

# EFFECT OF AQUEOUS EXTRACTS OF SOME MEDICINAL PLANTS ON *IN VITRO* GROWTH OF *Botryodiplodia theobromae* pat.

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## ABSTRACT

The development of *Botryodiplodia theobromae* Pat, was evaluated in chloramphenicol potato dextrose agar (cPDA) medium amended with aqueous extracts of eight plants used in Nigerian ethnomedicine. Linear mycelial growth in media containing unautoclaved or autoclaved extract was measured at 12 h interval for 48 h, while dry weight was determined after 48 h of incubation. Stroma production was determined after 7 days of incubation. *Cassia alata* was most inhibitory to the fungus and was followed by *Azadirachta indica*, *Alchornea cordifolia* and *Baphia nitida* respectively. *Cyathula prostrata*, *Vernonia amygdalina*, *Euphorbia hirta* and *Zingiber officinale* promoted radial mycelial growth of the pathogen. Autoclave heat treatment did not affect the inhibitory action of *C. alata* on the linear growth but reduced the efficacy of the leaf extract on dry weight and stroma production. Autoclaved extracts of *A. cordifolia*, *A. indica*, *E. hirta* and *C. alata* supported more stroma production, heat treatment did not stimulate production of more stroma on media containing extracts of *C. alata*, *V. amygdalina* and *Z. officinale*. The inhibition of mycelial growth and stroma production by unheated aqueous extracts of some of the test plants suggests that effective control of the pathogen with extracts of medicinal plants is possible.

**Key words:** *Botryodiplodia theobromae*, medicinal plants, aqueous extracts, *in vitro* growth inhibition.

## INTRODUCTION

*Botryodiplodia theobromae* Pat. is a ubiquitous opportunistic pathogen prevalent in the warm tropic, causing huge losses in the yield and quality of several crops ( Fajola and Nwufu 1985, Weerasinghe and Naqvi 1985, Meah *et al.* 1991, Lutchmeah and Santchum 1991). It is the most important tuber-rotting fungus of yam (*Dioscorea* sp.), a crop that is grown by millions of smallholder farmers in the tropics. The fungus is also associated with tip end rot of banana and plantain (*Musa* sp) in the field and in storage (Lutchmeah and Santchum 1991, Pasberg-Gauhl and Gauhl 1996). *B. theobromae* can be effectively controlled with chemical synthetic fungicides (Ekundayo 1984). However, the high cost, scarcity, and absence of pesticide monitoring and 'Safe-Use' initiatives make the

use of synthetic chemical control of the pathogen undesirable for farmers in Nigeria.

Researches into alternative ways to control plant pathogens, particularly using extracts of medicinal plants have been reported (Awuah 1989, Singh *et al.* 1993, Ejechi *et al.* 1997). Successful inhibition of the growth activity of the fungus by extracts of locally grown or indigenous medicinal plants would provide alternative ways of managing the pathogen on host plants. Besides being available in the country, the use of plant extracts to reduce the development of the pathogen would be practical for farmers since studies have shown that higher order plant products are non-phytotoxic and are easily biodegradable (Fawcett and Spencer 1970).

This investigation was undertaken to study

the *in vitro* fungitoxic effect of plants used in Nigerian ethnomedicine (Ogunlana and Ramstael 1975, Abimbola-Schipe 1986, Gill 1992) on the development of *B. theobromae*, a pathogen isolated from *Musa* tissue (Pasberg-Gauhl and Gauhl 1996).

## MATERIALS AND METHODS

**Source of fungus:** The culture of *B. theobromae* CE 19-2 was obtained from the International Institute of Tropical Agriculture, Ibadan. The fungus was routinely maintained at room temperature ( $30^{\circ} \pm 2^{\circ}$  C) on acidified Oxoid Potato Dextrose Agar (APDA).

**Source of medicinal plants:** Leaves of *Alchornea cordifolia* (Christmas bush), *Azadirachta indica* (neem), *Baphia nitida* (cam wood), *Cassia alata* (ringworm plant), *Cyathula prostrata*, *Euphorbia hirta* (asthma weed), and *Vernonia amygdalina* (bitter leaf) were collected from Choba in Obio Akpor Local Government Area of Rivers State, Nigeria. The rhizome of *Zingiber officinale* (ginger) used in the study was purchased from Choba market.

### Preparation of aqueous leaf extracts:

Aqueous extracts of the medicinal plants were prepared following the procedure described by Bashar and Rai (1992). Five hundred g of washed fresh plant material rhizome was crushed with a manual grinder and mixed with deionised water in the ratio 1:1 (w/v), then squeezed through four layers of cheesecloth and centrifuged at 5,000 rpm (model 7704, Griffin and George Ltd., Great Britain) for 20 min. The supernatant was collected and 200 ml added to 800 ml Potato dextrose agar medium (PDA). The PDA amended with the extract was autoclaved at  $121^{\circ}$  C,  $1.03 \text{ kg cm}^2$  for 15 min. After cooling to  $40^{\circ}$  C, chloramphenicol sulfate (500  $\mu\text{g/ml}$ ) was incorporated into the molten PDA medium to inhibit bacterial contamination during incubation (Awuah 1989). This preparation is hereafter referred to as Autoclaved Extract. A second portion of each extract (200 ml) was also incorporated into autoclaved molten agar medium

to which 500  $\mu\text{g/ml}$  chloramphenicol sulfate was added; hereafter referred to as Unautoclaved Extract. A 40 % extract medium contained 400 ml aqueous extract and 600 ml of cPDA. The control medium, which consisted of cPDA in which an equivalent volume of sterile distilled water was added, was also dispensed into 100-mm diameter sterilized Petri plates.

### Evaluation of anti-fungal activity of extracts:

The anti-fungal activity of the extracts were evaluated by monitoring the growth of the fungus on chloramphenicol PDA (cPDA) plates amended with extracts and inoculated with a 7 mm mycelial disks cut from the edge of a two-day old culture of *B. theobromae*, incubated in the dark at room temperature ( $28^{\circ}$  to  $30^{\circ}$  C). Each treatment was replicated four times. Linear growths at 12 h intervals were recorded for 48 h, after which the initial size (7 mm) of mycelial disk used to inoculate the plates was deducted in order to determine the actual growth of the fungus.

The dry weight of the fungus mycelium was determined after 48 h of incubation in the dark at room temperature. Each culture was cut into four sections, placed in glass jars containing about 100ml water and autoclaved for three min to melt the agar. The mycelial mat was rinsed in

three changes of distilled water and transferred to a pre-weighed foil then oven dried at  $90^{\circ}$  C, overnight. The weight of the dried fungus thallus together with the foil was determined after oven drying. The dry weight of the fungus mycelium was derived by subtracting the weight of the foil from the weight of the foil and dried mycelium.

Chloramphenicol PDA amended with 40 % extracts was used to determine antifungal effects of the medicinal plants on stroma formation by the fungus. Petri plates were inoculated with 7 mm mycelial disks from the edge of two-day-old cultures and incubated under darkness for 48 h then the cultures were exposed to 24 h light at room temperature for an additional five days, to stimulate stroma production. The stroma produced in each of the four replicated plates was enumerated. Chloramphenicol PDA plates without extracts served as control

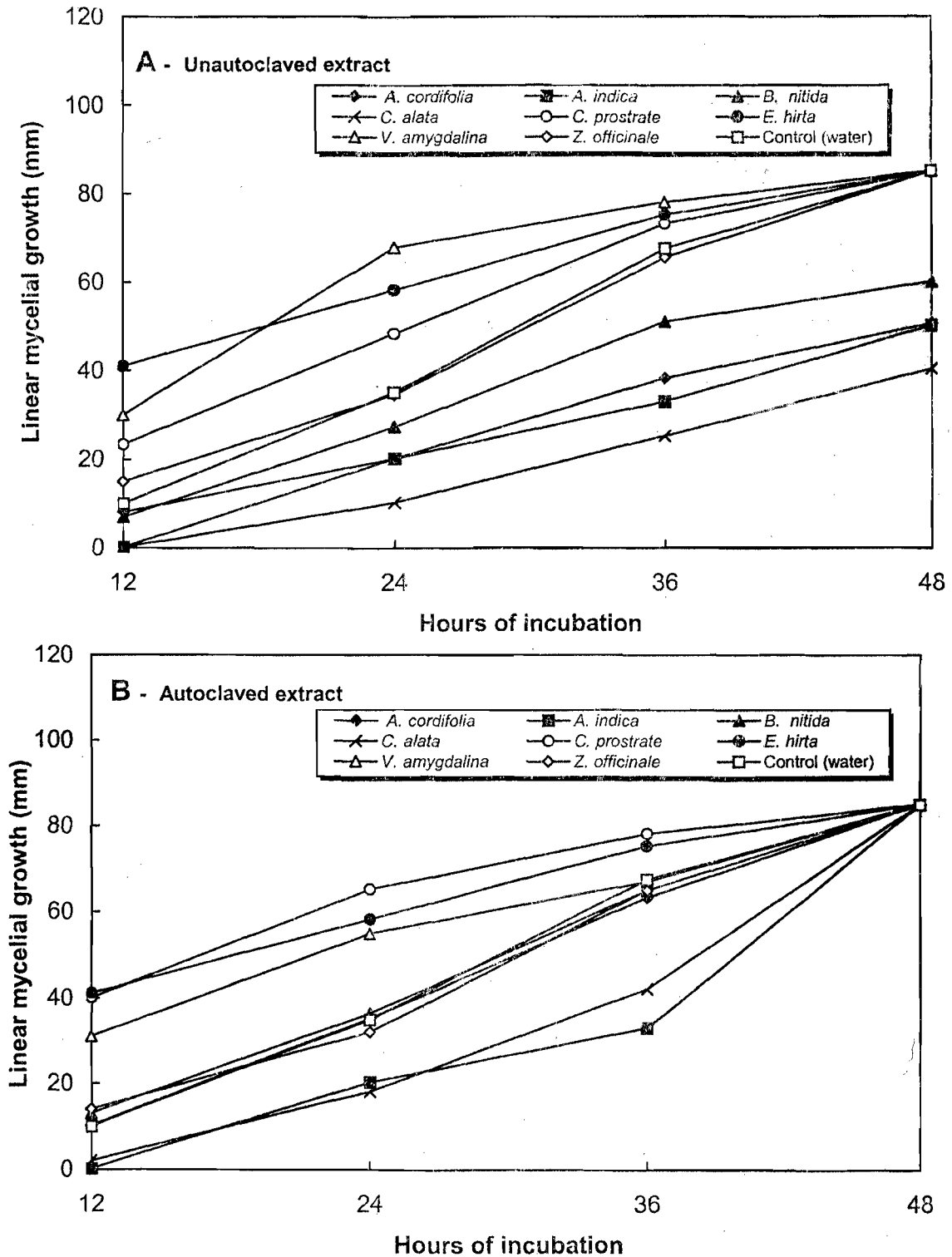


Figure 1: Effects of aqueous extracts of medicinal plants on linear mycelial growth of *Botryodiplodia theobromae* at different incubation periods.

Table 1. Effects of aqueous extracts of Nigerian medicinal plants on mycelial dry weight and stroma production by *Botryodiplodia theobromae*.

Source of Extract	Unautoclaved Extract		Autoclaved Extract <sup>b</sup>	
	Dry wt (mg) <sup>a</sup> at 48 hours	% stroma formation after 7 days <sup>c</sup>	Dry wt (mg) <sup>a</sup> at 48 hours	% stroma formation after 7 days <sup>c</sup>
<i>Alchornea cordifolia</i>	30ab <sup>d</sup>	0	110e	8.33
<i>Azadirachta indica</i>	30ab	0	90cd	5.21
<i>Baphia nitida</i>	40b	0	80c	0
<i>Cassia alata</i>	20a	0	40a	0
<i>Cyathula prostrata</i>	90d	8.33	160g	11.41
<i>Euphorbia hirta</i>	110e	0	130f	15.63
<i>Vernonia amygdalina</i>	130f	0	120ef	0
<i>Zingiber officinale</i>	60c	0	60b	0
Control (water)	60c	100	60b	100

<sup>a</sup>Choramphenicol PDA (cPDA) amended with 20% leaf extract of the first seven plants and rhizome extract of *Zingiber officinale*.

<sup>b</sup>cPDA amended with extract was autoclaved for 15 min at 121°C, 1.03 kg cm<sup>-2</sup>.

<sup>c</sup>cPDA amended with 40% extract was used

<sup>d</sup>Value are means of 4 replicates. Values within the same column followed by different letters are significantly different ( $P \leq 0.05$ ), according to Duncan's New Multiple Range Test.

Data on linear growth and mycelial dry weight were subjected to analysis of variance (ANOVA) to determine significant differences. Means were compared using Duncan's Multiple Range Test ( $p \leq 0.05$ ).

## RESULTS AND DISCUSSION

Leaf extracts of the medicinal plants promoted as well as inhibited the growth of *B. theobromae* (Fig. 1). Of the eight medicinal plants, *Cyathula prostrata*, *Vernonia amygdalina* and *Zingiber officinale* showed promotory effect on the linear growth when compared with the control. Four of the unautoclaved leaf extracts inhibited mycelial growth of *B. theobromae* throughout the 48-h incubation period (Fig. 1A), with *Cassia alata* being most inhibitory followed by *Azadirachta indica*, *Alchornea cordifolia* and *Baphia nitida*, respectively. All four leaf extracts significantly reduced the growth of *B. theobromae* when compared with the control.

Fig.1B further shows the extent of inhibition of the growth of *B. theobromae* leaf extracts as affected by autoclave heat treatment.

The heat treatment affected the inhibitory activity of *C. alata*, *A. cordifolia* and *B. nitida* within the first 24-h of incubation because growth of the fungus was more. The reduced effect was not apparent after 36 h of incubation, since the fungus grew well in all plates amended with the extract of these four medicinal plants regardless of the heat treatment. On the contrary, heat treatment did not affect the activity of the three medicinal plants that promoted the growth of the fungus, *C. prostrata*, *V. amygdalina* and *Z. officinale*.

The effect of the aqueous extract on the dry weight of *B. theobromae* is shown in Table 1. The inhibitory effect of the unautoclaved extracts of *A. cordifolia*, *A. indica* and *B. nitida* were comparable. In contrast, the extracts of *C. prostrata*, *Euphorbia hirta*, *V. amygdalina* and *Z. officinale* increased the dry weight of the fungus in the following order: *V. amygdalina* > *E. hirta* > *C. prostrata* > *Z. officinale* = control.

Heat treatment significantly affected the inhibitory effect of the extracts on the dry weight.

With the exception of *C. alata*, the extracts of the other seven medicinal plants increased the fungal dry weight above that of the control. This indicates that autoclaving adversely affected the fungitoxicity of the extracts of *A. cordifolia*, *A. indica* and *B. nitida* because the unautoclaved comparable treatments still reduced the fungal mass.

With the exception of *C. prostrata*, all the unautoclaved extracts inhibited stroma production (Table 1). Ninety-six stroma (100 %) were produced on the extract-free (control) plates while only eight (8.33 %) were produced on plates amended with the extracts of *C. prostrata*. When the extracts were heat treated, *A. cordifolia*, *A. indica* and *V. amygdalina* further supported production of stroma although the amount was still very low (5.21 to 15.63 %) when compared with the control treatment (100 %). In contrast, heat treatment did not affect the efficacy of *B. nitida*, *C. alata*, *V. amygdalina*, and *Z. officinale* in inhibiting stroma production *in vitro*.

The eight medicinal plants whose antifungal activities were investigated are used in Nigerian ethnomedicine for the treatment of various ailments of bacterial and fungal origin (Abimbola-Sodipe 1986, Gill 1992, Ogunlana and Ramstael 1975). There have been reports of toxic effects of several African medicinal plants against human pest (Frieburghaus *et al.* 1980), human pathogens (Fabry *et al.* 1996, Rabe and Van-Staden 1997), plant pests (Clarke and Appleton 1996), and plant pathogens (Auwah 1989, Ejechi *et al.* 1997). But only Ejechi *et al.* (1997) reported on the inhibitory effects of some species of Nigerian plants against *B. theobromae*. Therefore, the present findings provide added support to the work of Ejechi *et al.* (1997) who found that the growth of *B. theobromae* can be inhibited by crude extracts of some Nigerian medicinal plants.

Both the heat-treated and non-heat treated extracts of four of the medicinal plants studied showed antifungal activity against the growth of *B. theobromae*. The activities of three of these plants, *A. cordifolia*, *B. nitida* and *C. alata*

were reduced by the autoclave heat treatment while that of *A. indica* was not. The antifungal activity of these extracts against the growth of *B. theobromae* has not been reported. Zeringue and Bhatnagar (1994) noted that blender maceration and boiling of leaves of *A. indica* reduced its activity. They observed that most of the antifungal activity was attributed to the presence of volatile antifungal C2 to C9 monounsaturated aldehydes found in the leaves. These aldehydes were lost during maceration or boiling. Since manual grinding before the heat treatment also macerated the leaves, it is possible that some of these aldehydes may also have been lost during preparation. Furthermore, the present study showed that there are other antifungal heat-resistant compounds in the leaves of *A. indica*. Fabry *et al.* (1996) also reported that the extract of *A. indica* was active against *Candida* and *Aspergillus* species, and Singh *et al.* (1993) found that the extract of *A. indica* gave good control of disease of banana fruits infested with *B. theobromae*.

*Zingiber officinale* rhizome extract did not inhibit or stimulate the growth of the fungus. However, Marallo-Rejesus and Punzalan (1997) found the volatile oil of *Z. officinale* toxic to the golden snail (*Panacea* sp.). It is also possible that *B. theobromae* maybe more sensitive to the refined extracts of *Z. officinale*. Other studies have shown that organic extracts are more toxic than aqueous or crude extract (Frieburghaus *et al.* 1980).

The complete inhibition of stroma production by *B. theobromae* in PDA amended with 40% leaf extracts of seven of the medicinal plants tested suggests that effective control of the reproductive stage of this ubiquitous, pathogenic fungus might be possible. Further studies on effects of these leaf extracts on sporulation and spore germination of the fungus should be established. Although the focus of the reported investigations were on the antifungal activity of these medicinal plants, the results obtained in this study indicate that some crude leaf extracts may promote the growth of some fungi while inhibiting others. Thus, care must be taken in the use of

leaf extracts for the control of pathogenic fungi, particularly when they occur in association with other pathogens on the host surface.

In conclusion, aqueous leaf extracts of four plants used in Nigerian ethnomedicine, *A. cordifolia*, *A. indica*, *B. nitida* and *C. alata* were found to inhibit the linear mycelial growth, fungal dry weight, and stroma production of *B. theobromae*, *in vitro*. Of these plants, *A. indica* has been most extensively and intensively studied because of its broad antimicrobial and biotoxic properties. The antifungal activity of *C. alata* was comparable in efficacy to *A. indica*. Since *A. indica* contains specific volatiles with known fungicidal property (Zeringue and Bhatnagar 1994), there is also the possibility that *A. cordifolia*, *B. nitida* and *C. alata* may contain compounds with antifungal properties, which may not be soluble in water.

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