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EVALUATION OF LEAF EXTRACTS OF FIVE PLANT SPECIES AGAINST RED ROT PATHOGEN OF SUGARCANE (*Saccharum officinarum*).

[1]Adebola, M. O., [2]Aremu, M.B. and [1]Gana, P.
[1]Federal University of Technology, Department of Plant Biology Minna
[2]National Cereal Research Institute, Badeggi, Nigeria
***Corresponding author's email:** adebolamo@gmail.com. Phone:08033821297

ABSTRACT

The research was carried out to evaluate the *in vitro* effects of five botanicals extracts; *Argomone mexicana, Hyptis suaveolens, Corchurus olitorius Cymbopogon citratus and Acalypha wilkesiana* on mycelial growth of *colletotrichum falcatum,* the pathogen of red rot disease of sugarcane with methanol and aqueous extratants, at concentrations of 150mg/ml, 200mg/ml and 250mg/ml using food poison techniques. The results on phytochemical analysis revealed that these botanicals contained alkaloids, saponins, glycosides, flavonoids and steroids. At 250mg/ml concentration of methanol extracts, 100% inhibition in mycelial growth were recorded in both *C. citratus* and *C. olitorius* which were significantly different ($P<0.05$) from other botanicals; *A. wilkesiana* (88.72%) and *H. suaveolens* (82.56%). The least inhibition (47.68%) was recorded in *A. mexican.* In aqueous extracts, at 250mg/ml concentration, 41.87% mycelial growth inhibition of *C. falcatum* was recorded in *C. citratus,* followed by *A. wilkesiana* (24.42%) and least inhibition in *H. suaveolens* (15.66%), while *C. olitorus and A, mexicana* has no effect. Also the inhibition increased significantly with increase in concentrations *from* 150mg/ml to 250mg/ml of the botanicals in either of both extractants. In comparing the efficacy of the two solvents (methanol and aqueous) used in the study, it was observed that methanol extracts at different concentrations inhibited the mycelial growth of *C. falcatum* better than the aqueous. However, in all, *C. citratus* gave maximum inhibition which was statistically different ($P<0.05$)from other botanicals. On this note, these botanicals are recommended for the field trial on the control of red rot disease of sugarcane.

Keywords: Extracts, Red rot pathogen, Phytochemicals, Leaf extracts, Sugar cane

INTRODUCTION

Sugarcane *(Saccharum officinarum)* is the world's economically important crop which is cultivated on 20.42 million hectare across the world with an estimated total production of 1,333 million metric tons (Alwala *et al.*, 2006). It is an important cash crop which besides serving as food also fulfils requirements of energy and feedstock for industry (Martin *et al.*, 2002). Chemically, it consists of 70% water, 14% fibre, 13% sucrose and 2-7% soluble impurities. Juice is extracted for the production of edible sugar (Da Costa *et al.*, 2011). Moreover, efficient photosynthetic mechanism

of sugarcane enables it to fix almost 2 - 3 % of radiant solar energy and transform it into green biomass. This efficient photosynthetic capability also allows it to show a high coefficient of CO_2 fixation as compared to the moderate climate zone; thereby contributing to the decrease of the greenhouse effect (Alwala *et al*., 2006).

However various biotic and abiotic factors are responsible for its low yield. Many types of bacterial, fungal, viral and nematodal diseases of sugarcane have been reported worldwide(Agnihotri,1996). Among fungal diseases, red rot disease (Causal agent: *Colletotrichum falcatum;* perfect stage *Glomerella tucumanensis*) is the most devastating and threatening disease. It can reduce cane weight by up to 29% and loss in sugar recovery by 31% (Mohanraj *et al*., 2002; Shama and Tamta, 2015). First symptom of the disease is seen usually after rainy season when the plant growth ceases and sucrose formation begins. The external symptoms appear firstly on the leaves. A slight discolouration and drooping are observed that leads to the withering of the entire tip progressing down the margins at the initial stage. With the passage of time, the infection originates as a dark reddish area on the leave midrib which elongates rapidly, forming blood red lesions whose margins become darker. The centre becomes raw dull grey coloured in older lesions and the lesions are covered with a powdery mass of conidia on formation of the pathogen fructifications. The canes become shriveled with shrinked and longitudinally wrinkled rind (Meziane *et al*., 2005). If the affected canes are split opened, the inter-nodal tissues will be found to be reddened in longitudinal direction in one or more internodes usually proceeding towards the base. The reddening is more conspicuous in the vascular bundles and progresses towards the pith of the cane. The reddening is interrupted transversely by the white postules; an important characteristic that distinguishes the red rot disease from any other physiological stress as any injury leads to the reddening of tissue in sugarcane.

Various chemicals especially fungicides are used to control the red rot disease. Because of the negative public perceptions about the use of synthetic chemicals, resistance to fungicides among fungal pathogens, and high development cost of new chemicals. The uses of plant-derived products as disease control agents are the alternative control method needed, since these products have low mammalian toxicity, less hazardous to ecosystem and wide public acceptance (Alwala *et al*., 2006). In view of the above facts, this research was investigated to control red rot pathogen of sugarcane using aqueous and methanol leaf extracts of five plant species namely, *Argomone mexicana, Hyptis suaveolens, Corchurus olitorius Cymbopogon citratus and Acalypha wilkesiana.*

MATERIALS AND METHODS

Collection and identification of the plants

The fresh leaves of *Argomone mexicana, Hyptis suaveolens, Corchurus olitorius Cymbopogon citratus and Acalypha wilkesiana* were collected from Doko in Lavun Local Government Area, Bosso campus, Federal University of Technology, Minna and Bosso in Bosso Local Government

Area of Niger State. The plants were identified and authenticated from the herbarium of Department of Biological Sciences, Federal University of Technology Minna, Niger State using the documented literatures and monographs.

Table 1 The family, common namesand the parts used of five botanicals collected from different locations in Niger State.

Botanical names	Families	Common names	Part used
Argomone mexicana	Papaveraceae	Prickly Poppy	Leaf
Hyptis suaveolens	Lamiaceae	Vilayati tulsi	Leaf
Corchurus olitorius	Tiliaceae	Mallow/ ewedu	Leaf
Cymbopogon citratus	Poaceae	Lemon grass	Leaf
Acalypha wilkesiana	Euphorbiaceae	Copper leaf	Leaf

Preparation of Aqueous and Methanol Leaf Extracts of Different Plant Species

The fresh leaves of *Argomone mexicana, Hyptis suaveolens, Corchurus olitorius Cymbopogon citratus and Acalypha wilkesiana* were air dried for fifteen days. Both dried materials were crushed using mortar and pestle, later pounded into powder form; and sieved with a mesh of sized 0.05mm. This was done to enhance the penetration of the extracting solvents into the cell, thereby facilitating the release of active ingredients (Ahsidi *et al.*, 2012). The micronized sample was used for the extraction. The micronized sample (12.5g) was added to 100ml distilled water in 250ml size conical flasks. The conical flasks were plugged with cotton wool and the mixture was allowed to settle down and left overnight (24hours) in the refrigerator. The extracts were filtered off using cotton with No1 Whatman filter paper (Ahsidi *et al.*, 2012).

The filtrates were concentrated by evaporation using a rotator evaporator at 40°C to concentrate each of the extracts. The procedure was repeated using methanol as extractants (Sofowora, 1993). The amount of sample obtain was recorded and stored in a well-corked bottle for further analysis.

Phytochemicals Screening of Leaf Extracts of Five Plants Species

Phytochemical test was conducted to qualitatively verify the presence or absence of secondary metabolites from the extract of the plants. A small portion of the extracts was subjected to phytochemical analysis using standard methods for presences of alkaloids, saponins, proanthocyanins, steroids, flavonoids, tannins and terpenoids (Sofowora, 1993; Ogukwe *et al.*, 2004; Hassan *et al.*, 2004; Falodun *et al.*, 2011)

Isolation, Purification and Maintenance of Red Rot Pathogen

Twenty diseased sugarcane stems were collected from the sugar field of National Cereal Research Institute Badegi in Kaccha Local Government Area of Niger State. The pathogen was isolated from the diseased tissues (Plate 1) of the infected sets of sugarcane stem by tissue segment method. The tiny pieces (2-3 mm) were cut and washed vigorously with distilled water and then surface sterilized with 0.1% mercuric chloride ($HgCl_2$) for 30-40 seconds. The tissues were washed with sterile distilled water and dried on aluminum foil paper, after which the tissues were inoculated into the Potato Dextrose Agar (PDA) and incubated at 28 ± 2°C for 48-72hr. The pathogen was further purified by subsequent sub culturing to obtain pure culture isolate (Adegoke and Adebayo, 2009; Prince and Prabakaran, 2011; **Nikhil and Sahu, 2014**). Identification of *Colletotrichum falcatum* was based on the observed morphological features taking note of the growth rate and pattern on agar plates colony size, colour and shape of spores. Stock culture of the isolate was maintained in McCartney bottle slants and stored at 4°C in a refrigerator for subsequent use.

Pathogenicity Test of *Colletotrichum falcatum*

The inoculum was prepared by taking five discs of *C. falcatum* culture in PDA using 5mm cork borer into a beaker containing 15ml of sterile distilled water and were shaken for two weeks using shaker machine to homogenize the fungal growth. Ten (10) ml of the inoculum was inoculated into the stem of fresh healthy sugar cane obtained from the field and incubated at 27±2°c for 48-72hrs. After which the fungal was re-isolated and identified (Agrios, 2005).

Evaluation of aqueous and Methanol Leaf Extracts of Five Plant Species against test pathogen

Different concentration of 250, 200 and 150 mg/ml of *Argomone exicana, Corchurus olitorius Hyptis suaveolens, Cymbopogon citratus and Acalypha wilkesiana* leaf extracts were prepared using sterile distilled water (Adegoke and Adebayo, 2009). Poison food technique by (Ambika and Sujatha, 2015) was employed to screen the antifungal efficacy by introducing 2ml of each extracts (of different concentrations) into a sterile petridish before 10ml of PDA was poured. A well (5mm diameter) was bored at the center of poison PDA medium using sterile cork borer (size: 5mm) and 5mm of *C. falcatum* culture was taking using 5mm cork borer and inoculated into the well and incubated at 28±2°c for 48- 72hrs. After which the plates were observed for the inhibition of fungal growth by measuring the diameter of the growth from the point of inoculation using a ruler graduated in millimeters (mm). A control was prepared without introduction of any extracts. Three replicates of each inoculation were made.

Inhibition percentage

The inhibition percentage was calculated measuring the radial growth on the control and amended plates, using the following formula (Herlapur *et al*., 2007):

$$I\% = \frac{(C1 - C2)}{C1} X\ 100$$

Where, I% = Inhibition percentage of pathogen growth, C_1 = average radial growth in control plate and C_2 = average radial growth in plates amended with sample of extracts.

Statistical Analysis

The experiment was carried out following Complete Randomized Block Design (RBD) with three replicates of each treatment. All experimental data were subjected to analysis of variance (ANOVA).The means of mycelial inhibition of all treatments were separated using New Duncan Multiple Range Test at $P<0.05$.

RESULTS

The results of pathogenicity (Fig.1) confirmed the fungus isolated was *Colletotrichum falcatum*, the causative organism of the red rot disease of sugar cane. The phytochemicals analysis (Table 2) revealed that alkaloids and saponins were present in all the leaves of five plant species examined, while flavonoids, glycosides steroids and were present in all the plants except in *C. olitorius, C. citratus,* and *A. mexicana* respectively

Table 2: Phytochemical components of leaf extracts of five plants species

Botanical	Alkaloids	Flavonoids	Glycosides	Saponins	Steroids
H. suaveolens	+	+	+	+	+
A. mexicana	+	+	+	+	-
C. olitorius	+	-	+	+	+
A. wilkesiana	+	+	+	+	+
C. citrates	+	+	-	+	+

+ = present, - = absent.

***In vitro* growth inhibition of *C. falcatum* in methanol leaf extracts.**

The results on the *in vitro* growth inhibition of *C. falcatum* (Table 3) by five plant extracts; *H. suaveolens, A. mexicana, C. olitorius, A. wilkesiana* and *C. citratus* using food poisoned showed

that the inhibition varied significantly with different botanicals at different concentrations of 250mg/ml, 200mg/ml and 150mg/ml. At 250mg/ml, maximum inhibition (100%) in mycelial growth was recorded in both *C. citratus* and *C. olitorius* which were statistically different ($P<0.05$) from other botanicals; *A. wilkesiana* (88.72%) and *H. suaveolens* (82.56%). The least inhibition (47.68%) was recorded in *A. mexican*

At 200mg/ml concentration, 100% inhibition in mycelial growth was recorded in *C. citratus, A. wilkesiana* (82.56%), *C. olitorius* (81.40%), *H. suaveolence* (75.58%) and least inhibition in mycelial growth (24.42%) was recorded in *A. mexicana.* At 150mg/ml concentration, 90.69% inhibition in mycelial growth was recorded in *C. citratus, A. wilkesiana* (70.93%), *C. olitorius* (73.26%), *H. suaveolence* (70.93) and least inhibition in mycelial growth (16.28%) was recorded in *A. mexicana. C. citratus* showed maximum inhibition which was statistically different from other botanicals and control (Figs. 2 and 3)

Table 3: *In vitro* growth inhibition of *C. falcatum* by methanol leaf extracts of five different plant species at different concentrations.

Plant leaf	Mycelial growth (mm)			Inhibition of growth (%)		
extracts	150mg/ml	200mg/ml	250mg/ml	150mg/ml	200mg/ml	250mg/ml
Control	86.00 ± 0.00^{d}	86.00 ± 0.00^{e}	86.00 ± 0.00^{e}	0.00^{a}	-	-
H. suaveolens	25.00 ± 0.58^{b}	21.00 ± 0.58^{c}	16.00 ± 0.58^{c}	70.93^{c}	75.58^{b}	82.56^{b}
A. Mexicana	72.00 ± 0.58^{c}	65.00 ± 0.58^{d}	45.00 ± 1.15^{d}	16.28^{b}	24.42^{a}	47.68^{a}
C. olitorius	23.00 ± 1.15^{b}	15.00 ± 0.58^{b}	0.00 ± 0.00^{a}	73.26^{c}	81.40^{c}	100.00^{c}
A. wilkesiana	$25.00\pm s0.00^{b}$	15.33 ± 0.33^{b}	7.67 ± 3.93^{b}	70.93^{c}	82.56^{c}	88.72^{b}
C. citrates	8.00 ± 0.58^{a}	0.00 ± 0.000^{a}	0.00 ± 0.00^{a}	90.69^{d}	100.00^{d}	100.00^{c}

Means with the same superscript along the same column are significantly different at $P<0.05$.NDMT

***In vitro* growth inhibition of *C. falcatum* in aqueous leaf extracts.**

Table 4 showed the *in vitro* effects of aqueous extracts of five botanicals, the results revealed that at 250mg/ml concentration, 41.87% mycelial growth inhibition of *C. falcatum* was recorded in *C. citratus*, followed by *A. wilkesiana* (24.42%) and least inhibition in *H. suaveolens* (15.66%), while *C. olitorus and A. mexicana* has no effect. At 200mg/ml concentration, 33.72% mycelial growth inhibition was recorded in *C. citratus, A. wikesiana* (18.60%) and least inhibition in mycelia growth (3.49%) was recorded in *H. suaveolens*, while *C. olitorus and A. Mexicana* showed no inhibitory effect. At 150mg/ml concentration, 30.23% mycelial growth inhibition was recorded in *C. citratus* and least inhibition in mycelia growth (10.47%) was recorded in *A. wikesiana* and no inhibition in mycelia growth was recorded in *H. suaveolens, C. olitorus and A. Mexicana*(Figs.4 and 5).

Table 4: *In vitro* growth inhibition of *C. falcatum* by aqueous leaf extracts of five different plant species at different concentrations.

Plant leaf extracts	Mycelia growth (mm)			Inhibition of growth (%)		
	150mg/ml	200mg/ml	250mg/ml	150mg/ml	200mg/ml	250mg/m
Control	86.00±0.00^{c}	86.00±0.00^{d}	86.00±0.00^{d}	00.00 ^{a}	00.00	00. 00
Hyptis suaveolens	86.00±0.00^{c}	83.00±0.58^{c}	73.00±1.15^{c}	00.00 ^{a}	3.49^{a}	15.66^{a}
Argemone Mexicana	86.00±0.00^{c}	86.00±0.00^{d}	86.00±0.00^{d}	00.00 ^{a}	00.00	00. 00
Corchorus olitorius	86.00±0.00^{c}	86.00±0.00^{d}	86.00±0.00^{d}	00.00 ^{a}	00.00	00.00
Acalypha wilkesiana	77.00±0.00^{b}	72.00±1.15^{b}	65.00±0.58^{b}	10.47^{b}	18.60^{b}	24.42^{b}
Cymbopogon citrates	62.00±1.15^{a}	57.00±0.00^{a}	51.00±0.58^{a}	30.23 ^{c}	33.72^{c}	41.87^{c}

Means with the same superscript along the same column are not significantly different at P<0.05.NDMT

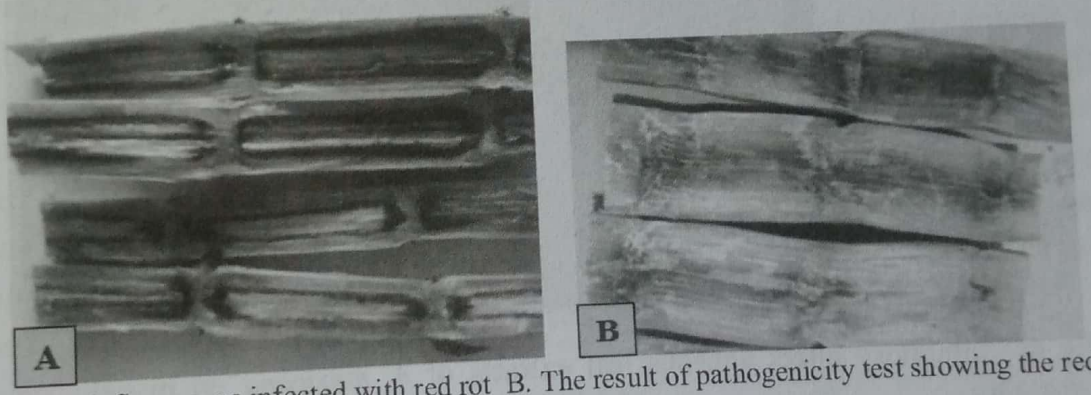

Fig 1: A Sugarcane infected with red rot B. The result of pathogenicity test showing the red rot symptom of sugar cane stem

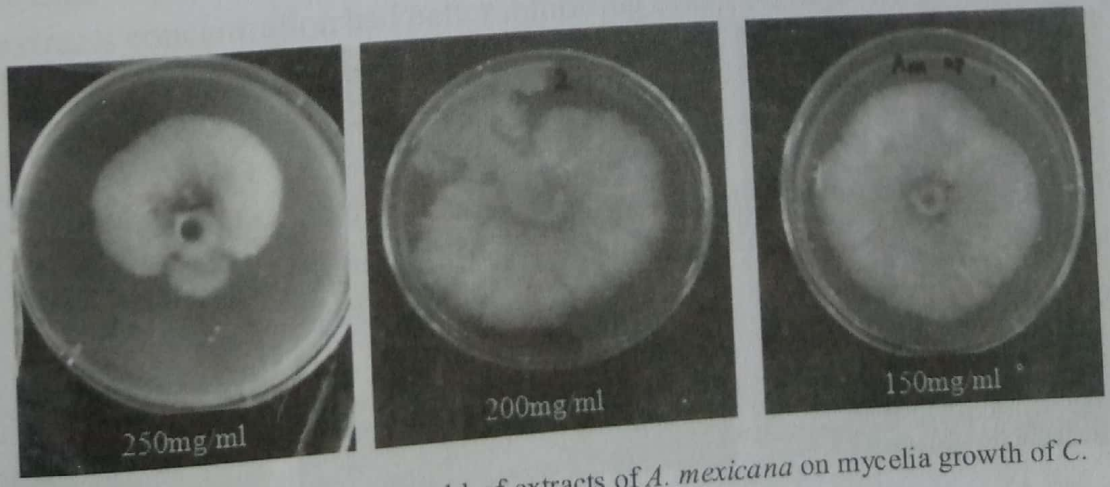

Fig 2: Inhibitory effect of methanol leaf extracts of *A. mexicana* on mycelia growth of *C. falcatum*

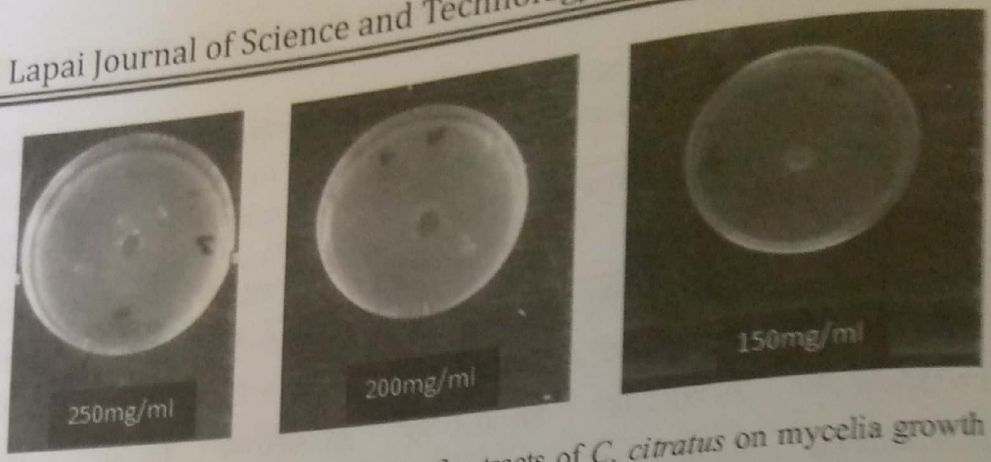

Fig 3: Inhibitory effect of methanol leaf extracts of *C. citratus* on mycelia growth of *C. falcatum*.

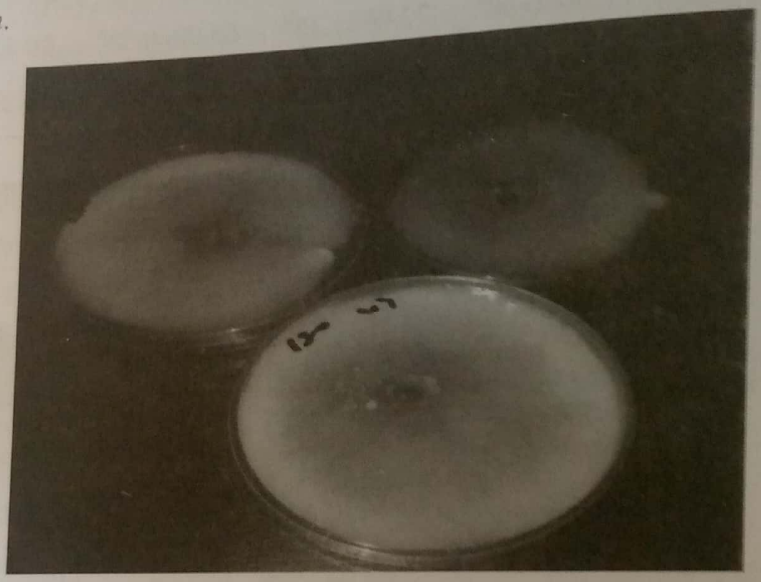

Fig 4: Inhibitory effect aqueous leaf extracts of *A. mexicana* on mycelia growtg of *C. falcatum.*

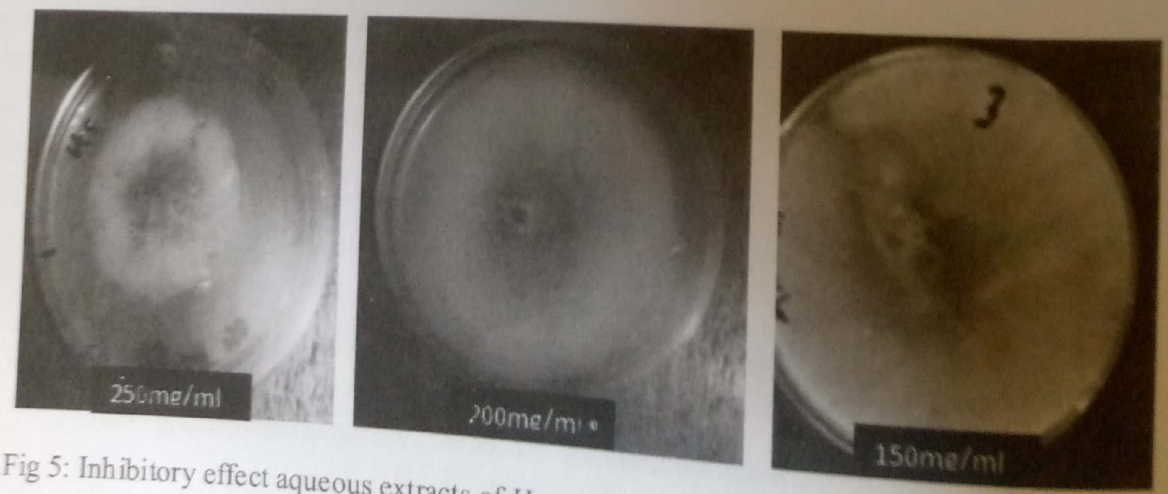

Fig 5: Inhibitory effect aqueous extracts of *H. s*

DISCUSSION

The results showed that high concentration of methanol extracts of the botanicals inhibited the growth of *C. falcatum* in sugar cane. **These results were similar to the findings of Prince and Prabakaran (2011) who observed that high concentration of plant extracts using ethanol solvent play maximum antifungal activity against the pathogen tested. Nikhil and Sahu, (2014) also reported that high concentration of plant extracts viz;** Ginger, Garlic, Turmeric, Onion and, Ocimum inhibits the growth of *C. falcatum* in sugar cane. Ahmed *et al.* (2007) also reported that the application of plant extracts such as *Curcuma domestica* and *Dutura metel* played an important role in the inhibition of *C. falcatum* in sugarcane. Also methanol leaf extracts of *C. citratus* and *C. olitorius* at high concentrations inhibited the mycelia growth of *C. falcatum*. *A. wilkesiana* also showed a good response in inhibiting the growth of the tested fungus. *C. citratus* was seen to perform best both in the aqueous and methanol extracts.

The aqueous leaf extracts of *C. citratus* showed inhibitory effect on mycelial growth of *C. falcatum* at all level of concentrations than other plant extracts. This result was similar to the findings of Ambika and Sujatha(2015), who reported that aqueous extracts of *Sargassum myricocystum* at high concentration inhibit the growth of mycelial of *C. falcatum*.

Among the extracts tested, methanol extract showed better antifungal activity in inhibiting mycelia growth of the fungus. However, both aqueous and methanol extracts of the botanicals exhibited antifungal activities on the test fungus. This was similar to the findings of Abera *et al.* (2011) who reported the antifungal potential of aqueous and ethanol extracts of eight different plants species (*Hogenia abyssinica, Allium sativum, Phytolacca dodcandera, Croton macrostachyus, Maesa lanceolata, Eucalyptus globules, Eucalyptus citriodera* and *Lippia adoensis) in vitro* and *in vivo* against *Colletotrichum kahawae*. This study also indicated that the inhibitory effect of the extracts depended on the type of plant species and solvents used.

CONCLUSION

In conclusion, high concentration of the botanicals used in this study inhibits the growth of *C. falcatum*. *C. citratus* showed maximum inhibition in both methanol and aqueous leaf extracts. Methanol leaf extracts concentration had better inhibiting effect on mycelia growth of *C. falcatum* than aqueous leaf extracts.

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