abiotic stresses. *A. esculentus*, *A. hybridus*, *S. melongena*, *C. sativus* and *V. unguiculata* particularly suffer from several fungal diseases of fruits, stems and roots (Agrios, 2009). *C. cucurbitarum* is a pathogenic soil-borne fungus responsible for fruit and blossom rot among cucurbits. Infections have been reported in *S. melongena*, *Momordica subangulata* Blume subsp. *renigera*, *Lablab purpureus*, and *Pisum sativum* (Das *et al.*, 2017). Signs of infection on fruits or leaves include water-soaked, necrotic lesions, which progress rapidly under ideal conditions. As the fungus begins to produce spores, affected tissues become dark grey-brown and hairy as a result of the superficial sporangia. Earlier researchers (Kwon *et al.*, 2001) described the morphology of *C. cucurbitarum*, and reported that the fungus produces whitish mycelia and monosporous sporangia on the lesions. Authors found the fungus isolated from lesions produced white to pale yellowish-brown mycelia with scattered monosporous sporangia on potato dextrose agar (PDA) plates. Kuo *et al.* (1999) isolated *C. cucurbitarum* on lima bean and described the morphology of the fungus. The colonies of the *C. cucurbitarum* grown on potato dextrose agar

at 25 °C was white at the early stage of growth with abundant aerial mycelium, but at later growth stages turn pale yellow.

Previous studies have demonstrated that *C. cucurbitarum* has a wide host range such as *Cucurbita pepo*, *Crotalaria spectabilis*, *Brassica chinensis*, *Hibiscus syriacus*, *Lactuca sativa*, and some common weeds. Symptoms expressed by these host plants include flower blight, leaf wet rot, flower blight, stem blight, blossom blight, and fruit soft rot (Park *et al.*, 2016; Ryu *et al.*, 2023; Pornsuriya *et al.*, 2017; Alfenas *et al.*, 2018; Garcia-Estrada *et al.*, 2023; Žerjav and Schroers, 2019). This present study aimed to morphologically characterize three pathotypes of *C. cucurbitarum* and assess their host range on selected commonly cultivated vegetable crops.

## 2.0 Materials and methods

### 2.1 Experimental site

This research work was carried out at the Botanic Garden, University of Calabar, Calabar. All laboratory investigations were carried out in Botany Postgraduate Research Laboratory.

### 2.2 Source of pathogen

The three pathotypes of *C. cucurbitarum* used in this study were isolated from okra (*A. esculentus*), Amaranthus (*A. hybridus*) and eggplant (*S. melongena*) leaves grown in the Agricultural Farm of the Faculty of Agriculture, University of Calabar, Calabar, Cross River State.

### 2.3 Preparation of culture medium

Potato dextrose agar (PDA) was used as the culture medium. Medium was prepared by dissolving 39 g of commercial Potatoes dextrose agar in one litre of distilled water and autoclaved at 121 °C for 15 minutes. The medium was allowed to cool to about 40 °C before dispensing into sterile glass Petri-dishes.

### 2.4 Culturing and purification of pathogen

The pathotypes of *C. cucurbitarum* were cultured following the method of Umana and

Ikotun (2000). Sections (3 mm<sup>2</sup>) of infected leaves of *A. esculentus*, *A. hybridus* and *S. melongena* were aseptically taken. These were surface sterilized using 70 % ethanol, and rinsed severally with distilled water. The sterile sections were inoculated into the prepared PDA in Petri dishes and incubated at 27 ± 1 °C. Cultures were examined daily for growth and on the third day, sub-cultured to obtain pure cultures. The isolates were stored as stock culture in Mckaney bottle as agar slant and stored in a refrigerator at 4 °C for further use.

### 2.5 Identification of isolates

Isolated fungal pathotypes from *A. esculentus*, *A. hybridus*, *S. melongena* were microscopically identified using the identification guides of the International Mycological Institute, and according to method of Hashem and Farag (2005) and Barnet and Hunter (2006). The isolate from Amaranthus leaves were labeled (A1), isolates from Okra leaves (O2) and isolate from eggplant (E3).

### 2.6 Pathogenicity test

The pathogenicity test was carried out according to Umana and Ikotun (2000). Pathogenicity test of the different isolates of *C. cucurbitarum* was confirmed by following Koch's postulates. Healthy leaves of *V. unguiculata*, *C. sativus*, *S. melongena*, *A. esculentus*, and *A. hybridus* were surface sterilized with 70 % ethyl alcohol. Wounds were created using a sterile inoculating needle to facilitate the entry of the fungal pathotypes. A spray inoculation with spore suspension from a four-day old pure culture of each isolate was aseptically transferred into the wounds on leaves of the test plants and covered with transparent polyethylene bags to prevent moisture loss and entry of other pathogens. Uninoculated potted plants were used as control. Daily observations were made for rot symptom development and spread of pathogen on the leaves. The symptoms of wet rot based on the description given by Awurum and Uchegbu (2013) and Akwaji *et al.* (2014) were observed

in the potted experiment. Re-isolation of the pathogen from the artificially inoculated leaves yielded some pathotypes A1, O2 and E3 identical to the original cultures in Petri-plates.

### 2.7 Cultural characterization of the pathotypes

The cultural characteristics of *C. cucurbitarum* pathotypes was carried out by growing them on Potatoes Dextrose Agar (PDA), and observation made on the nature of growth and sporulation of the pathotypes.

### 2.8 Morphological characterization of the pathotypes

The morphological characters of the pathotypes of *C. cucurbitarum* were studied by preparing slide from pure culture of the pathotypes. The slides were stained with lactophenol in cotton blue and observed for the mycelial characters, sporulation and characteristics of the Sporangiohores, using an Olympus microscope and observed under 40x and 100x magnifications in oil emersion.

### 2.9 Preliminary host range test

A potted plant experiment of test plants was conducted for each plant to assess their susceptibility to wet rot disease caused by the three isolated pathotypes (A1, O2 and E3) of *C. cucurbitarum*. The host range of the three pathotypes of *C. cucurbitarum* was investigated using fifteen plants. The plants, their accession number and source are presented in Table 1.

### 2.10 Preparation of growth medium

The top soil, obtained from the Botanical Garden of University of Calabar, was sieved through a 0.5 x 0.5 cm wire mesh to remove large objects and root fragments, was used as the growth medium (Biodgett and Swart, 2002). Sieved soil was sterilized at 160 °C in a hot air oven (Model: DHG 9053A).

### 2.11 Seed inoculation technique

Healthy, certified and surface sterilized seeds of test plants were inoculated according to the method of Markson *et al.* (2014). The seeds were inoculated using a four-day old sporulating pure

culture of *C. cucurbitarum* pathotypes. A spore suspension ( $1 \times 10^6$ ) spores per ml of distilled water) of 0.05ml was taken aseptically with the help of a 5 ml sterilized syringe and was distributed into sterile Petri plates and the seeds

were inoculated by rolling the seeds in the spore suspension. Five inoculated seeds were sown in thirty-centimeter (30cm) diameter polyethylene pots containing 5kg of sterilized soil, and replicated thrice.

**Table 1:** Vegetables used for preliminary host range test and their sources

Name	Common name	Accession number	Source
<i>V. unguiculata</i>	Brown seed cowpea	07k-313-33	IART
<i>Zea mays</i>	Maize	07k-292-10	IART
<i>Corchorus olitorus</i>	Bush okra	04k-212-25	IART
<i>Solanum lycopersicum</i>	Tomatoes	10GRS1558117	CADP
<i>Citrullus lanatus</i>	Water melon	10GRS21980021835	CADP
<i>C. sativus</i>	Cucumber	10GRS22020091728	CADP
<i>Daucus carota</i>	Carrot	10GRS21985111701	CADP
<i>S. melongena</i>	Eggplant	5GRS22958021803	CADP
<i>Latuca sativus</i>	Lettuce	10GRS22369121739	CADP
<i>Allium cepa</i>	Onions	5GRS1879509733	CADP
<i>A. hybridus</i>	Green leaf	10GRS21277041786	CADP
<i>A. esculentus</i>	Okra	10GRS21955111746	CADP
<i>Arachis hypogaea</i>	Groundnut	07k312-12	IART
<i>Cucumis melo</i>	Melon	07k412-30	IART
<i>Solanum tuberosum</i>	Potato	07k512-10	IART

- CADP (Calabar Agricultural Development Project); IART (Institute of Agriculture Research and Training)

## 2.12 Experimental design

The experiment was laid out as 4x3 factorial experiment fitted into a Completely Randomized Design (CRD) with each treatment replicated three times.

## Data collection and analysis

The various data for the study were collected based on foliar infection on the one hundred and ninety-two polyethylene bags of test plants inoculated with the pathotypes at four weeks after planting. A sterilized inoculating needle was used to pierce the leaves of the test plants and a 5ml syringe was used to aseptically

transfer the spore concentration of  $5.0 \times 10^6$  ml was introduced into the wounds created by the needle. The wounds were covered with transparent polyethylene bags and observed daily for infection. Mean values were taken from measurement of three replicates. All data were statistically analyzed using the Gen Stat Package (2014) version 8.1. The means were separated using Fishers Least Significant Difference (FLSD) at 5 % probability level.

## 3.0 Results

The results obtained from the *in vitro* studies on the morphological and cultural characteristics of

the pathogen, host range, symptomatology and management of *C. cucurbitarum* pathotypes causing wet rot disease of five vegetables in Calabar, Southern Nigeria; carried out at the Department of Botany, University of Calabar, Calabar - Nigeria are presented below.

### 3.1 Cultural characterization of pathotypes *C. cucurbitarum*

The growth of the pathotypes of *C. cucurbitarum*, as studied using Potatoes Dextrose Agar (PDA) medium showed that isolate A1 from *A. hybridus* sporulated within 22 hours. The growth of isolate O2 from *A. esculentus* was rapid. It sporulated within 20 hours. The growth of the isolate E3 from *S.*

*melongena* was rather slow with few mycelia and it sporulated after 28 hours (Table 2).

### 3.2 Morphological characterization of pathotypes *Choanephora cucurbitarum*

The morphological characteristics of the three isolates of *C. cucurbitarum* on prepared slides viewed under the Olympus microscope (Model: 08100000) revealed that the isolate from Amaranthus leaf (A1) had a monosporous sporangiophore that was slender and 121  $\mu\text{m}$  long, each branch bore a head of sporangium containing spores (Figure 1). The sporangium was ovoid (35  $\mu\text{m}$  in diameter) and dark brown in colour. Isolates from okra (O2) had hyaline, unbranched mycelium of 156  $\mu\text{m}$  long, without septations (Figure 2).

**Table 2:** Growth period of the three pathotypes of *C. cucurbitarum* in culture on PDA

Isolates codes	Source	Growth period (hours)
A1	From infected Amaranthus leaf	22.00
O2	From infected Okra flower	20.00
E3	From infected eggplant	28.00

- Values are means of three replicates. A1= isolate from *A. hybridus*; O2 = isolate from *A. esculentus*; E3 = isolate from *S. melongena*.

The sporangiophore bearing sporangium was non-septate and 41 $\mu\text{m}$  in diameter, apically brown at maturity, indistinctly straight with drooping multi-sporous sporangia that were subglobose in shape. The Sporangiophore from which the sporangium arose was long slender with vesicle, which bore as spores (conidia). *C. cucurbitarum* isolates from eggplant (E3) had a

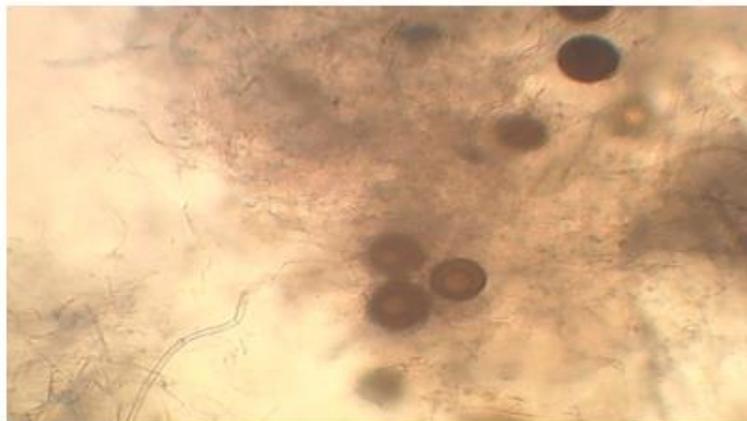
colony that was cottony, with scanty white mycelium (Figure 3). The mycelium was 115  $\mu\text{m}$  long, non-septate, unbranched and hyaline. Conidia were smooth walled and dark brown in colour, typically obovate. Sporangiophore was non-septate, straight, and 38 $\mu\text{m}$  in diameter. Table 3 shows a morphological description of the pathotypes.



**Figure 1:** Photomicrograph of *C. cucurbitarum* isolated from *A. hybridus*



**Figure 2:** Photomicrograph of *C. cucurbitarum* isolated from *A. esculentus*.



**Figure 3:** Photomicrograph of *C. cucurbitarum* isolated from *Solanum melongena*.

**Table 3:** Morphological characteristics of spores and sporangiophores

Feature	A1 (Amaranthus)	O2 (Okra)	E3 (Eggplant)
Mycelium	Hyaline, unbranched, non-septate	Hyaline, unbranched, non-septate	Hyaline, scanty white, non-septate
Sporangiophore	Monosporous, long, slender	Long, slender, vesiculate	Long, straight, non-septate
Sporangia	Ovoid, dark brown	Subglobose, drooping, apically brown	Not clearly described
Conidia (spores)	Not detailed	Present, borne on vesicle	Smooth-walled, obovate, dark brown
Colony appearance	Not detailed	Not detailed	Cottony growth

### 3.3 Pathogenicity test of three pathotypes of *C. cucurbitarum* (Symptomatology)

The symptoms of wet rot appeared 24 hours after artificial inoculation on the five test plants. Symptoms on Cowpea, Cucumber and Amaranthus appeared as water-soaked lesions on the leaves of the vegetables, and further expanded causing wet rot of the leaves, stem and fruits (Figure 4, 5, 6). In the advanced stage of disease development, the invaded portions became covered with luxuriant fluffy white growth of the fungus consisting of mycelium

and sporangiospores along with sporangia which appeared as minute black headed pin-like structures around the leaves resulting in complete decay of the leaves, apical buds of flower and fruits of the plants. In okra and eggplants, symptoms appeared as water-soaked lesion on the leaves and later became necrotic and increased in size and then coalesced to cause wet rot. Affected tissues became shrunken and disintegrated, causing toppling down of the leaves, peduncles, and stem. The details of the symptoms are shown in Figure 7 and 8.



**Figure 4:** Wet rot of *V. unguiculata* leaf showing white dense mycelium.



**Figure 5:** Wet rot of *C. sativus* leaf showing chlorosis, yellowish brown soft rot



**Figure 6:** Wet rot of *S. melongena* leaf showing brown soft rot.



**Figure 7:** Wet rot of *A. esculentus* leaf showing chlorosis, yellowish brown soft rot.



Figure 8: Wet rot of *A. hybridus* leaf showing white dense mycelium.

### 3.4 Preliminary Host range test

Out of fifteen species of vegetables employed in the host range test, only eight species germinated and five were established and used in the study. The species that germinated included, *V. unguiculata* (Brown Seed Cowpea), *Zea mays* (Maize), *Solanum lycopersicum* (Tomato), *C. sativus* (Cucumber), *S. melongena* (Eggplant), *Allium cepa* (Onions), *C. lunatus* (Water melon), *Solanum tuberosum* (Potato). The five established species were, *A. hybridus* (Green leaf), *A. esculentus* (Okra), *S. melongena* (Eggplant), *C. sativus* (Cucumber), and *V. unguiculata* (Brown seed cowpea). The results (Figures 4-8) of the host range test showed that the vegetables were susceptible to the pathotypes of *C. cucurbitarum*.

### 4.0 Discussion

Host range and pathogenicity are key factors in a disease circle. Host range of a pathogen encompasses the plant species that are not resistant but susceptible to the pathogen or pathotypes of the pathogenic organism. While the term pathogenicity is the potential capacity of certain species of microbes to cause a disease in their host (Kenny, 2010). In this study, it was observed that the three pathotypes of the fungus: *C. cucurbitarum* isolated from infected leaves of *A. hybridus*, *A. esculentus* and *S. melongena* were pathogenic to *V. unguiculata*, *A. hypogaea*, *C. sativus*, *S. tuberosum*, *C. melo*, *S. melongena*, *A. hybridus*, *A. esculentus*. The results of the

preliminary host range test showed that all the vegetables were susceptible to the three pathotypes. The uninoculated plants (control) performed significantly better than the plants that were inoculated using seed and seedling. The results obtained clearly indicated that *C. cucurbitarum* (Berk and Ravenel) Thaxt causes wet rot of *V. unguiculata*, *A. hypogaea*, *C. sativus*, *Solanum tuberosum*, *Cucumis melo*, *S. melongena*, *A. hybridus*, *A. esculentus* in Calabar, Cross River State.

*Choanephora* wet rot has been reported in Florida by Raid and Palmateer (2006) and in Malaysia by Siddiqui (2006) and Siddiqui *et al.*, (2008) which corroborated with the work of Ziedan (2012). The ability of *C. cucurbitarum* to infect the five test plants shows a wide host range nature of the pathogen as reported by Abel-Motaal *et al.* (2010) and Saroj *et al.* (2012). The present finding agrees with the work of Kacharek *et al.*, (2003) who reported that *C. cucurbitarum* (Berkeley and Ravenel) has a wide host range in some vegetable families including, Cucurbitaceae, Solanaceae, Leguminaceae, Malvaceae and Amaranthaceae. Umana *et al.* (2014) also listed *V. unguiculata*, *A. esculentus*, *S. melongena*, *C. sativus* and *A. hybridus*, whereas Kacharek *et al.* (2003) and Umana *et al.* (2014) mentioned squash, pumpkin, pepper, pea, bean, millet, rice and sorghum as hosts of *C. cucurbitarum*. *A. hybridus*, *A. esculentus*, *S. melongena*, *C. sativus*

and *V. unguiculata* were susceptible from seedling to flowering stage, damaging the entire buds, flower stalk and stem tissue leading to wilt and abortion of young infected fruits.

The findings of this study also align with Li *et al.* (2023) who highlighted the pathogen is economically significant as infections lead to significant preharvest and postharvest losses, especially in tropical and subtropical regions where vegetables are important to both subsistence farming and commercial agriculture. For instance, crops like okra, pepper, tomato, and pumpkin, when infected, often suffer from soft rot of flowers and fruits, drastically reducing their marketability and shelf life. This not only affects farmers' income but also threatens food security and increases dependency on fungicides, raising production costs and environmental concerns (Chen *et al.*, 2022).

Furthermore, researchers have made different conclusions on the significance of pathogens with a wide host range. Diseases can spread more easily across different plant species, leading to higher rates of infection and disease outbreaks in agricultural settings or natural ecosystems. Managing a pathogen with a wide host range can be more challenging because it may require control strategies that are effective across multiple plant species, which can be complex and costly (Woolhouse and Gowtage-Sequeria, 2005). If a pathogen affects a wide range of plant species, it can potentially impact food security and agricultural sustainability (Duffy *et al.*, 2006). Again, the host range of a pathogen affects how diversification and speciation occur, shifting among hosts in response to biotic and abiotic environmental variation, or the ability to persist in the environment between epidemics (Moury *et al.*, 2017).

## 5.0 Conclusion

*C. cucurbitarum* was identified as the causal agent of wet rot in several plants including *V. unguiculata*, *A. hypogaea*, *C. sativus*, *S.*

*tuberosum*, *C. melo*, *S. melongena*, *A. hybridus*, *A. esculentus* in Calabar, Cross River State. The capacity of *C. cucurbitarum* to infect the aforementioned plants demonstrated the broad host range characteristic of this pathogen. The symptoms of wet rot manifested subsequent to deliberate inoculation on the test plants. For instance, Cowpea, Cucumber, and Amaranthus exhibited water-soaked lesions on their leaves which eventually progressed to wet rot affecting the leaves, stems, and fruits. Conversely, symptoms on *C. sativus* and *A. esculentus* leaves presented as chlorosis and yellowish-brown soft rot. A pathogen with a wide host range not only plays a significant role in plant disease epidemiology but also has the potential to cause substantial economic losses through decreased crop yields, heightened disease management expenses, and potential trade restrictions on affected crops. Beyond hygiene measures, rotate with non-host crops to break the pathogen life cycle, as well as use of crop varieties or accessions that show resistance or tolerance to isolated pathotypes of *C. cucurbitarum* are recommended management approaches. Bio-control agents like *Trichoderma harzianum*, *Bacillus subtilis*, or *Pseudomonas fluorescens*, which have shown efficacy in suppressing soil-borne and floral pathogens can also be applied.

## Conflict-of-interest disclosure

Authors have declared there is no conflict of interest.

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