Evaluation of Leaf Extracts of Four Plant Species Against Rice Blast Pathogen (*Magnaporthe oryzae)* (T. T. Hebert) M. E. Barr.

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ABSTRACT

Rice (*Oryza sativa*) is one of the most popular food crops in Nigeria. Its successful production has been drastically affected by blast disease caused by *Magnaporthe oryzae*. *In vitro* control of the pathogen by four medicinal plants *(Carica papaya, Azadirachta indica, Calotropis procera* and *Anacardium occidentale)* was assessed in this study. The extracts of the plants were prepared using water and methanol, and agar well diffusion method was used to assess the toxicity of each extract. The pathogen was isolated from rice infected with blast disease. The results revealed the presence of one or more phytochemicals in each of the plant extracts. Among these were alkaloids, tannins, flavonoids, saponin, anthocyanin and phenol. All the extracts inhibited mycelia growth of *M. oryzae*. The potency of all the extracts increased with increasing concentration in the order; 50mg/ml <100mg/ml <150mg/ml. The inhibitions by methanol extracts were higher and significantly different ($P \geq 0.05$) from aqueous extracts. At the highest concentration tested (150mg/ml), *A. occidentale* and *C. procera* gave the highest inhibitions (99. 0mm and 98. 6mm respectively) which were not significantly different ($P \leq 0.05$) but different from *C. papaya* and *A. indica (*89. 1mm and 90. 4mm respectively). However, in all, *A. occidentale* aqueous and methanol extracts gave the highest percentage growth inhibition of the pathogen at all levels of concentrations tested while *C. papaya* aqueous and methanol extracts though effective were the least. Therefore, field trials of these four medicinal plants on the control of rice blast disease are recommended since they are easy to obtain and the extracts could easily be made via a simple process of maceration or infusion, they could be cheaper substitute for conventional drugs in controlling rice blast disease.

Key Words: Blast, Potency, Extract, Phytochemicals, Pathogen. *Magnaporthe oryzae*

INTRODUCTION

Rice (*Oryza sativa*) is the most important human food crop in the world, directly feeding more people than any other crop. In 2012, nearly half of world's population; more than 3 billion people relied on rice every day (International Rice Research Institute: IRRI, 2013). Global rice production was more than triple between 1981 and 2010, with a compound growth rate of 224% per year, this increase was slightly greater than that for wheat; 202% per year (IRRI, 2013).

However, the bountiful harvest of rice, despite its high production rate is hampered by some pathogenic fungi causing diseases such as rice blast, rice rotten neck, rice seedling blight, blast and ryegrass blast. Rice blast, because of its capacity to reduce yields between 11% and 30%, is currently the most important disease of rice worldwide and each year it is estimated to destroy enough rice to feed more than sixty (60) million people (Kurashasi 2014). The blast is caused by *Magnaporthe oryzae* which is known to occur in 85 countries Worldwide (Wilson and Talbot, 2009). The symptoms of rice blast include lesions that can be found on all parts of the plant including leaves, leaf collars, necks, pedicels, panicles, and seeds (TeBeest *et al.*, 2007).

Control of rice blast has depended on some cultural practices, multiple applications of fungicides and development of cultivars tolerant to this disease. Effective and long term control of blast can be achieved by applying recommended fungicides at their commenced time interval. However, repeated application of fungicides had led to reduced efficacy of the fungicides due to a gradual loss of sensitivity in the target pathogen population (Nascimento *et al.*, 2000; Xin *et al.*, 2012). It has also contributed to greater production costs and environmental pollution. Therefore, the search for alternative to chemical products such as the use of natural biocides of plant origin is the most promising outlet for a safe and sustainable agriculture (Akujobi *et al.*, 2004). One hundred and nineteen (119) secondary metabolites derived from plants are used globally as drugs. Fifteen percent (15%) of all angiosperms have been investigated chemically out of that 74% of pharmacological active plant derived components such as phytochemicals, vitamins and minerals were discovered (Okwu and Ekwe, 2003; Govindarajan *et al.*, 2008). Therefore, this study investigated the *in vitro* activities of *Anacardium occidentale* L. (cashew), *Carica papaya* L. (Pawpaw), *Calotropis procera* (Aiton) W. T. Aiton (Sodom apple) and *Azadirachta indica* A. Juss (neem) extracts for the control of *M. oryzae*.

MATERIALS AND METHODS

Collection of Materials

The fresh leaves of *C. papaya* (Pawpaw), *A. indica* (Neem) and *C. procera* (Sodom apple); and stem bark of *A. occidentale* (Cashew) were collected in clean polythene bag between February and March, 2016 at Bosso campus of the Federal University of Technology, Minna, Nigeria. The plants were authenticated at the herbarium of Biological Sciences Department at Federal University of Technology, Minna, Nigeria. .

Preparation and Extraction of Plant Materials

The fresh plant parts were washed and air dried for fifteen days. All dried materials were crushed using mortar and pestle and later pounded into powdered form and sieved with a muslin cloth of mesh size 0. 05mm. The micromised samples were then used for extraction purpose. Fifty (50) grams of the dried powder was packed with thimble and then subjected to extraction with 98. 9% ethanol in soxhlet apparatus. The collected extracts were concentrated by evaporation at room temperature.

Phytochemical Screening of Leaves and Stem Bark

A preliminary phytochemical screening of the samples for the presence of phytochemical compounds was performed using the methods described by Hassan (2006).

Isolation of Pathogens

The rice blast pathogen (*Magnaporthe oryzae*) was isolated from the infected leaves of rice plant by tissue segment. The tiny pores (2-3mm) was vigorously washed with tap water and then rinse and surface sterilized with 15% hypochlorite for 30 seconds. The leaves were washed with sterile distilled water, dried on aluminum foil, inoculated in Potato Dextrose Agar (PDA) and incubated at 27 ± 2 °C for 72 hrs. The pathogen was further purified by subsequent sub-culturing to obtain pure cultures (Prince and Prabakaran, 2011). Identification of the pathogen was based on the observed morphological features taking note of the growth rate and pattern on agar plates, colony size, colony colour and shapes of spores. Stock culture of the isolate was maintained in McCartney bottle slants and stored at 4°C in refrigerator for subsequent use (Adebola and Amadi, 2010).

Pathogenecity Test

Five (5)mm of *M. oryzae* culture was taken using 5mm cork borer from Potato Dextrose Agar into a beaker containing 15ml (200g/L) sterile Potato Dextrose Broth (PDB), This was shaken for five days using shaker (Table top Model) at

28±2°C and 95 rpm for 30 minutes. The suspension of each was separately added to a polythene bag filled with 300g of sterilized rice, 70ml distilled water and sealed. The bags were opened after 48hrs and the contents were spread on trays under aseptic condition and incubated further for 3 days at 28±2°C for initiation of spore production. The suspension of *M. oryzae* was prepared by blending rice substrate with distilled water, 20% pectin and ammonium diphosphate (2:1:1) at pH 5. 5 for 10 seconds in a blender and filter through cheese cloth. The final concentration of *M. oryzae* suspensions was adjusted to 10^8 propagules/ml. The suspensions were separately stored in a container and put in a bigger container placed with ice block at 4°C for further use (Adebola and Amadi, 2012). The resulting filtrate was used to spray five (5) healthy randomly arranged potted rice plants while the control was sprayed with distilled water.

Screening of botanicals for antifungal potency on *M. oryzae*

Twenty (20) mm of sterile PDA in Petri dishes was inoculated with the pathogen. After 72hrs, well of 5mm diameter was cut on the plate using 5mm diamater cork borer. The cut agar disc was carefully removed with sterile forceps, after which the well was filled with different plant extracts. The control was set up by substituting sterile distilled water for plant extracts. Three replicates of each treatment were made. The plates were allowed to stand for one hour at 4°C in the refrigerator to allow for diffusion of extracts into Potato Dextrose Agar. The plates were then incubated at 28±2°C and the zone of inhibition was measured after 24hrs (Okigbo and Ogbonnaya, 2006). The inhibition percentage was determined thus: $(R_1-R_2)/R_1X100$. Where R_1= Radial growth of pathogen in control and R_2 = the radial growth of pathogen in the test plate (Adebola and Amadi, 2010).

Statistical Analysis

The experiment was carried out in a Randomized Complete Block Design (RCBD) with three replicates of each treatment. All data were subjected to Analysis of Variance (ANOVA) and Duncan's New Multiple Range test was used to separate the treatment means at $p\leq0.05$ or 5%. level of significance.

RESULTS

Pathogenecity test

The result of pathogenecity test with lesions that are found on all parts of the plant including leaves, leaf collars, necks, pedicels, panicles, and seeds established *M. oryzae* as the causative organism of the blast on rice plant.

Phytochemical Properties

Phytochemical analysis (Table 1) revealed the presence of alkaloids, saponins, tannins, anthocyanin, phenol and flavonoids in the four plant extracts. Alkaloids, saponins, tannins and flavonoids were found in all the plant extracts while *C. papaya* did not contain anthocyanin and phenol; and only phenol was not found in *A. occidentale*.

Antifungal Activities of the Extracts on Mycelia Radial Growth of *Magnaporthe oryzae*

The results of *in vitro* screening of different concentrations of the plant extracts on mycelia radial growth of *M. oryzae* (Table 2) revealed that the potency of the extracts from different plants increased with increased concentration. In *A. indica* extract, the reduction of mycelia growth was in the order of 20. 5mm>14. 0mm>8. 6mm respectively and were significantly different (P<0. 05). This same trend was observed with the extract from *A. occidentalis*. In *C. papaya* and *C. procera* extracts, the reduction in mycelia growth followed the same trends, but there was no significant difference (P<0. 05) between 50mg/ml and 100mg/ml concentrations.

The inhibitory effecs of methanol extracts of on mycelia growth of *M. oryzae* (Table3) ranged from 0. 0mm to 15. 9mm. The trends in reduction of mycelia growth was also in the order 50mg/ml<100mg/ml<150mg/ml of the concentration irrespective of the plant extracts tested. However, in *A. Occidentale* extract, at 150mg/ml concentration did not inhibit fungal mycelia growth.

Effects of Plant Extracts on Percentage Growth Inhibition of *M. oryzae*

The results on the screening of extractants on percentage growth inhibition of *M. oryzae* revealed that both methanol and aqueous extracts of the plants tested at any of the three concentrations (50mg/ml, 100mg/ml and 150mg/ml), gave significant growth inhibitions (Figures 1, 3 , 5 and 7). The percentage growth inhibition of methanol extracts were significantly different (P<0. 05) from aqueous extracts. However, in *A occidentale* extract there was no significant difference (P<0. 05) at 150mg/ml (Fig. 4. 7) while at this same aqueous extract concentration, *C. procera* extract gave better and significantly different (P<0. 05) percentage growth inhibition than methanol extract.

The effects of extract concentration on percentage growth inhibition of *M. oryzae* followed the same trend (50mg/ml<100mg/ml<150mg/ml) as observed with mycelia radial growth reduction (Fig. 2, 4, 6 and 8). At 150mg/ml of *C. papaya* extracts, the

highest and significant percentage growth inhibition of *M. oryzae* was observed in both aqueous and methanol extracts. In *A. indica* extract, there was no significant difference (P<0. 05)in the three concentrations used (Fig. 2). In methanol extracts of *C. procera* and *A occidentale*, there was no significant difference in percentage growth inhibition among the three extract concentrations (P<0. 05).

The comparison of the effects of various aqueous plant extracts on percentage growth inhibition of *M. oryzae* (Fig. 9) revealed that at 50mg/ml, *A. occidentale* gave the highest percentage growth inhibition of the pathogen (83. 1mm) which was significantly different (P<0. 05). However, there was no significant difference between *A. indica, C. procera* and *C. papaya* (78. 3mm, 78. 7mm and 77. 7mm respectively). At 100mg/ml, the percentage growth inhibition was not significantly different (P<0. 05) between *A. Indica* and *A. occidentale* (84. 4mm and 86. 7mm respectively). , and also between *C. procera* and *C. papaya* (80. 4mm and 79. 5mm respectively). At the highest concentration tested (150mg/ml), *A. occidentale* and *C. procera* gave the highest inhibitions (99. 0mm and 98. 6mm respectively) which were not significantly different (P<0. 05) but different from *C. papaya* and *A. indica* (89. 1mm and 90. 4mm respectively).

Similarly, *A. occidentale* gave the highest percentage growth inhibition of the pathogen in the three concentrations of methanol plant extracts (Fig. 10) that were significantly different (P<0. 05)from other plant extracts. However, in all, *C. papaya* aqueous and methanol extracts gave the least percentage growth inhibition of the pathogen at all concentrations tested while *A. Occidentale* aqueous and methanol extracts were the highest.

DISCUSSION

The results of this study showed the presence of one or more phytochemicals such as alkaloids, saponin, tannin, anthocyanins, phenol and flavonoids in the four medicinal plants screened. The result was in agreement with earlier work of Falodun *et al.* (2011), who reported some of these metabolites in different plants extracts.

The medicinal plant extracts screened in this study were found to show varying degrees of antifungal activities against *M. oryzae*. The methanol and aqueous extracts showed a wide range of percentage growth inhibition of the pathogen. This confirmed the earlier reports by Tibiri *et al.* (2010) that the leaf extracts of *Entada africana*showed a higher percentage of growth inhibition and that the antimicrobial constituents

of the plants are preferentially concentrated in the leaves.

The differences observed in antifungal activities of the extracts may be attributed to the differences in solubility of the metabolites in water or alcohol or to the presence of inhibitors in the fungitoxic principles as earlier reported by Okigbo and Ogbonnaya (2006). The antimicrobial, antifungal and antioxidant activities of flavonoids, tannins, saponin, steroids and phenolsas antimicrobial agents could inhibit the growth of microorganisms by precipitating the microbial protein and thus depriving them of nutritional proteins++ needed for their growth and development (Ishida *et al.*, 2009; Raymond *et al.*, 2010; Owoycle *et al.*, 2008 and Obasi *et al.*, 2010).

The antimicrobial activities were found to increase with increase in extract concentrations. The results of *in vitro* effects of aqueous extract concentrations on mycelia radial growth of *M. oryzae* revealed that the potency of the extracts from different plants increased with increased concentration. This was in agreement with Mahmoud *et al.* (2011) who reported an increase potency of aqueous ethanol and ethyl acetate extracts from *Azadirachta indica* leaves on growth of some human pathogens (*A. flavus, A. fumigatus, A. niger, A. terreus, Candida albicans* and *Microsporum gypseum*) with increase in extract concentration. Also, Abiola *et al* (2014) reported same with *A. indica* seed oil on four fungi namely; the species of *Fusarium, Rhizopus, Curvalaria,* and *Aspergillus.*

Anacardium occidentale aqueous and methanol extracts gave the highest percentage growth inhibition of *M. Oryzae* in this study. This was in line with Silva *et al.* (2014) who assessed the effect of *A. occidentale* tree bark ethanol extract on *Staphylococcus aureus* (resistant and sensitive to methicillin) and found it to be effective against all the test organisms. More over, Lidyawita and Sudarsono (2013) reported antifungal activities of boiled *A. occidentale* bark on *Candida albicans* in acrylic resins and attributed to tannins, gallic acid, anacardic acid and phenolic compounds it contains.

Carica papaya aqueous and methanol extracts gave the least percentage growth inhibition on *M. oryzae*in this study. This was in agreement with Chavez- Quintal (2011) who investigated the antifungal activity in ethanolic extracts of *C. papaya* L. cv maradol leaves and seeds; and reported a moderate inhibition of *Fusarium* spp. and a weak inhibition on *Colletotrichum gloeosporiodes.*

Calotropis procera aqueous and methanol extracts gave better percentage growth inhibition on *M. Oryzae* in this study. This result confirmed the report of Nenaah and Ahmed (2011) that the

aqueous and organic solvent extracts and the latex of *Calotropis procera* showed considerable antibacterial and antifungal activities against the microorganisms.

Conclusion

This research has shown a range of antifungal activities of the extracts from the four medicinal plants screened against *M. oryzae* and provided support to some traditional uses of these medicinal plants. Therefore, since these plants are easy to obtain and the extracts could easily be made via a simple process of maceration or infusion, they could therefore be cheaper substitute for conventional drugs in controlling various plant diseases.

Table 1: Phytochemical constituents of the medicinal plants screened

Plants pecies	Alkaloids	Saponins	Tannin	Anthocyanins	Phenol	Flavonoids
C. papaya	+	+	+	-	-	+
A. indica	+	+	+	-	+	+
C. procera	+	+	+	-	+	
A. occidentale	+	+	+	+	-	+

Key: + = present - = absent

Table 2: *In vitro* effects of aqueous leaf extracts concentration of four botanicals on mycelia growth (mm) of *M. oryzae*

Plant extracts	*Mean radial growth (mm) 50mg/ml	100mg/m	1150mg/ml
S. D. W (R1)	90.0±0.01		
A. indica	20.50±0.32c	14.00±0.02b	8.60±0.02a
C. papaya	20.00±0.22b	18.40±0.30b	9.80±0.01a
C. procera	19.10±0.01b	17.60±0.11b	1.20±0.12a
A. occidentale	15.20±0.12c	12.00±0.01b	1.30±0.02a

*Mean of three replicates. Values of means followed by the same superscript along the same row are not differ at P<0.05
SDW= Sterile Distilled Water

Table 3: *In vitro* effects of methanol leaf extracts concentration of four botanicals on mycelia growth(mm) of *M. oryzae*

Plant extracts	Mean radial growth (mm) 50mg/ml	100mg/ml	150mg/ml
S. D. W (R1)	90.0±0.01		
A. indica	13.00±0.22c	10.00±0.21b	4.7±0.12a
C. papaya	15.9±0.12b	13.4±0.01b	9.1±0.03a
C. procera	12.80±0.13c	9.1±0.03b	5.1±0.10a
A. occidentale	2.7 ±0.11b	1.5±0.03b	0.0±0.02a

*Mean of three replicates. Values of means followed by the same superscript along the same row are not differ at P<0.05 SDW= Sterile Distilled Water

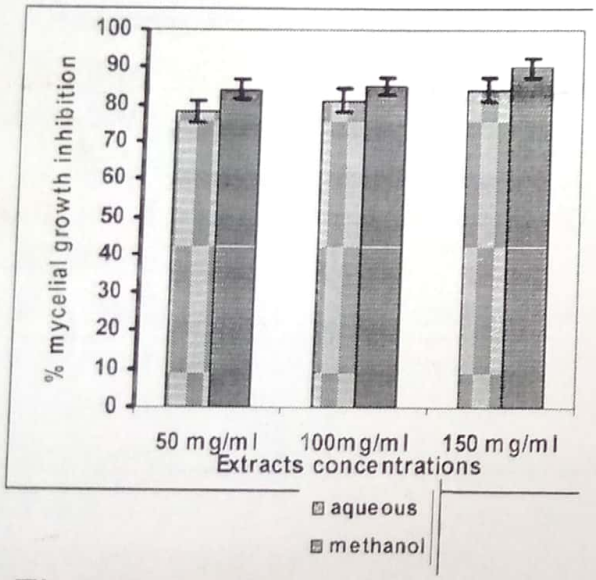

Fig.1 Effects of different concentrations of *A. Indica* On percentage growth inhibition of *M. oryzae*

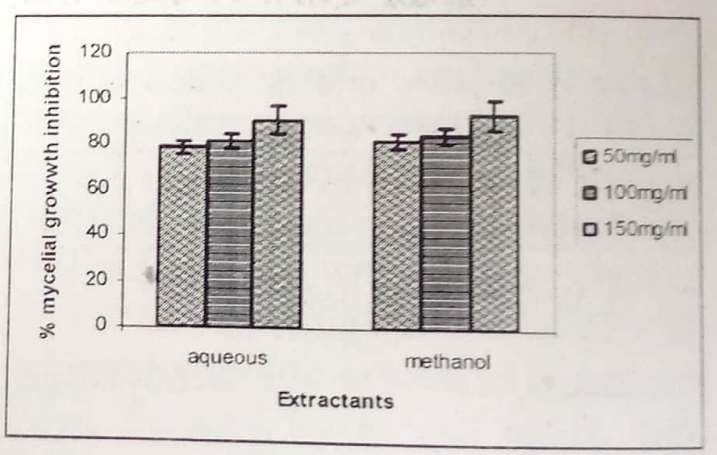

Fig. 2 Effects of extractants of *A. indica* on percentage growth inhibition of *M. oryzae*

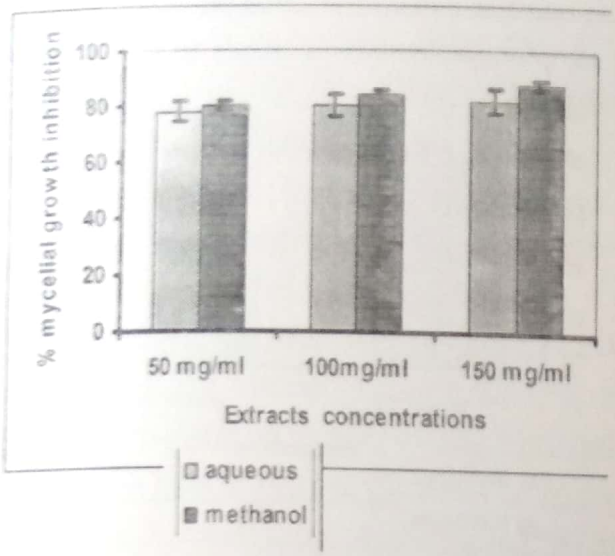

Fig. 3: Effects of different concentrations of *Carica papaya* percentage growth inhibition of *M. oryzae*

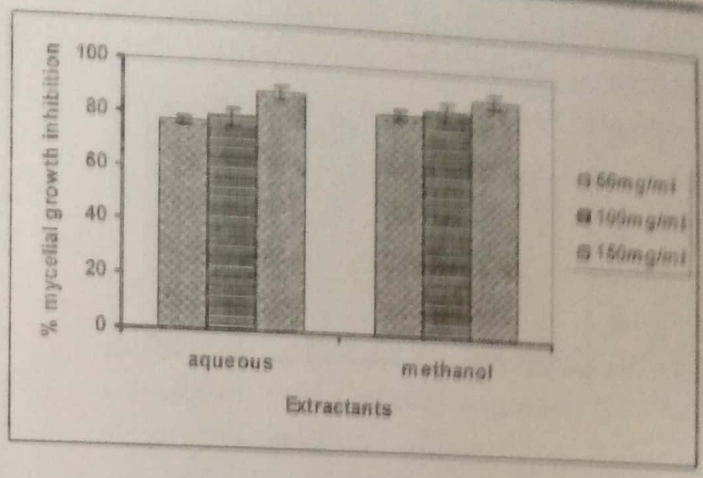

Fig.4: Effects of extractants of *Carica papaya* on percentage growth inhibition of *M. oryzae*

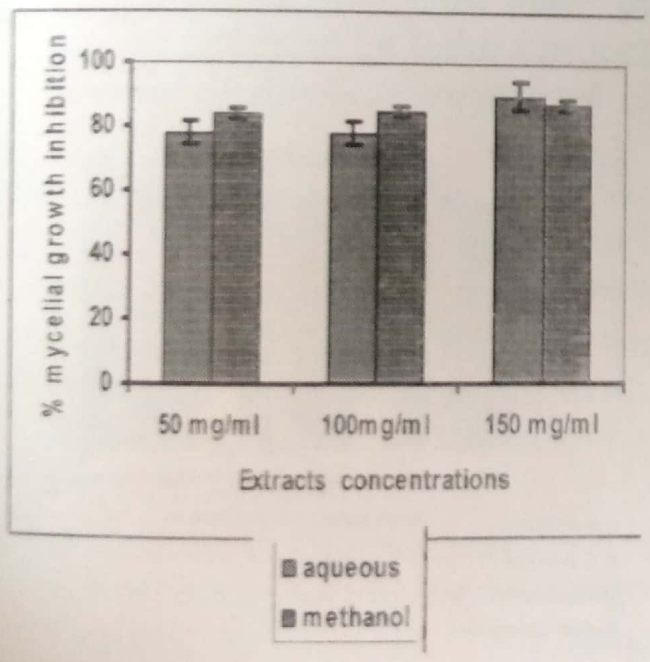

Fig. 5: Effects of different concentrations of *Clatropis procera* on percentage growth inhibition of *M. oryzae*

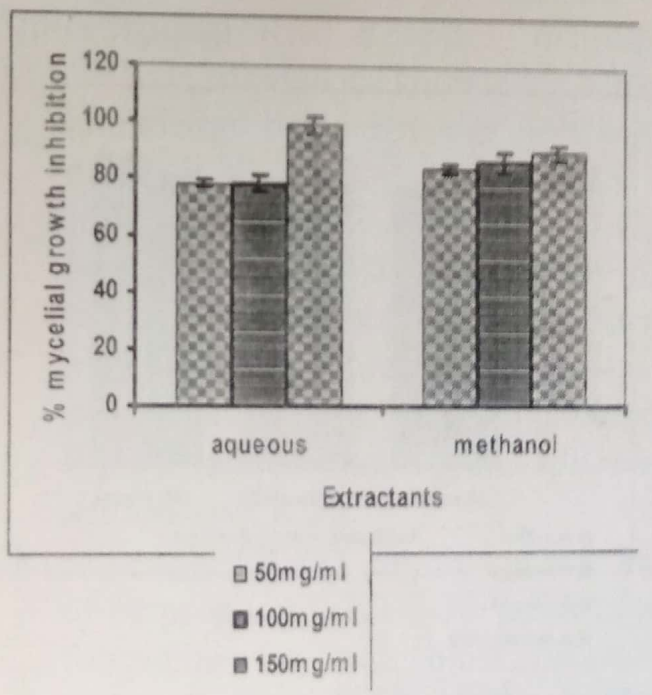

Fig.6: Effects of extractants of *Clatropis procera* on percentage growth inhibition of *M. oryzae*

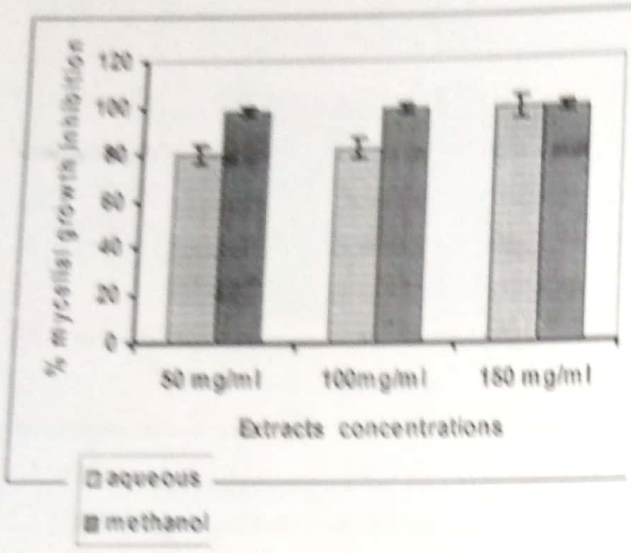

Fig. 7: Effects of different concentrations of *Anacadium occidentale* on percentage growth inhibition of *M. oryzae*

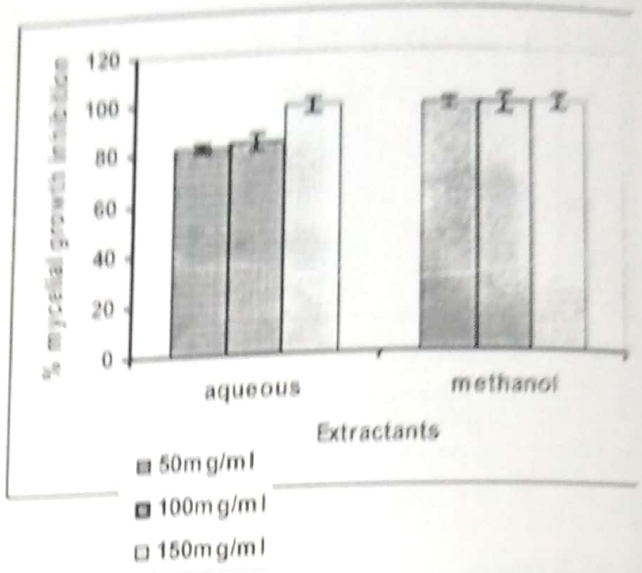

Fig.8: Effects of extractants of *Anacadium occidentale* on percentage growth inhibition of *M. oryzae*

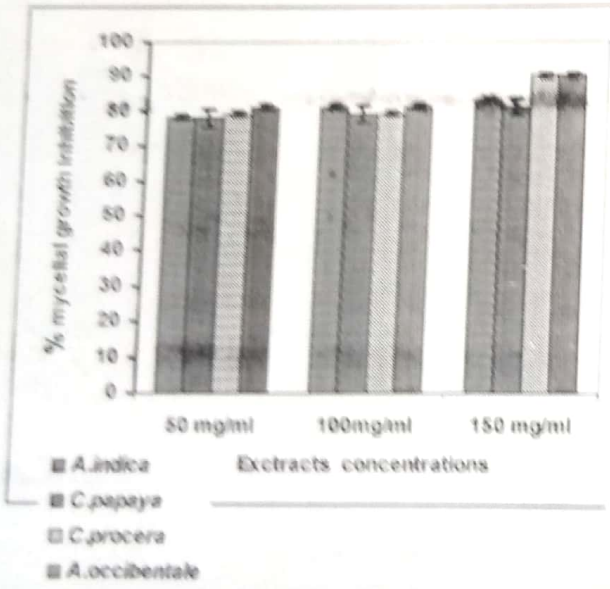

Fig. 9: Effects of different aqueous plant extracts on percentage growth inhibition of *M. oryzae*

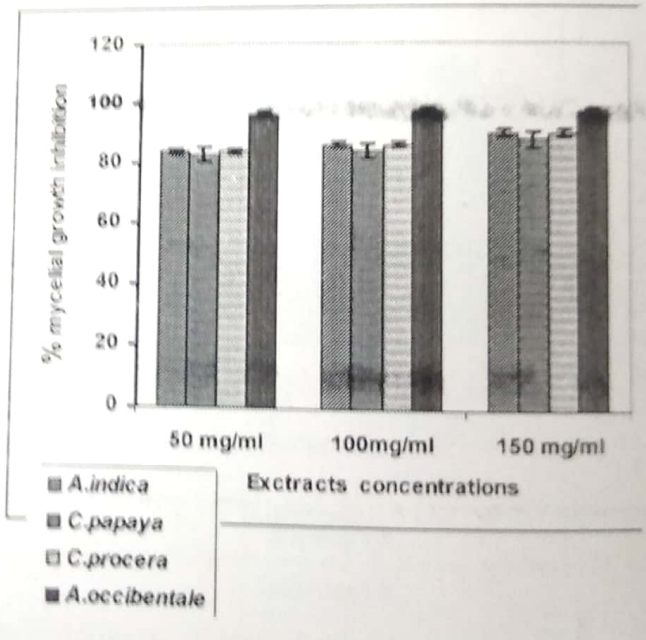

Fig.10: Effects of different methanolic plant extracts on percentage growth inhibition of *M. oryzae*

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