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Comparative Studies On The Effect Of Aqeous And Methanolic Extracts Of Some Botanicals On Growth And Sporulation Of Colletotrichum Graminicola

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Abstract: ***C. graminicola is responsible for Sorghum anthracnose. It is the most threatening grain, leaf and stalk disease of sorghum. The effect of different concentrations of aqueous and methanolic plant extracts of 9 medicinal plants and benomyl were evaluated against the fungus in vitro. The extracts exhibited antifungal activity in a dose dependent manner. In both extract types, 10% Allium sativum was found to be most effective in inhibiting mycelial growth, next to benomyl, one of the recommended fungicides for the control of the fungus. Allium sativum at all concentrations completely inhibited sporulation. With the exception of Azadirachta indica, Eucalyptus camuldulensis and Vernonia amygdalina, aqueous extracts showed less control of C. graminicola than with methanolic extracts.***

Keywords: Colletotrichum graminicola, aqueous and methanolic plant extracts

I. INTRODUCTION

Sorghum is the dietary staple of more than 500 million people in more than 30 countries. In Africa, it is processed into a very wide variety of attractive and nutritious traditional foods such as semi-leavened bread, couscous, dumplings and fermented and non-fermented porridges. Sorghum is also the grain of 21st century Africa (Taylor, 200).

Colletotrichum species are among the important plant fungal pathogens in tropical and sub-tropical countries responsible for causing disease syndrome called anthracnose (Shivaprakash *et al.*, 2011). It attacks a wide range of agro-economically important crops such as sorghum resulting in annual losses of huge amounts of money in revenue (Korsten and Jefries, 2000).Inclusion of *Colletotrichum* species as 8th rank among 10 fungal pathogens is sufficient to prove its devastating effect on crops, herbaceous and woody plants (Dean *et al.*,2012).

C. graminicola is the causative agent for anthracnose disease of sorghum. All parts of the sorghum plant are susceptible to the disease. The pathogen can cause leaf blight, stalk rot and root rot of seedlings (Gwary *et al.*, 2004). Losses caused by *C. graminicola* can range from 30-70% depending on which organ is infected (Bonzi *et al.*, 2012).

Management strategies for sorghum anthracnose include chemical means (Asala, 2001), host plant resistance (Gwari *et al.*, 2001), cultural practices (Marley, 2004) and integrated management using host plant resistance, date of planting and cultural practices together with seed treatment (Marley, 2004). Unfortunately however, quality seeds of improved varieties do not reach small scale farmers who used their saved seeds without chemical treatment. Apart from being unaffordable to

small scale farmers, the continuous, inappropriate and non discriminative use of chemicals is known to cause undesirable effects such as residual toxicity, development of resistance, environmental pollution, health hazards to human and animals and increased expenditure for plant protection (Harish *et al.*, 2008). To seriously address these problems, attention is now focused on developing environmentally safe, long lasting and effective biocontrol methods for the management of plant diseases. Several researchers have reported the use of plant extracts to be a reliable alternative to the use of chemicals in the effective management of diseases (Donli and Dauda, 2003; Potphode, 2004; Tasiwal, 2008; Jayalakshmi, 2010; Zarafi and Moumoudou 2010).

In this study, the *in vitro* antifungal activities of aqueous and methanolic extracts of some plants were investigated against growth and sporulation of *C. graminicola*.

II. MATERIALS AND METHODS

ASSESSMENT OF THE POTENTIAL OF SOME PLANT MATERIALS FOR THE CONTROL OF C. GRAMINICOLA.

The following plant extracts were used (Table 1).

Botanical name	Common name	Family	Plant part used
Allium cepa L.	Onion	Amaryllidaceae	Bulb
Allium sativum L.	Garlic	Amaryllidaceae	Cloves
Azadirachta indica	Neem	Meliaceae	Leaves and seeds
Vernonia amygdalina	Bitter leaf	Asteraceae	Leaves
Moringa oleifera	Moringa	Moringaceae	Leaves and seeds
Eucalyptus camaldulensis	Eucalyptus	Myrtaceae	Leaves
Cymbopogon citratus	Lemon grass	Poaceae	Leaves

Table 1: Plants, Family and the plant parts used Preparation of aqueous leaf/bulb extracts (Akinbode and Ikotun, 2008)

Fresh leaves and bulbs of each plant were collected and surface sterilized in 70% ethyl alcohol for 1 min and then washed three times in sterile distilled at an interval of 5 minutes after which 100g were crushed into pulp with a sterile pestle and mortar and then suspended in 100 ml of sterile distilled water to give 100% solution. The mixture was agitated for 2 minutes, stored overnight at room temperature after which each was filtered through sterile cotton cloth into sterile conical flasks to give the aqueous extracts.

PREPARATION OF SEED POWDERS (OBI AND BARRIUSO-VARGAS, 2013)

The seeds of the some of the test plants (as indicated in Table 1) were collected, air dried for 5 days, decorticated and thereafter crushed to powder using sterilized mortars and pestles. 100g of the seeds were crushed into pulp with a sterile pestle and mortar and then suspended in 100 ml of sterile distilled water to give solution 100%. The mixture was agitated for 2 minutes, stored overnight at room temperature after which each was filtered through sterile cotton cloth into sterile conical flasks to give the aqueous seed powder.

To obtain a concentration of 1, 2.5, 5 and 10%, 1, 2.5, 5 and 10 ml of the extract was pipetted into 100 ml of cooled potato dextrose agar and then dispensed into petri dishes into which a drop of lactic acid had been added.

PREPARATION OF METHANOLIC EXTRACTS

The plant materials (150g) each were extracted at 70°c with methanol using soxhlet apparatus. Each sample was placed in a clean, dry thimble and put inside the extraction flask of the apparatus. 500ml of methanol was added and allowed to operate for three hours. The obtained extracts from each sample were evaporated on a water bath at 45^0c to remove excess alcohol.

ASSESSING THE EFFECT OF AQUEOUS AND METHANOLIC PLANT EXTRACTS ON COLONY DIAMETER OF *C.GRAMINICOLA.*

Inoculum discs of 5mm in diameter were obtained from the edge of a 5 day old culture of *C. graminicola* on PDA and placed face down at the center of each of the different plant extracts amended agar plates. Un-ammended plates served as control. Each treatment was replicated 5 times. For comparison, benomyl was used following the same procedure. Colony diameter was measured after 10 days.

Percent Growth Inhibition (PGI) was calculated using the formula given by Vincent (1947):

$$PGI = \frac{(C-T)}{C} \times 100$$

Where;

PGI = Percentage Growth Inhibition

C = Average diameter of mycelial colony of control (mm)

T = Average diameter of mycelial colony of treatment (mm)

III. RESULTS

IN VITRO EVALUATION OF AQUEOUS PLANT EXTRACTS ON GROWTH AND SPORULATION OF C.GRAMINICOLA.

EFFECT OF DIFFERENT CONCENTRATIONS OF AQUEOUS PLANT EXTRACTS ON MYCELIAL INHIBITION

The results in Figure 1 indicate that apart from benomyl (which is a recommended fungicide), *Allium sativum* had the greatest inhibitory effect on the growth of the fungus while *Moringa oleifera* – both leaf and seed extracts had the least effect among the plant materials. The differences between the plant extracts were significant. The inhibition was dosage dependent – the higher the dosage, the greater the growth inhibition. The differences between the effects of the concentrations within each plant extract were also significant.

EFFECT OF DIFFERENT CONCENTRATIONS OF AQUEOUS PLANT EXTRACTS ON CONIDIAL COUNT

Data presented in Table 2 indicate that *Allium sativum* was the most effective plant extract, inhibiting conidia production at all concentrations. It was as effective as benomyl which is one of the recommended fungicides for the control of sorghum anthracnose caused by *C. graminicola*. The differences between the effects of the various aqueous plant extracts on the number of conidia were significant. The effects of all extracts are dosage dependent, that is, the higher the concentration, the lower the number of conidia produced except for *Allium sativum* where the lowest concentration brought about 100% inhibition of conidial production.

EFFECT OF DIFFERENT CONCENTRATIONS OF METHANOLIC PLANT EXTRACTS ON MYCELIAL INHIBITION

The results of this study are presented in Figure 2 and show that *Allium sativum* extract was the most effective of the methanolic plant extracts for the control of anthracnose of sorghum followed by *Azadirachta indica* seed extract. The least effective was *Eucalyptus camuldulensis* extract. All the extracts reduced the growth of the pathogen. The effect of all the extracts were dosage dependent, that is, the higher the dosage, the greater the inhibition of mycelial growth of the pathogen.

EFFECT OF DIFFERENT CONCENTRATIONS OF METHANOLIC PLANT EXTRACTS ON CONIDIAL COUNT

The results in Table 3 indicate that *Allium sativum* was the most effective plant extract, inhibiting conidia production at all concentrations. All materials tested in this experiment exhibited significant reduction in sporulation of the pathogen. There was no significant difference in sporulation between 1% *Moringa oleifera* leaf powder and 5% *Eucalyptus camuldulensis* and between 2.5% *Moringa oleifera* leaf powder and 5% *Vernonia amygdalina*. Furthermore, whilst *Allium sativum* at 1% with mycelial inhibition of 56.36% and 65.68% at 2.5% completely inhibited sporulation, some sporulation was observed in 5% and 10% *Azadirachta indica* seed extract with mycelial inhibition of 57.50% and 68.50% respectively.

COMPARISON BETWEEN THE EFFECT OF AQUEOUS AND METHANOLIC PLANT EXTRACTS ON GROWTH OF PATHOGEN

The results are presented in Figures 3, 4, 5 and 6. Figure 3 represents the effect of aqueous and methanolic plant extracts on growth of *C. graminicola* on amended PDA plates. The figure represents the percentage inhibition of mycelial growth. The results show that the methanolic extracts of the plant materials was more effective than the aqueous extracts except for *Azadirachta indica* leaf powder, *Vernonia amygdalina*, *Eucalyptus camuldulensis* and *Cymbopogum citratus* aqueous extracts which were more effective in reducing mycelial growth than their methanolic extracts. The differences between the effects of the two types of extracts on mycelial growth were not significant.

Figure 4 represents the comparative effect of the two types of plant extracts on sporulation (conidial count). The results show that all extracts significantly reduced sporulation of *C. graminicola* compared to the control without any extracts. Of the two extracts, the methanolic extract was more effective in reducing sporulation than the aqueous extract of the same plant materials except *Moringa oleifera* seed extract. The differences were however not significant.

Figure 5 shows the comparative effects of the level of concentration of aqueous and methanolic extracts on mycelial growth. The figures used in the graphic representations are averages across the different levels of concentrations. This figure shows that the aqueous extracts of *Allium sativum*, *Azadirachta indica* leaf powder, *Vernonia amygdalina*, *Eucalyptus camuldulensis* and *Cymbopogum citratus* were more effective in reducing growth of the pathogen than the methanolic extract. The differences were however not significant.

Figure 6 shows the comparative effects of the level of concentration of aqueous and methanolic extracts on sporulation. The figures used in the graphic representations are averages across the different levels of concentrations. This figure indicates that both the aqueous and methanolic extracts of *Allium sativum* totally inhibited sporulation and both were as effective as benomyl which is a recommended fungicide for the control of the pathogen. For the other plant materials, the methanolic extracts were more effective in reducing sporulation than aqueous extracts except for *Moringa oleifera* seed extract. The differences were however not significant.

Extracts	Extract conc(%)	Conidial count (x10^5 conidia/ml)
Allium sativum	1	0.00^{s}
	2.5	0.00^{s}
	5	0.00^{s}
	10	0.00^{s}
Azadirachta indica leaf powder	1	13.24^{i}
	2.5	11.45^{l}
	5	9.69^{o}
	10	7.89^{q}
Azadirachta indica seed extract	1	11.55^{l}
	2.5	10.28^{n}
	5	8.86^{p}
	10	7.28^{r}
Moringa oleifera leaf powder	1	14.40^{g}
	2.5	12.50^{k}
	5	11.34^{l}
	10	9.49^{o}
Moringa oleifera seed extract	1	15.98^{d}
	2.5	14.29^{g}
	5	12.92^{j}
	10	11.29^{l}
Vernonia amygdalina	1	16.09^{d}
	2.5	14.45^{g}
	5	12.58^{k}
	10	11.29^{l}
Allium cepa	1	13.57^{h}

	2.5	12.87^{j}
	5	11.02^{m}
	10	10.18^{n}
Eucalyptus camuldulensis	1	16.56^{c}
	2.5	15.08^{f}
	5	13.76^{h}
	10	12.76^{jk}
Cymbopogum citratus	1	17.51^{b}
	2.5	16.56^{c}
	5	15.45^{e}
	10	14.34^{g}
Benomyl	0	0.00^{s}
Control	0	21.09^{a}
S. E. ±		0.255

Means in each column with same superscript are not significantly different at $P \geq 0.05$ using Duncan's Multiple Range Test.

Table 2: Effect of different concentrations of aqueous plant extracts on conidial count

Extracts	Extract conc(%)	Conidial count (x10^5 conidia/ml)
Allium sativum	1	0.00^{v}
	2.5	0.00^{v}
	5	0.00^{v}
	10	0.00^{v}
Azadirachta indica leaf powder	1	11.93^{l}
	2.5	10.01^{p}
	5	8.46^{r}
	10	6.85^{t}
Azadirachta indica seed extract	1	10.86^{n}
	2.5	9.58^{q}
	5	8.13^{s}
	10	6.53^{u}
Moringa oleifera leaf powder	1	13.54^{gh}
	2.5	11.99^{kl}
	5	10.44^{o}
	10	9.31^{q}
Moringa oleifera seed extract	1	16.22^{c}
	2.5	14.50^{e}
	5	13.11^{i}
	10	11.77^{l}
Vernonia amygdalina	1	15.39^{d}
	2.5	13.79^{g}
	5	11.95^{kl}
	10	10.5^{o}
Allium cepa	1	13.43^{h}
	2.5	12.26^{jk}
	5	11.19^{m}
	10	10.22^{op}
Eucalyptus camuldulensis	1	16.48^{c}
	2.5	14.66^{e}
	5	13.65^{gh}
	10	12.52^{j}
Cymbopogum citratus	1	17.39^{b}
	2.5	16.43^{c}
	5	15.52^{d}
	10	14.18^{f}
Benomyl	0	0.00^{v}
Control	0	21.09^{a}
S. E. ±		0.297

Means in each column with same superscript are not significantly different at $P \geq 0.05$ using Duncan's Multiple Range Test.

Table 3: Effect of different concentrations of methanolic plant extracts on conidial count

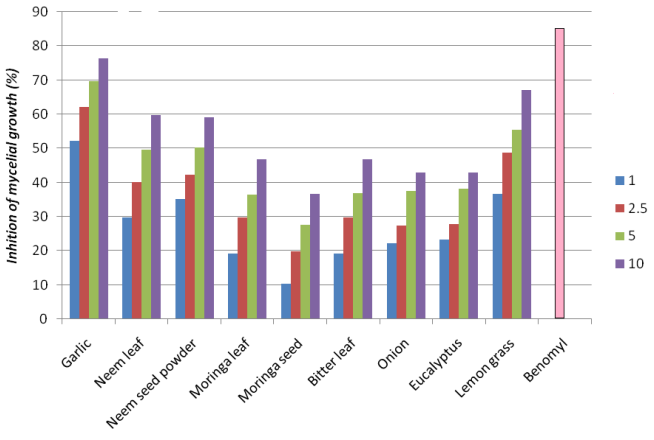

Figure 1: ***in vitro*** **evaluation of different concentrations of aqueous plant materials for control of** ***C. graminicola***

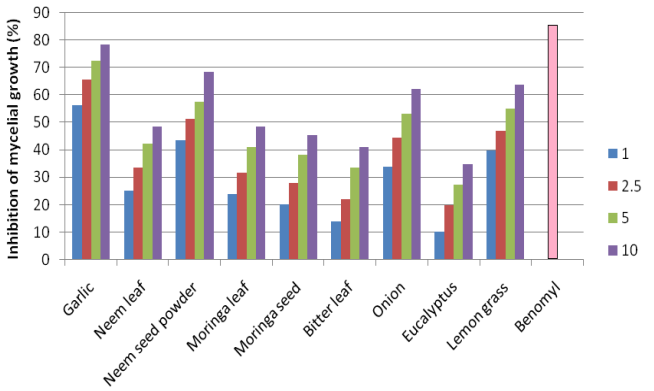

Figure 2: ***in vitro*** **evaluation of different concentrations of methanolic plant extracts for control of** ***C. graminicola***

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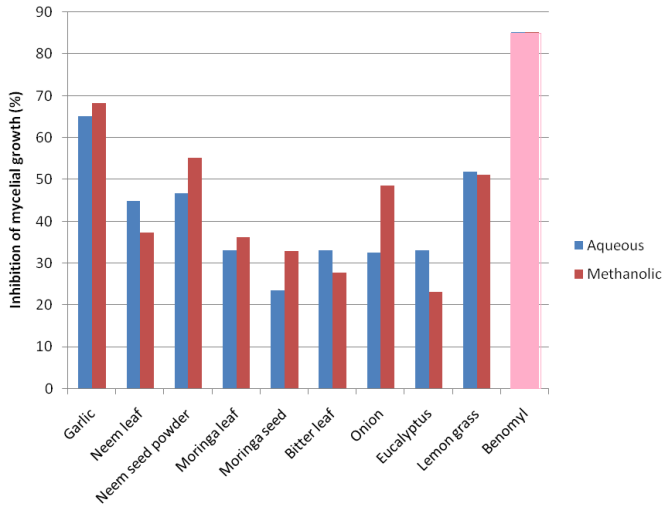

Figure 3: Comparative effect of aqueous and methanolic plant extracts on mycelial inhibition

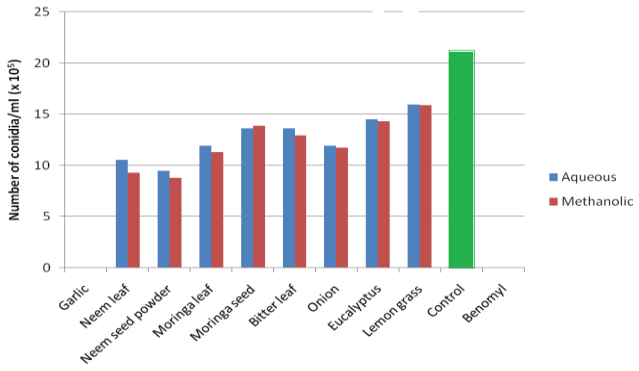

Figure 4: Comparative effect of aqueous and methanolic extracts on sporulation

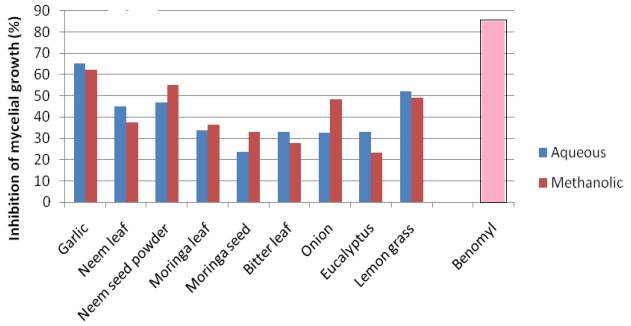

Figure 5: Comparative effect of levels of concentration of aqueous and methanolic extracts on mycelial inhibition

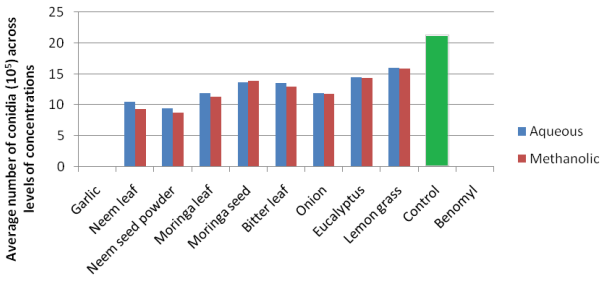

Figure 6: Comparative effect of levels of concentration of aqueous and methanolic extracts on sporulation

IV. DISCUSSION

Preliminary assessment of the *in vitro* antimicrobial potential of aqueous and methanol extracts of these plants confirmed significant antifungal activity. However, the inhibitory potential of the extracts were found to vary with specific plant species that is variability in chemical constituents of the plant as well as the solvent used for extraction.

All the treatments were found to be significantly superior over the control. *A. sativum* showed the highest antimicrobial effect followed by *A. indica* seed extract. The results support the observation of Sopanrao (2010) who found *A. sativum* bulb extract and *A. indica* seed extract to be most effective against *C.graminicola.* Similar success was reported by Potphode (2004), Haralpatil (2006) and Shovan *et al* (2008) who found *A. sativum* extract to be very effective in controlling the anthracnose pathogen *Colletotrichum* species in different crops. The better efficacy of *A. sativum* over the other plant extracts may be traced to allicin, a highly reactive compound formed when allinase catalyses its synthesis from allin. Other phytochemicals present in *A. cepa* and *A. sativum* are alkanoids, cardiac glycosides, terpenes and steroids, resin (Gazuwa *et al.*, 2013).

The test fungus in this study was more sensitive to *A. indica* seed extract than *A. indica* leaf powder. According to Zakaria (2010), the bioactive compounds of *A. indica* are the terpenes, of which azadirachtin in the leaves are known to rank second to the seed in terms of the active ingredient. The degree of success achieved with *A. indica* extracts have also been buttressed by other works (Amadioha and Obi, 1998; Onifade, 2002; Obi and Barriuso-Vargas, 2013).

Results obtained also demonstrated the superiority of *Moringa oleifera* leaf powder. This is consistent with those obtained by other investigators who reported antifungal activities of *Moringa* plant extracts against several pathogen (Adandonon *et al*, 2006; El-Mohamedy *et al.*, 2013; Akinbode and Ikotun, 2008). *Moringa* seeds contain some crystalline alkaloids, fatty acids, proteins, glycosides and niazirin said to be responsible for antimicrobial activities (Adandonon *et al.*, 2006). This might explain the findings in the current study.

Also established in this investigation is the sensitivity of *C.graminicola* to *Cymbopogon citratus*. In an evaluation on sorghum, *C.citratus* completely inhibited the growth of *C.graminicola* and *Phoma sorghomi* causing seed and

seedling rots in the plant (Bonzi *et al.*, 2012). Obi and Barriuso-Vargas (2013) demonstrated that *C.citratus* proved effective in inhibiting *C.destructivum* both *in vitro* and *in vivo*. Somda *et al.*, (2007) also reported *C.citratus* as exhibiting the best control effect on *C.graminicola* than *Eucalyptus* and *A.indica* seed extract. *C.citratus* leaves contain tannins, phlobatannins, flavanoid, cardiac glycoside, kellerkilliani (Joshua *et al.*, 2012). The effect of *Vernonia amygdalina* was less inhibitory than most of the plant species tested, *Eucalyptus camaldulensis* being the least effective. This was also the findings of Okigbo and Emoghene (2003) who observed that of all the three plant extracts tested against Mycosphaerella fijiensis, *V.amygdalina* was the least inhibitory.

When sporulation was the parameter for measurement of the effectiveness of the test materials, the results showed the most effective plant extract was *A.sativum* in which sporulation was completely inhibited and was as effective as benomyl which is one of the recommended fungicide for the control of *C.graminicola.* The more inhibitory a plant material is to sporulation the better it is for disease management and the opposite is true, that is, plant extracts that encourage sporulation should not be used in plant disease management because they increase the disease incidence and severity thus increasing production of spores which subsequently spread to new areas and crops (Hedge *et al.*, 2014).

The results of the methanolic plant extracts followed the same trend as that of the aqueous extracts. However, plant materials extracted in organic solvent (methanol) provided more consistent antimicrobial activity compared to aqueous extracts and hence more effective in inhibiting growth and sporulation except in the case of *A.indica* leaves, *V.amygdalina* and *E.camaldulensis* where the aqueous extracts were found to be more effective (Figures 4, 5 and 6). Similar findings were also observed in other investigations with aqueous extracts of *Eucalyptus* and bitter leaf exhibiting more antimicrobial activity than the methanol extract (Okigbo and Emoghene, 2003).These results generally confirmed the evidence that methanol is a better solvent for more consistent extraction of antimicrobial substances from plants compared to other solvents (Talibi *et al.*, 2013).Furthermore, Onifade (2000) found water extracts of *A.indica* leaves more effective in reducing spore germination and growth of *Colletotrichum* sp than other solvents. The possible explanation for this superiority is that organic solvents may have reacted with the active ingredients in the neem leaf forming inactive compounds. According to Somchit *et al.*, (2003), the extract efficiency displayed by solvents is dependent on the nature of the active ingredients so that methanol extracts are not always superior. It has been reported that methanol has capacity to solubilize bioactive compounds from the plants substances. Water however is a universal solvent used to extract plant products with antimicrobial activity. Nearly all of the identified antimicrobial compounds from plants are aromatic or saturated organic compounds, thus water is among other solvents that are most commonly used for preliminary investigations of antimicrobial activity in plants (Gurjar *et al.*, 2012). According to Amadioha and Obi (1999), effectiveness of the extracts largely depends on the type of solvent used in the extraction procedure, the age of the plant, method of extraction and time of harvesting. Tagoe *et al.*, (2009) on the other hand, concluded that the choice between aqueous and solvent extraction depends on the plant material being studied. Whereas aqueous extraction was most effective in inducing anti fungal properties of lime, ethanol based extraction was best for ginger while both methods had similar effects on anti fungal properties of garlic.

Though methanolic plant extracts proved to be more effective than the aqueous plant extracts, there are some limitations to the use of methanol at the level of the subsistence farming under which most of the sorghum in Nigeria is produced. These include:

- ✓ The cost of methanol
- ✓ The complicated procedure for extraction

These make extraction with water more attractive especially in view of the fact that the differences between the two methods of extraction were not significant. For both methods of extraction, the effect of the extracts was dosage dependent, that is, the higher the concentration, the more inhibitory the extracts.

Highest antifungal activity in this study was demonstrated by the standard control, benomyl. This is probably because the fungicide is in its pure state and has refined processes that have established it as a standard antibiotic (Prescott *et al.*, 2002).

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