

DETERMINATION OF PATHOGENICITY OF *CHOANEPHORA CUCURBITARUM* (BERKELEY AND RAVENEL) THAXT, AMONGST COMMONLY CULTIVATED VEGETABLES IN CALABAR, CROSS RIVER STATE, NIGERIA

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ABSTRACT

Choanephora cucurbitarum is a plant pathogenic fungus with a wide host range. The fungus was isolated from rotting fruits of *Capsicum anuum*, after it was observed that some vegetables were infected in the farm. The role of infection courts on severity was determined by inoculating the pathogen into soil, leaves and stems of the test plants. The test plants used were *Abelmoschus esculentus*, *Amaranthus* sp. *Cucumis sativus* and *Vigna unguiculata*. Determinants of pathogenicity were plant height, leaf reduction, fresh and dry weight. Results showed that the fungus was pathogenic to the test plants. A significant reduction in height, leaves, fresh and dry weight of the test plants was observed when compared with the control. The greatest effect of the pathogen was on the inoculated stem of the test plants with a mean of 42.94. The least effect was observed on infected soil with a mean of 92.99. *Cucumis sativus* showed the least effect by the pathogen with a mean of 84.18, while *Abelmoschus esculentus* showed the greatest effect with a mean of 44.59. The pathogen showed the greatest effect on leaves of *Cucumis sativus* with a mean of 20.45, while the least effect was on *Vigna unguiculata* with a mean of 36.77. Fresh weight ranged from 3.35g to 37.40g, Dry weight 2.15 to 7.90g as compared with the control which had a fresh weight of 7.0g to 57.25g and 3.8g to 11.90g for dry weight. Symptoms such as leaf blight, blight of the shoot apex, soft rot of stems, die back and decay of *Vigna unguiculata* pods were observed.

Keywords: *Choanephora cucurbitarum*, Pathogenicity, plant height, leaf reduction, fresh and dry weight.

INTRODUCTION

Choanephora cucurbitarum is a facultative saprobe that belongs to the sub division zygomycotina, order: mucorales and family choanephoraceae. It is a fungal plant pathogen and has a wide host range (Abel-motaal *et al.*, 2010).

According to Sikora (1998), host range is defined as the various kinds of host plant that may be attacked by a pathogen, and the term pathogenicity is the potential capacity of certain species of microbes to cause a disease in their host (Kenny, 2010).

Choanephora cucurbitarum is a plant pathogenic fungus causing fruit rots, flower rot and leaf blights on a variety of plants including squash, pumpkin, pepper, pea and bean (Kacharek *et al.*, 2003). This fungus is known to

attack several other crops which include cereals such as millet, rice and sorghum. The fungus also causes pod blight known as wet rot, blossom blight and whisker rot (Kacharek *et al.*, 2003). This disease is also common on squash and southern pea but occurs on the floral parts of many types of plants (Afolabi, 1994). It causes blossom blight of pepper, die back, wet rot and soft rot of stems or side shoots of chillier plants (Maeda *et al.*, 2010).

The fungus is more successful under humid conditions and thrives best at a temperature of 25°C and relative humidity of about 100%. A temperature of about 31°C stimulates the production of large sporangia but unfavorable for conidia formation (Umana and Ikotun, 2000).

Due to the menace caused by this fungus on vegetables as observed in the farm during the 2011/2012 farming season in Calabar, Cross River State, Nigeria. It became

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necessary to investigate the pathogenicity of the fungus using four (4) test plants (*Abelmoschus esculentus*, *Amaranthus* sp, *Cucumis sativus* and *Vigna unguiculata*) commonly cultivated in Calabar. This will help farmers select crops during mixed crop farming to avoid epidemics that may be caused this fungus.

MATERIALS AND METHODS

Source of Materials/Processing: Rotten fruits of *Capsicum annum* were collected from the research farm of Department of Crop science, Faculty of Agriculture, Forestry and Wild life Resources Management of the University of Calabar, Nigeria for isolation purpose. Garden soil was obtained from the Botanic Garden of Department of Botany, University of Calabar, Nigeria and was sterilized using wet steam sterilization for three hours a day for three days. Potted seedlings of *Vigna unguiculata*, *Abelmoschus esculentus*, *Amaranthus* sp and *Cucumis sativus* raised from nursery were used in this research and were six weeks old before inoculation (Abel-mortaal *et al.*, 2010).

The experiment was carried out at the Green House of Department of Crop Science, Faculty of Agriculture, Forestry and Wild Life Resources Management and the Laboratory of Department of Botany, Faculty of Science both of the University of Calabar, Nigeria.

Source of Fungal Pathogen and Morphological Identification:

The fungus used in this research work was isolated from rotting fruits and stems of *Capsicum annum* collected from the research farm of Department of Crop Science, Faculty of Agriculture, Forestry and Wild Life Resources Management of the University of Calabar, Nigeria. Cut sections of the diseased assay plants were surface sterilized with 70% sodium hypochlorite (bleach) solution for 1min and rinsed quickly in 3 changes of sterile distilled water, blotted dry on Whatman's No. 1 filter paper and placed on Potato Dextrose Agar (PDA) in Petri dishes. Four (4) sections were inoculated per Petri dish. The plates were incubated at $28 \pm 1^\circ\text{C}$ until fungal growth was noticed. After 5 days, the different isolates were subcultured on freshly prepared PDA to obtain their pure culture. Isolated fungi were microscopically (Olympus optical, Phillipines) identified as far as possible using the identification guides of the International Mycological Institute, Kew and of Barnett and Hunter (1998), Alexopolous and Mins (1989). Stock cultures of these fungi were stored in agar slant bottles for subsequent use.

Pathogenicity Test:

The test fungus was ascertained using Koch's postulate (Abel-mortaal *et al.*, 2009). To test pathogenicity of the isolated fungus, some pieces of mycelia and spores were re-inoculated into potted healthy host plants (*Vigna unguiculata*, *Amaranthus* sp, *Abelmoschus esculentus* and *Cucumis sativus*) that were grown in sterilized garden soil in a green house.

On a wet healthy host leaf, an infection court was created by scrapping with a sterile needle. A piece of mycelium of the pathogenic fungus was inoculated on wound created and thereafter covered with a transparent polyethylene bag for 24 hours. In the control wound was created and only a piece of gelled PDA was introduced.

For the spore inoculation, a spore suspension was produced using the method of (Abel-mortal *et al.*, 2009). With a sterile hypodermic syringe equipped with a 20 gauge hypodermic needle, approximately 5×10^5 spores were inoculated on the abaxial surface of wet healthy host leaves, stems and soil by spraying to run-off level. The leaves, stems and soil was covered with transparent polyethylene bags and allowed to stay for 24 hours. Spores measurement was done with a haemocytometer. The control experiment was carried out with sterile distilled water without spores. All the experimental setups were observed for symptom development. The experiments were replicated three (3) times each.

Determination of Height of Plants and Leaf Reduction:

Height and leaf reduction of the test plants was determined using the method of (Madunagu *et al.*, 2006). Height and leaf reduction of the test plants (*Abelmoschus esculentus*, *Amaranthus* sp, *Cucumis sativus*, and *Vigna unguiculata*) was measured in Centimeters (cm) using a measuring rule from the first (1st) to twentieth (20th) day after inoculation with the fungus based on infection period, infection courts and on daily bases.

Fresh and Dry Weight Determination: The test plants were carefully uprooted after eight weeks of inoculation and weighed in grams (g) to determine the fresh weight of the different samples (Sikora, 1998) using an electronic weighing balance (Casio, Japan). These was then dried in an oven until constant dry weights were obtained. The actual dry weight was then measured in grams (Sikora, 1998) using an electronic weighing balance (Casio, Japan).

Statistical Analysis: Analysis of data involved the use of

analysis of variance (ANOVA), separation of means and least significant difference (LSD) at 5% probability level.

RESULTS

Identification of pathogen: *Chanephora cucurbitarum* (Fig. 1) was implicated in the disease, so it was the pathogen used in this study.

Results of Pathogenicity Test: Results from this study showed that *Choanephora cucurbitarum* was pathogenic to the test plants (*Abelmoschus esculentus*, *Amaranthus* sp, *Cucumis sativus* and *Vigna unguiculata*). Symptoms observed from this study during the infection period included: lesions which turned necrotic and appeared dried out, water soaked lesions appeared on the leaves, margins of leaf tip were blighted (leaf blight), blight of the shoot apex, Soft rot of stems, die back and decay of cowpea pods. Also flower and flower buds turned dark and wilted, as the fungus began to produce spores, affected tissues became dark grey-brown and hairy as a result of the superficial sporangia.

Result also showed that infection courts played a significant role in the pathogenicity of the fungus amongst the test plants. The least effect was observed on *Cucumis sativus* when it was grown on infected soil. However, leaf and stem inoculation for the same plant (*Cucumis sativus*) revealed high susceptibility of the plant to the pathogen. A least effect was also observed on *Abelmoschus esculentus*, when it was grown on infected soil, while leaf and stem inoculation revealed high susceptibility of the same plant (*Abelmoschus esculentus*) to the pathogen. Same effect was again observed with *Amaranthus* sp and *Vigna unguiculata*. Results, therefore, reveals the consistency of the fungus in causing diseases in these plants.

Effect of the Pathogen on the Height of the Test Plants: Result from this study, showed that the infection period by the fungus played a significant role in height reduction of the test plants when compared with the control (Table 1). Based on the infection courts (leaf,

stem, Soil), the least effect of infection period was observed on the height of *Amaranthus* sp (leaf inoculation 55.37). Stem (45.66) and soil inoculation (44.29) for the same plant (*Amaranthus* sp) showed a decrease in height when compared with the control (70.14). The greatest effect by the pathogen was on *Abelmoschus esculentus* (stem inoculation (27.24) and on *Vigna unguiculata* when it was grown on infected soil (28.83). Leaf and stem inoculation for the same plants (*Abelmoschus esculentus* and *Vigna unguiculata*) also showed a decrease in height. A least effect by the pathogen was also observed on the height of *Cucumis sativus*, when it was grown on infected soil. Leaf and stem inoculation for the same plant (*Cucumis sativus*) showed a decrease in height when compared with the control. However, irrespective of infection by the pathogen, a continuous growth was observed on the test plants.

Result (Table 2), showed that the infection courts also played a significant role in height reduction of the test plants. Based on the infection courts (leaf, stem, soil), the greatest effect was observed with leaf and stem inoculation (48.11 and 42.91 on Day 20) The least effect was observed when the test plants were grown on infected soil (54.01) as compared with the control which had a mean height of (106.75 on Day20).

Result (Table 3), showed that the pathogen had a significant effect on the height of the test plants on daily bases, when compared with the control. A significant effect on the height of the test plants was observed from the 1st to 20th day after infection by the pathogen. *Cucumis sativus* showed the least effect in terms of height reduction on daily bases (Mean height ranged from 76.60 to 89.20 from the 1st to 20th day respectively). The greatest effect was observed on *Abelmoschus esculentus* (38.99 to 45.67) as compared with the control (80.01 to 106.75 from the 1st to 20th day).

Table 1. Effect of infection period on the height of the test plants (cm).

Infection courts	<i>Amaranthus</i> sp	<i>V. unguiculata</i>	<i>C. sativus</i>	<i>A. esculentus</i>
Leaf	55.37	43.26	39.29	45.86
Stem	45.66	45.38	53.46	27.24
Soil	44.29	28.83	96.66	37.68
Control	70.14	86.93	147.32	67.57
LSD	5.33			

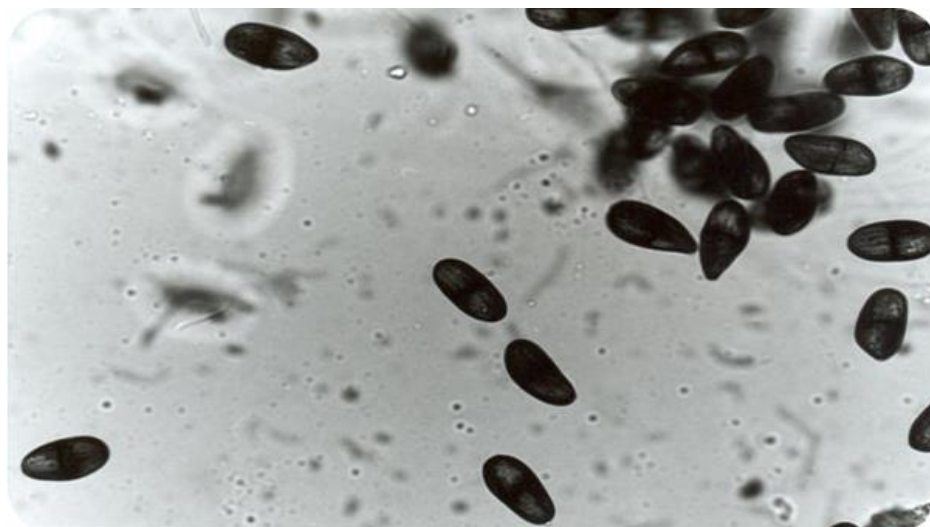


Figure 1. Photomicrograph of *Choanephora cucurbitarum* × 400 showing its conidia.

Table 2. Effect of the different inoculation courts on the height of the test plants (cm).

Infection courts	Day 1	Day 4	Day 7	Day 10	Day 14	Day17	Day 20
Leaf	41.60	43.33	45.03	45.78	46.40	48.21	48.11
Stem	37.55	41.96	43.56	43.56	45.06	45.95	42.91
Soil	48.42	49.69	50.86	51.73	55.18	53.16	54.01
Control	80.31	85.13	98.38	91.50	96.50	101.38	106.75
LSD	0.67	0.70	0.70	0.70	0.67	0.68	0.68

Table 3. Effect of the pathogen on the height of the test plants on daily bases (cm).

Test plants	Day 1	Day 4	Day 7	Day 10	Day 14	Day 17	Day 20
<i>Amaranthus sp</i>	48.06	49.78	51.88	51.93	55.23	57.58	59.48
<i>V. unguiculata</i>	44.24	47.85	50.01	50.88	52.45	54.83	57.44
<i>C. sativus</i>	76.60	81.21	83.44	84.89	86.28	87.66	89.20
<i>A. esculentus</i>	38.99	41.26	43.50	44.88	49.19	48.64	45.67
Control	80.31	85.13	91.50	96.50	98.38	101.38	106.75
LSD	0.67	0.70	0.70	0.70	0.67	0.68	0.68

Effect of the Pathogen on the Leaves of the Test Plants:

Table 4 showed that the infection period by the pathogen played a significant role in leaf reduction of the test plants, when compared with the control. Based on the infection courts (leaf, stem, soil), it was observed that the greatest effect of the pathogen was on the inoculated stem of *Abelmoschus esculentus* (14.86) inoculated leaf of *Vigna unguiculata* (13.47), and on the inoculated leaf of *Cucumis sativus* (13.50). The least effect was observed when *Amaranthus sp* and *Cucumis sativus* were grown on infected soil, inoculated stem of *Vigna unguiculata* and inoculated leaf of *Abelmoschus*

esculentus when compared with the control.

Table 5 showed that the greatest effect of the fungus on leaf reduction (on daily bases: 1st to 20th day) after inoculation was on the inoculated leaf and stem of the test plants. The least effect was observed when the test plants were grown on infected soil (13.88 to 28.50). It was also observed that the greatest effect of the pathogen (leaf reduction) of the test plants was on *Cucumis sativus* (14.00 to 24.50). The least effect was on *Vigna unguiculata* (22.75 to 51.50) as presented in (Table 6) when compared with the control (19.75 to 52.63).

Table 4. Effect of infection period on the leaves of the test plants (cm).

Infection courts	<i>Amaranthus sp</i>	<i>V. unguiculata</i>	<i>C. sativus</i>	<i>A. esculentus</i>
Leaf	25.07	13.47	13.50	22.50
Stem	19.00	44.36	17.00	14.86
Soil	25.57	20.64	21.57	15.71
Control	31.29	49.36	29.71	30.86
LSD	1.83			

Table 5. Effect of different inoculation courts on the leaves of the test plants on daily bases (cm).

Infection courts	Day 1	Day 4	Day 7	Day 10	Day 14	Day 17	Day 20
Leaf	12.88	16.63	24.63	24.63	26.50	29.25	32.63
Stem	15.75	21.75	24.36	25.13	24.38	26.38	28.88
Soil	13.88	17.75	19.50	19.50	21.63	25.38	28.50
Control	19.75	24.13	28.75	35.00	40.50	46.38	52.63
LSD	0.02	0.30	0.03	0.28	0.33	0.37	0.42

Table 6. Effect of the pathogen on the leaves of the test plants on daily bases (cm).

Test plants	Day 1	Day 4	Day 7	Day 10	Day 14	Day 17	Day 20
<i>Amaranthus sp.</i>	16.50	20.75	23.25	24.75	26.88	30.75	33.75
<i>V. unguiculata</i>	22.75	28.50	24.13	37.13	39.00	44.38	51.50
<i>C. sativus</i>	14.00	17.38	19.75	21.38	22.75	23.38	24.50
<i>A. esculentus</i>	9.00	13.63	17.13	21.00	24.38	28.88	32.88
Control	19.75	24.13	28.75	35.00	40.50	46.38	52.63
LSD	0.02	0.30	0.03	0.28	0.33	0.37	0.42

Effect of the Pathogen on the Fresh and Dry Weight of the Test Plants:

Result from this study showed that the pathogen played a significant role in reduction of fresh and dry weight of the test plants. It was observed that the pathogen recorded significant differences (reduction) on fresh and dry weight when compared with the control. Based on the infection courts (leaf, stem, soil), it was observed that the pathogen showed the greatest effect for fresh weight on the inoculated leaf, stem and soil of *Cucumis sativus* (with dry weight ranging from 1 to 5g), inoculated stem of *Amaranthus sp* (5g) when compared with the control which had a fresh weight of (60g) as shown in (Fig 2).

While the greatest effect for dry weight was on the inoculated stem of *Amaranthus sp* (2g), inoculated leaf, stem and soil of *Cucumis sativus* (1g), inoculated stem of *Amaranthus sp* (2g) and on *Vigna unguiculata* when it was grown on infected soil (4g) when compared with the control (12g) as shown in (Fig 3).

DISCUSSION

In this study, it was observed that the fungus *Choanephora cucurbitarum* isolated from rotting fruits of *Capsicum anuum*, was pathogenic to *Abelmoschus esculentus*, *Amaranthus sp*, *Cucumis sativus* and *Vigna*

unguiculata, This agrees with the findings of Kucharek *et al.*, (2003) who reported that *Choanephora cucurbitarum* (Berkeley and Ravenel) that has a wide host range in some vegetable families, Cucurbitaceae, Solanaceae etc. Pepper plants (*Capsicum anuum*) are susceptible from seedling to flowering stage, damaging the entire buds, flower stalk, stem and tissue leading to wilt and defoliation of young infected fruits.

Pathogenicity tests of the fungus isolated was conducted and production of symptoms as those observed in the field was used as confirmation of pathogenicity, Kenny (2010). In this study, it was observed that the fungus caused leaf blight, serious shoot disease of *Amaranthus sp* with the affected plant characterized by a blight of the shoot apex, this agrees with the findings of Ikediugwu (1981), Yu and Ko (1997) who reported that the shoot apex is often accompanied by a conspicuous curvature of the young stem. It was also observed that the fungus caused die back and leaf blight of *Vigna unguiculata*, *Abelmoschus esculentus* and *Cucumis sativus*, This agrees with the findings of Kucharek *et al.*, (2009), Lefebvre and Weimer (1989) on *Choanephora* blight (wet rot) and *Choanephora cucurbitarum* attacking cowpea.

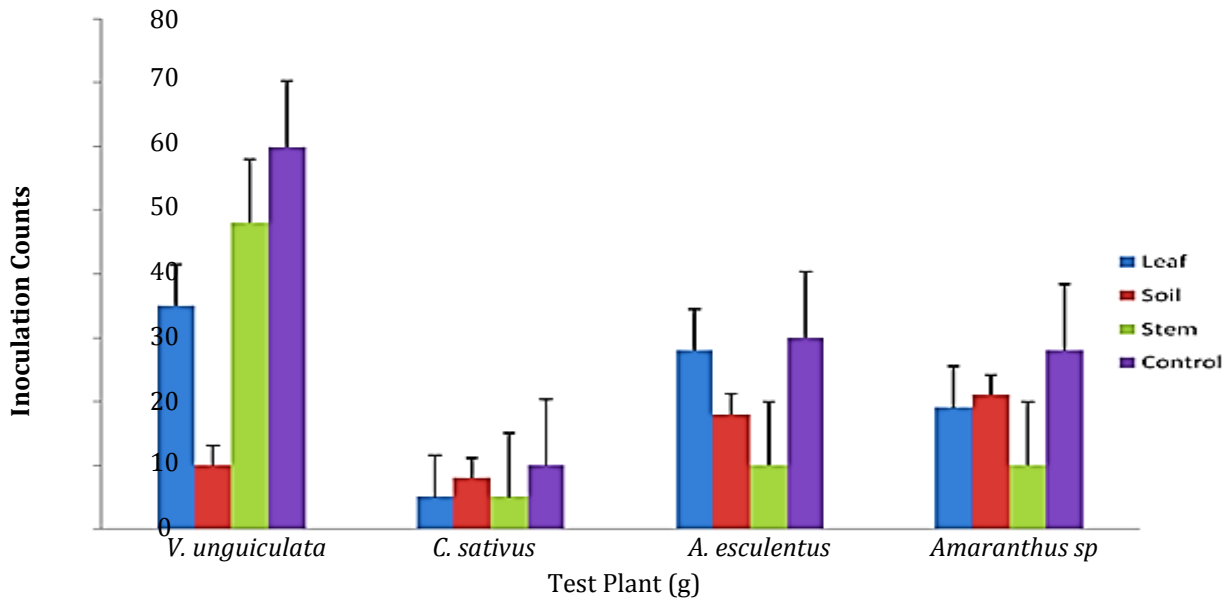


Figure 2. Effect of different inoculation courts on the fresh weight of the test plants.

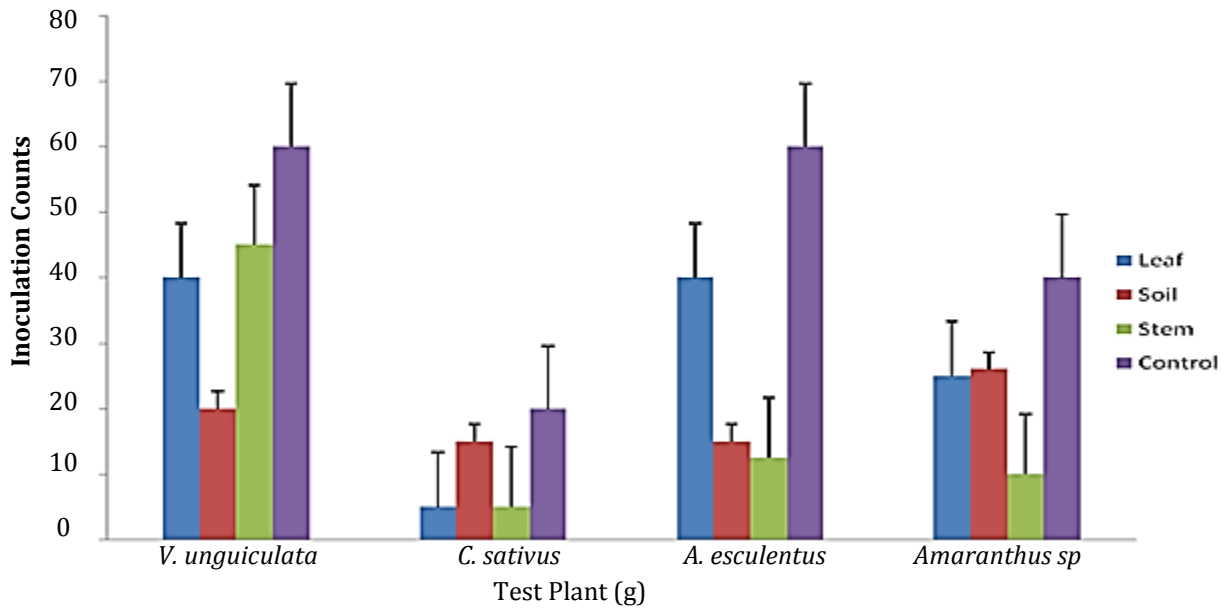


Figure 3. Effect of different inoculation courts on the dry weight of the test plants.

The fungus also caused soft rot of stems of *Abelmoschus esculentus*, *Amaranthus sp*, *Cucumis sativus* and decay of *Vigna unguiculata* pods, these findings is similar to that of Abel-mortaal *et al.*, (2009) on leaf spot disease of *Hyoscyamus muticus* and that of Wu and Chein (1980) on compatibility studies of *Choanephora cucurbitarum* isolated in Taiwan.

Results from this study showed that the pathogen caused significant reductions on the height, leaves, fresh and dry weight of the test plants, which agrees with that of Madunagu *et al.*, (2006) who reported a decrease in height and leaves of *Colocynthis citrillus* and *Cucurbita*

pepo infected with *Colletotrichum sp*, but disagrees with that of Makambila and Goma (1993) who reported increase in height of *Amaranthus sp* infected with *Choanephora cucurbitarum*.

In this study, the fungus was inoculated into leaves, stem and soil of the test plants, which is similar to that of Saroj *et al.*, (2012), Yu and Ko (1999) on wet rot of *Withania somnifera* and effect of *Choanephora cucurbitarum* infection on the growth of vegetables.

CONCLUSION

The study showed that *Choanephora cucurbitarum* was pathogenic to the test plants (*Abelmoschus esculentus*,

Amaranthus sp, *Cucumis sativus*, and *Vigna unguiculata*). Based on these findings, it is recommended that these plants should not be grown together during mixed crop farming. This is to avoid an epidemic that may be caused by the fungus. It is also important to carry out more epidemiological studies in Calabar, Cross River state, Nigeria on *Choanephora cucurbitarum*. A pathogenic fungus producing life threatening diseases on vegetables, and to educate farmers on the preventive and control measures especially mixed crop farming.

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