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In-Vitro Effect of some Commonly Found Botanicals on the Growth and Sporulation of Choanephora Cucurbitarum (Berkeley and Ravenel)

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Abstract
A soft rot infection of *Abelmoschus esculentus*, *Amaranthus hybridus* and *Vigna unguiculata* was observed in home gardens and Government farms in the 2010 cropping season. This disease caused remarkable yield loss in these crops. Due to residual effects of synthetic chemical control, it became necessary to test the potency of some botanicals on the growth and sporulation of the fungus as a control measure. The procedures involved isolation and identification of the fungus and potency trials of aqueous and ethanolic extracts of the botanicals on the assay fungus. The isolated fungus was confirmed as *Choanephora cucurbitarum*. The extracts were obtained from *Zingiber officinale* Roscoe, *Gmelina arborea* Roxb, *Chromolaena odorata Linnaeus* and *Azadirachta indica* A. Juss. Different concentrations of the extracts (0%, 10%, 20%, 30%, 40% and 50%) were used. With ethanol extract, there was complete inhibition of growth by all plant extracts and at all concentrations; and a little growth in aqueous extracts with *Azadirachta indica* showing the highest inhibitory effect, while *C. odorata* showed the lowest inhibitory effect on the first day, (*A. indica*, $0.1 \pm 0.0 \geq$ *G. arborea,* $0.1 \pm 0.0 >$ *Z. officinale,* $0.2 \pm 0.0 >$ *C. odorata,* 0.3 ± 0.0). On the last day, the level of inhibition was as follows *A. indica*, $0.4 \pm 0.0 >$ *G. arborea,* $0.6 \pm 0.0 >$ *Z. officinale,* $0.7 \pm 0.0 \geq$ *C. odorata* 0.7 ± 0.0. The inhibitory effect increased with increase in the percentage concentration of the extracts. The potency was also due in part to the phytochemical constituents of the plant extracts which was observed from the screening test that Saponins, Tannins, Alkaloids, Cardiac glycosides, Flavonoids, Reducing Compounds, Polyphenol, Phlobatannins, Anthraquinones and Hydroxymethyl anthraquinones were either present or absent.
Ke words: Botanicals, Cross-River, Phytochemicals, Extract, Susceptibility.

Introduction

Choanephora cucurbitarum (Berkeley and Ravenel) Thaxt. is a common fungus which is often implicated in the wet rot of many farm crops especially vegetables such as; *Abelmoschus esculentus* (L.) Moench, *Amaranthus hybridus* L., *Vigna unguiculata (L.) Walp* and Cucurbits such as *Cucumis sativus* L. *Cucumis melo* L. and *Telfaira occidentalis* (Jensen, 1995; Kucharek *et al.*, 2003; Ray, 2004, Jeffery, 2005; and Renner *et al*., 2007). It is also known to attack cereals such as Millet, rice and sorghum (Umana and Ikotun, 2000). It causes wet rot of *Capsicum annum* (pepper), fruit rot of vegetable crops (summer squash, *Telfaria occidentalis*., cottony blight of *Cucumis sativus L.* (cucumber) and pod rot of *Vigna sinensis* L. (Greuter *et al.,* 2000). According to Umana and Ikotun, 2000, isolates of the fungus grew readily and spontaneously in pure culture and the *in vitro* effect of agro-botanicals such as compost from rice straw and empty fruit bunch of oil palm suppressed the growth and sporulation of *Choanephora cucurbitarum*. Some medicinal plants such as; *Vernonia amygdalina* Delile, *Carica papaya L.* and *Ocimum gratissimum L.* have also proven to be very effective in inhibiting the growth and sporulation of fungi (Cuiller, 1982 ; Sofowara, 1993; Miliauskas *et al.*, 2004 ; and O'Hara *et al*., 1998).

Materials and methods

Source of fungal pathogen and morphological identification.

The fungus used in this research project 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 Wildlife 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 1 minute 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 ± 1^{o}C until fungal growth was noticed. After 5 days, the different isolates were sub-cultured on freshly prepared PDA to obtain their pure culture. Isolated fungi were microscopically (Olympus optical, Phillippines) identified as far as possible using the identification guides of the International Mycological Institute, Kew and of Barnet and Hunter, 1998). Stock cultures of these fungi were stored in Agar slant bottles for subsequent use.

Pathogenicity tests of the fungal isolates were conducted and production of symptoms, as those observed in the

field was used as confirmation of pathogenicity (Kenny, 2010).

Sources of plant materials.
The four plants used were; *Zingiber officinale*, *Gmelina arborea*, *Chromolaena odorata* and *Azadirachta indica*. The leaves of *G. arborea*, *C. odorata* and *A. indica* were harvested from the Botanic Garden of the Cross River University of Technology, Calabar, Nigeria while, *Zingiber officianale* rhizomes were bought from Watt market, Calabar. The methods of collection and treatment of plant samples were as recommended by Udo *et al*. (2006), The choice of plants for this work was based on the composition of multiple medicinal and biological properties, free radical scavenging actions, anti-inflammatory, antioxidant, anticarcinogenic abilities including inhibition of hydrolytic and oxidative enzymes, bacteria and fungi in line with Ernst and Pittler, 2000), Schmidt and Schilling, 2000), Miliauskas *et al.*, 2004), Igboh *et al*., (2009)

Preparation of Plant Extracts
The plants were collected, washed in distilled water and dried under room temperature for five (5) days. Dried plants were separately pulverized to powder with laboratory blender. Aqueous and ethanol extracts of all the test plants were prepared by extracting different weights (5, 10, 15, 20 and 25g) of each of the plants' powder with 50ml of the extracting solvents (AOAC, 1995). The mixture was allowed to stand for 48 hours before being filtered with Whatman No.1 filter paper. That for ethanol was allowed to stand for 24 hours only. The extracts were then stored in reagent bottles in the refrigerator as stock solution.

Susceptibility test
The extract percentage concentrations were prepared at 10,20,30,40 and 50% with distilled water and ethanol.

Dilution test procedures
About 2ml of each concentration was first poured into different sterile Petri dishes using sterile syringes. The sterilized potato dextrose agar (PDA) media was also poured into the plates containing the extracts after which the plate was gently swirled to ensure mixing. The media was allowed to solidify and with a sterilized No. 2 cork borer (5.5 mm in diameter), a disc of the matured culture was punched out, inoculated at the centre of plates and incubated at room temperature of 28±1ºC. As a control, the discs were inoculated on distilled water-agar mix instead of extracts-agar mix. Four (4) control plates were prepared. Growth measurement was done at 2 days interval for ten days (Udo et al., 2006).

Results
Identification of pathogen
Choanephora cucurbitarum (Figure1) was implicated in the disease so, it was the pathogen isolated, identified and used for the study.

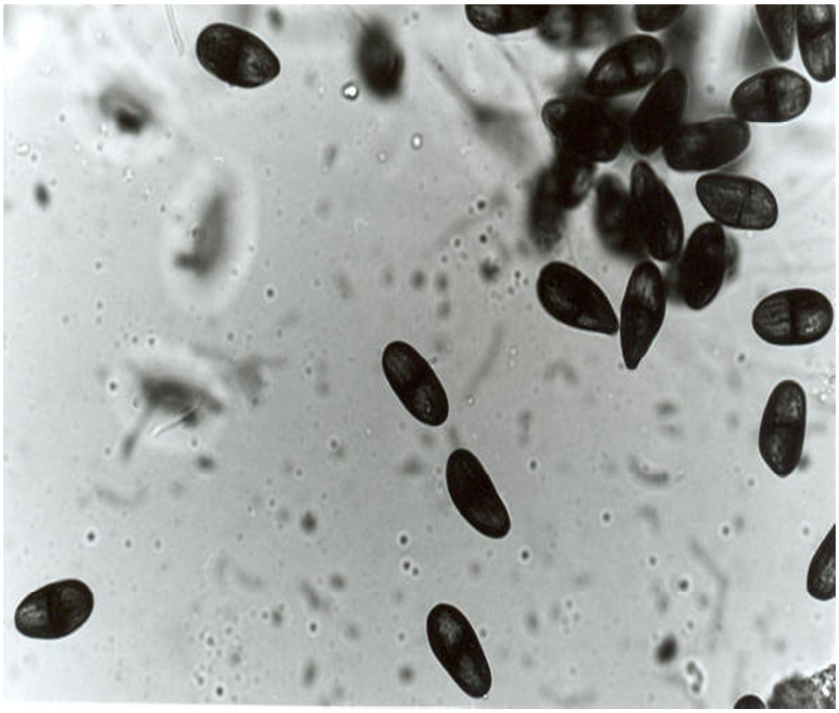

Figure 1: Photomicrograph of *Choanephora cucurbitarum* showing its conidia

Aqueous extract effect on mycelial growth

At 10% concentration, *Zingiber officinale* showed no inhibition from the 2nd -10th day. Mycelial growth increased as the number of days increased until the 10th day. There was a steady growth in the fungal mycelium at this concentration percentage for all the extracts except for *A. indica* that showed growth suppression from the 2nd to 6th day only.

All the plant extracts displayed potency on the organism from the 8th day except *Z. officinale*. Check on fungal growth was recorded for this plant extract between the 6th and 8th day only (Fig. 2).

At 20% concentration, nearly the same trend as for 10% was observed. However, some variations were observed. All the extracts except *Gmelina arborea* (6th day) checked the fungal growth from the 8th day. At this percentage concentration, *Zingiber officinale* was still the most potent (Fig. 3).

At 30% concentration, *Z. officinale* showed no further increment in growth from the 8th day. Growth was maintained at 2mm from the 8th to10th day. For *G. arborea,* no growth increase was observed between the 4th and 8th day but, there was a slight growth of about 1mm from the 8th day. *C. odorata* showed steady growth increase from the 2nd day to the 10th day. *A. indica* showed complete inhibition from the 2nd -10th day. 30% concentration of plant extract showed that *Azadirachta indica* had the highest inhibitory effect while *Chromoleana odorata* had the lowest as shown in Figure 4.

For *Z. officinale* at 40% concentration, growth was only observed between the 4th to 8th day after which death was recorded. *G. arborea* showed no growth from the 6th day. *C. odorata* recorded a slight growth from the 6th -8th day and this growth which was about 2mm was maintained to the 10th day. *A. indica* showed complete inhibition of mycelia growth from the 2nd -10th day. 40% concentration of plant extract on the fungus showed that *Azadirachta indica* had the highest inhibitory effect while *Chromoleana odorata* had the lowest (Figure 5).

At 50% concentration, only *Zingiber officinale* showed mycelia growth of the fungus from the 4th to 6th day and this was maintained to the 10th day. *Gmelina arborea*, *Chromoleana odorata* and *Azadirachta indica* showed complete inhibition of growth from the 2nd -10th day at this percentage concentration.(Figure 6).

It is observed that inhibitory effect increased with increase in the percentage concentration of the extracts and not basically on time.

Ethanolic extract effect

The four plant extracts in ethanol solution exhibited complete inhibition at 10, 20, 30 40 and 50% concentration from the first day of incubation to the tenth. No growth was observed.

Table 1: Effect of botanicals on the growth and sporulation of *Choanephora cucurbitarum*

Plant extract	Day 2	Day 4	Day 6	Day 8	Day 10
Zingber officinale	0.2^{b}	0.3^{b}	0.5^{a}	0.6^{a}	0.7^{a}
Gmelin arborea	0.1^{c}	0.3^{b}	0.4^{b}	0.5^{a}	0.6^{a}
Chromolaena odorata	0.3^{a}	0.4^{a}	0.5^{a}	0.6^{a}	0.7^{a}
Azadirachta indica	0.1^{c}	0.2^{c}	0.2^{c}	0.4^{b}	0.4^{c}
LSD	0.04	0.09	0.09	0.11	0.10

***Means values with same superscipt are not significantly different (p < 0.05 >) .**

Results of the effect of these botanicals on the growth and sporulation of *Choanephora cucurbitarum* , as seen in Table 1, showed that on the second day of incubation, the inhibitory effect of *A. indica* and *G. arborea* were not significantly different at P < 0.05. *A. indica* and *G. arborea* inhibited mycelial growth of *C. cucurbitarum* 0.1cm each, with *C. odorata* inhibiting growth of the fungus by 0.3cm. On the fourth day, *A. indica* had the highest inhibitory effect (0.2cm) on the fungus, with *Z. officinale* and *G. arborea* showing no significant difference on their inhibitory effect (0.3cm each) on *C. cucurbitarum*. Again, *A. indica* had the highest inhibitory effect (0.2cm) on the sixth day with *Z. officinale* and *C. odorata* showing the lowest inhibitory effect (0.5cm) on the mycelial growth of the fungus. on the eight day, *A. indica* showed the highest inhibitory effect of 0.4cm on the mycelial growth of the fungus, while *Z. officinale* and *C. odorata* both showed no significant difference in their inhibitory effect (0.6cm) on the mycelial growth of *C. cucurbitarum*. on the tenth day, *A. indica* inhibited the mycelial growth of *C. cucurbitarum* by 0.4cm, with *Z. officinale* and *C. odorata* showing no significant difference as both inhibited the mycelial growth the fungus by 0.7cm. It was thus observed that *A. indica* had the highest inhibitory effect on the mycelia growth of *Choanephora cucurbitarum* from the second to tenth day using LSD at p<0.05.

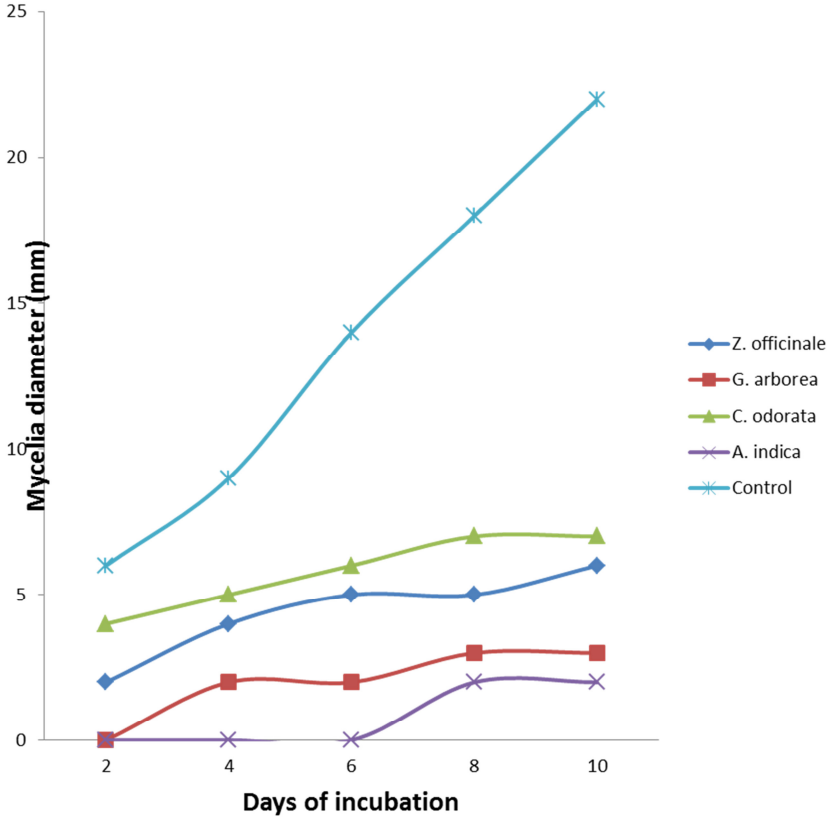

Figure 2: Effect of 10% concentration of plant extracts on the mycelia growth of isolate.

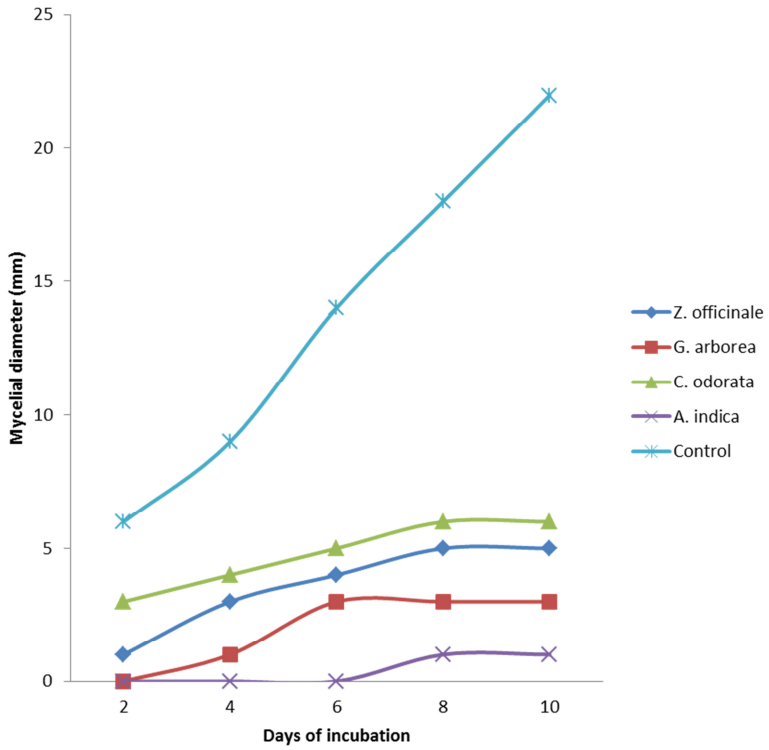

Figure 3: Effect of 20% concentration of plant extracts on the mycelia growth of isolate

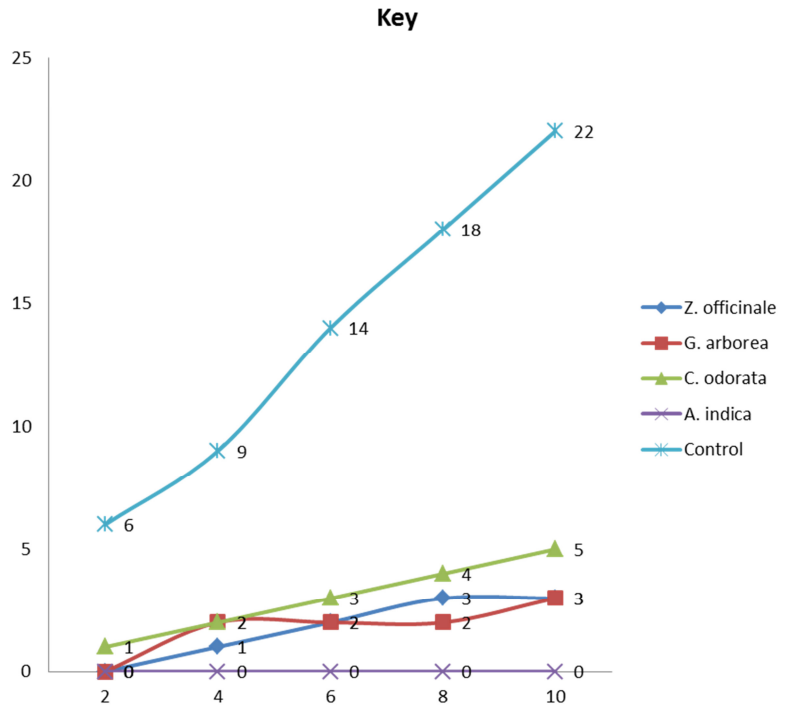

Figure 4: Effect of 30% concentration of plant extracts on the mycelia growth of isolate

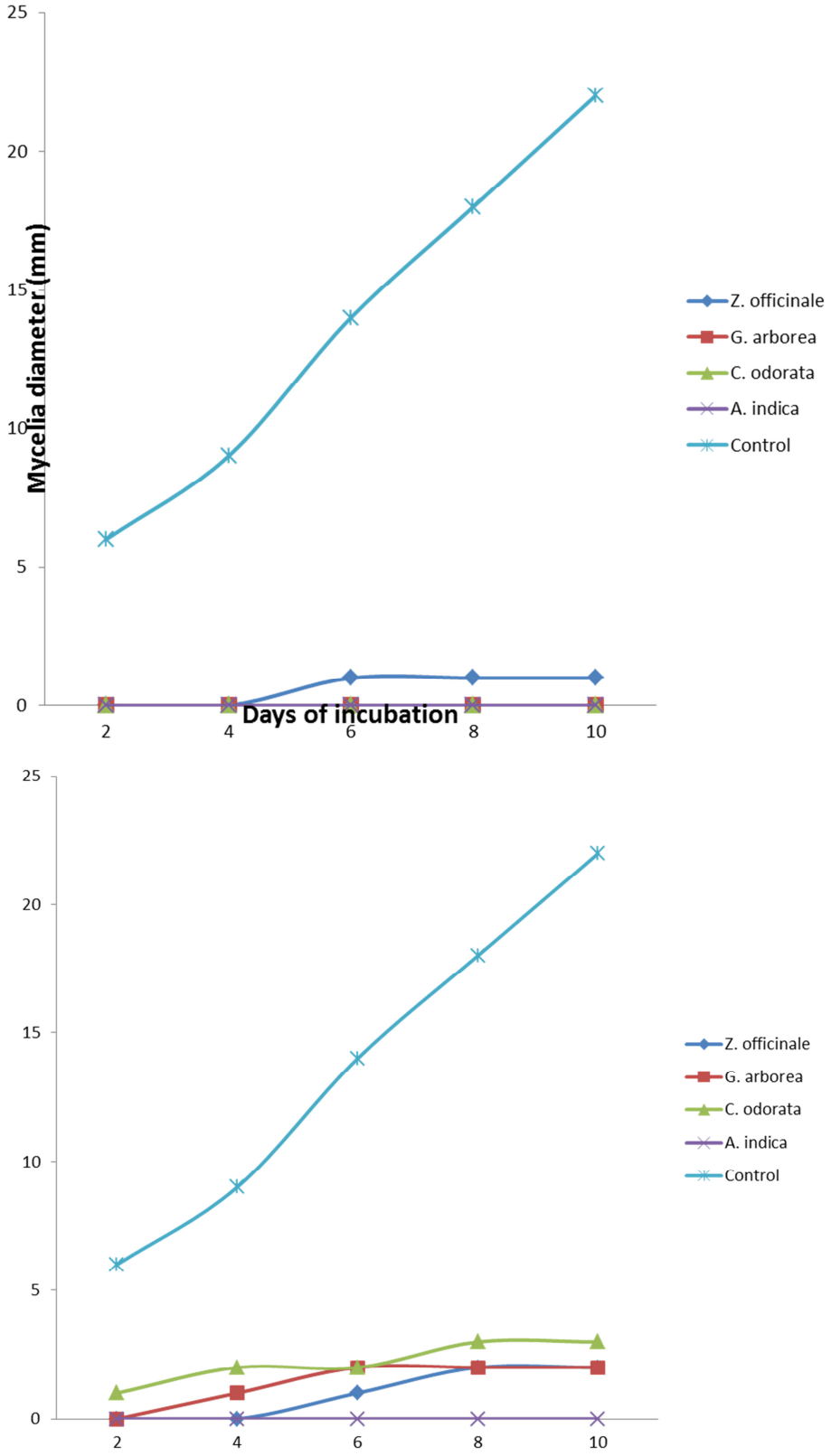

Figure 5: Effect of 40% concentration of plant extracts on the mycelia growth of isolate

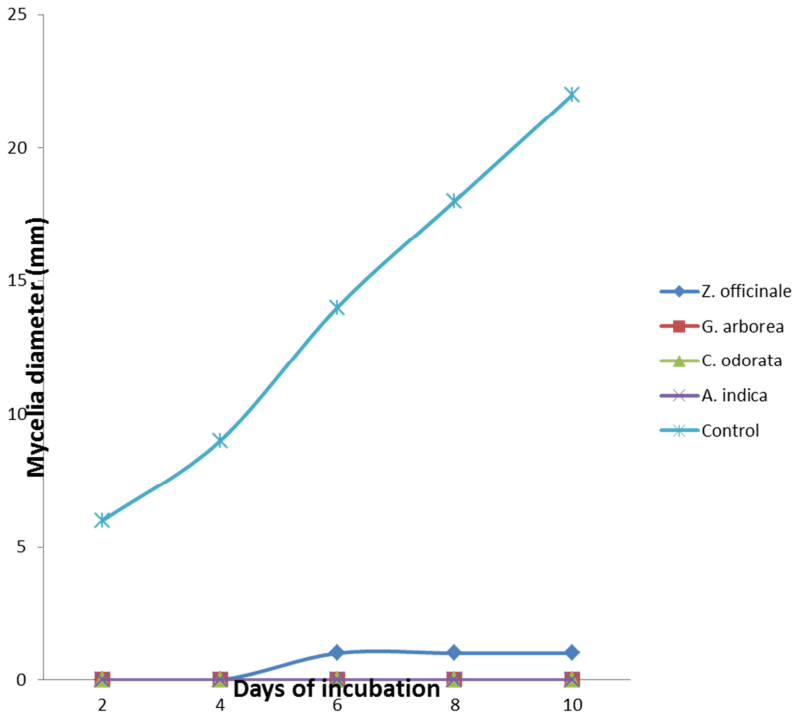

Figure 6: Effect of 50% concentration of plant extracts on the mycelia growth of isolate.

Discussion:

According to Jin-Hyeuk *et al* (2001) and Kucharek *et al.*, 2003), *Choanephora cucurbitarum (Berkeley and Ravenel) Thaxt.* has a wide host range in some vegetable families; Cucurbitaceae, Solanaceae, etc. Pepper plants (*Capsicum annuum*) are susceptible from seedling to early flowering stage, damaging the entire buds, flower stalk, stem, and tissue leading to wilt and defoliation of young infected fruits. Though cultural control measures have been employed but, no chemical control (fungicides) has been labeled for the disease (Siddiqui *et al., 2009*).

Although other effective medicinal plants have been worked on but, this study has shown that extracts of *Zingiber officinale* Roscoe, *Gmelina arborea* Roxb, *Chromolaena odorata* Linn and *Azadirachta indica* A. Juss inhibited the growth of *Choanephora cucurbitarum* Raimundo et al., 2007; El-Mahmood et al., 2010 and Witney, 2010) *Azadirachta indica* had the highest inhibitory effect from the second to the tenth day using LSD at $p<0.05$. Thus, *Azadirachta indica* was more potent (Ganguli,2002; Mohammed, 1999; Rasheed, 2002; Amrutha and Bhaskar, 2010 and Kim et al, 2010).

The potency in these plant extracts could be due to increase in concentration as proposed by Trease and Evans, 1979) who observed that the percentage inhibition of an isolated fungus increased with a corresponding increase in the concentration of the extract. Also, the inhibition of the growth of the fungi is as a result of the phytochemicals contained in the extracts.

CONCLUSION

Azadirachta indica had the highest inhibitory effect on the growth and sporulation of *Chaonephora cucurbitarum* while *chromolaena odorata* had the lowest inhibitory effect on the fungus when distilled water was used as extracting solvent, The ethanolic extracts of the four plants completely inhibited the fungus. Based on the results, the four plants can be utilized as phytofungicides to control the growth and sporulation of *Chaonephora cucurbitarum* on various economically important crops.

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