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EFFECT OF AQUEOUS EXTRACTS OF MEDICINAL PLANTS ON GROWTH OF *Fusarium moniliforme var subglutinans* WOLLENW. AND REINKING

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ABSTRACT

The growth of *Fusarium moniliforme* var *subglutinans* Wollenw. and Reinking was evaluated in chloramphenicol potato dextrose agar (cPDA) containing 20 %aqueous extracts of eight medicinal plants. Mycelia growth, was reduced in unautoclaved extracts of *Cassia alata, Baphia nitida*, *Euphorbia hirta*, *Vernonia amygdalina, Azadirachta indica*, and *Zingiber officinale,* with *B. nitida, V. amygdalina*, and *C. alata*, being the most effective. On the contrary, the unautoclaved extract of *Cyathula prostrata* did not affect the growth of the fungus. The antifungal activities of *B. nitida, A. indica, E. hirta, Z. officinale,* and *C. alata* were affected by autoclave treatment. Unautoclaved extracts of *C. alata, B. nitida, A. indica, V. amygdalina, A. cordifolia, E. hirta* and *Z. officinale* significantly ($p \leq 0.05$) reduced the dry weight of the fungus below the control. Autoclaving the extracts affected the fungitoxic activities of *C. alata, B. nitida,* and *V. amygdalina*, on the dry weight of the fungus. Sporulation was completely inhibited in cPDA medium amended with unautoclaved extracts of *C. alata, B. nitida, A. indica, V. amygdalina* and *Z. officinale.* However all the autoclaved extracts supported sporulation of the fungus. Conidia germination in full strength (w/v, 1:1) extracts was completely inhibited, whereas 90 % of the conidia germinated in sterile deionised water after 6 h of incubation. These findings suggest the potential role of the extracts in controlling the fungus.

Key words: *Fusarium moniliforme* var *subglutinans,* medicinal plants, aqueous extracts, *in vitro* growth inhibition.

INTRODUCTION

Fusarium moniliforme var subglutinans Wollenw. and Reinking is a common and worldwide pathogen of field crops, causing stalk, ear and kernel rots of maize, and producing mycotoxin in stored grain. The pathogen was associated with human esophageal cancer in Transkei, South Africa where contaminated grain was directly consumed (Morasas *et al.* 1981). Damicone *et al.* (1988) found that *F. moniliforme* isolated from corn stalk and dormant asparagus in the United States of America were cross-pathogenic and virulent in both crops grown in the country. In Nigeria, *F. moniliforme* causes heart rot (Ikotun and Ezomo 1996) and fruit tip rot (Pasberg-Gauhl and Gauhl 1996) of banana and plantain (*Musa* sp.). The most popular plantain variety 'Agbagba' is most susceptible to the pathogen (Ikotun and Ezomo 1996).

The chemical ethrel (ethephon) applied to unripe *Musa* fruits was found to reduce disease development by promoting early ripening and disease avoidance (Lutchmeah and Santchum 1991). Although synthetic chemicals control most field and storage plant pathogens, some causes several side effects and persists in the environment. Besides, the high cost, scarcity, and absence of pesticide monitoring in many developing countries including Nigeria, make the use of synthetic chemicals for the control of crop diseases undesirable for the farmers, many of which are small holders.

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Worldwide attention has been recently drawn to the protecting role of new agents from medicinal plants. Most of these agents exist as volatile and/or non-volatile compounds (Chaulfoun and Carvalho 1987,Bashar and Rai 1992, Zeringue and Bhatnagar 1994). A review of current literature revealed a paucity of information on research efforts into alternative ways of controlling *Fusarium* sp., particularly using extracts of medicinal plants (Narain and Satapathy 1977, Bera and Saha 1983, Bashar and Rai 1992, Singh *et al.* 1993, Ejechi *et al.* 1997)). Singh *et al.* (1993) noted that treatment of infested banana fruits with leaf extracts of medicinal plants gave good control of several fruit pathogens of banana fruits including *F. moniliforme*. But there has been no report on the control of *F. moniliforme* var *subglutinans* with medicinal plants used in Nigerian ethnomedcine. Other workers have shown that plants used in African ethnomedicine have antimicrobial activities (Khan *et al.* 1980, Awuah 1989, Fabry *et al.* 1996, Ejechi *et al.* 1997, Rabe and Van-Staden 1997).

The study reported was conducted to ascertain the effect of some Nigerian medicinal plants on the development of *F. moniliforme* var *subglutinans*, a pathogen causing fruit tip rot of banana and plantain in Nigeria. The test plants investigated are used in Nigerian ethnomedicine for the treatment of various ailments (Ogunlana and Ramstael 1975, Abimbola-Sodipe 1986, Gill 1992).

MATERIALS AND METHODS

Source of the fungus:

The isolate of *F. moniliforme* var *subglutinans* CE 24-1 used in the study, was received in a Potato dextrose agar (PDA) slant from the International Institute of Tropical Agriculture, Ibadan. It was originally isolated from *Musa* sp. Pure cultures of the fungus were routinely maintained in the dark at room temperature (28° to 30° C) on acidified PDA.

Source of medicinal plants:

Leaves of the medicinal plant, *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), which were used in the study, 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 crude extracts:

Crude extracts of the eight medicinal plants were prepared as described by Bashar and Rai (1992). Five hundred g of washed fresh leaf or 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 divided into two equal parts. Eight hundred ml of Oxoid potato dextrose agar (PDA) amended with 200 ml of each extract was autoclaved at 121° C, 1.03 kg cm^2 for 15 min. After cooling to 40° C, chloramphenicol sulfate (500 ppm) was incorporated into the molten potato agar medium (cPDA), to inhibit bacterial contamination during incubation (Awuah, 1989). This preparation is hereafter referred to as Autoclaved Extract. A second portion (200 ml) of each extract was incorporated into 800 ml autoclaved molten agar medium to which 500 ppm chloramphenicol sulfate was added; hereafter referred to as Unautoclaved Extract. About 20 ml of the amended medium was dispensed into 100 mm diameter sterilized glass petri plates and allowed to cool. 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.

Antifungal activity of extracts:

To evaluate the activity of each extract on the linear growth of the fungus, 7 mm mycelial disks cut from the edge of a 7-day old culture of *F. moniliforme* var *subglutinans* was inverted and placed in the middle of cPDA plates amended with unautoclaved or autoclaved extracts. The

plates were incubated on a laboratory bench at 28-30° C, in the dark. Chloramphenicol PDA plates amended with an equivalent amount of sterilized deionized water served as control. Each treatment was replicated four times. Linear growths, at 24 h intervals were recorded for 7 days, after which the initial size (7 mm) of mycelial disk used to inoculate the plates was deducted, to determine the actual growth of the fungus.

The dry weight of the fungal thallus was determined after 7 days. To harvest the thallus, each culture was cut into four sections, placed in a glass jar and autoclaved in about 100ml water for three min to melt the agar. The mycelial mat was then rinsed in three changes of distilled water, transferred to a pre-weighed foil and oven dried at 90° 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.

The effect of extracts on sporulation of the fungus was determined as follows. Conidia were washed off the surface of 7-day old cultures and adjusted to 8.0 x 10^4 conidia/ml after counting with a haemocytometer (WSI, Weber, England). Two drops of the spore suspension was spread plated on the surface of duplicate cPDA plates amended with the extracts and incubated at 28 to 30° C in the dark for 7 days. A duplicate set of plates containing an equivalent volume of sterile deionized water instead of extracts served as control. After incubation two agar disks were randomly cut from each culture, and placed in one ml sterile deionized water then centrifuged at 5,000 rpm to obtain a spore suspension. Spore counts were determined with a haemocytometer.

To determine the effect of the crude extracts on spore germination, triplicate 15 ml capacity tubes containing nine ml of a full strength preparation (1:1 w/v) of each extract was plugged with cotton ball wrapped in foil and autoclaved for 15 min at 121° C, 15 psi. Another set of triplicate tubes containing the extracts that were not autoclaved was similarly prepared. One ml of 8.0x 10^4conidia/ml suspension of *F. moniliforme* var *subglutinans* was used to inoculate each tube. The control tube containing 9 ml of sterile deionized water was also seeded with one ml of the conidia suspension. A drop of hydrogen peroxide was added to each tube to reduce the oxygen tension, after which the tubes were incubated for 6 h on the laboratory bench. Conidia were observed for germ tube formation under low power, using a haemocytometer. The number of spores in the central square, which produced germ tubes was divided by total number of spores in the central square and multiplied by 100.

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

RESULTS AND DISCUSSION

The rate of growth of *F. moniliforme* var *subglutinans* differed on media amended with the different extracts, although the general effect was inhibitory (Figure 1). Mycelia growth was reduced in the unautoclaved media amended with extracts of *Cassia alata, Baphia nitida, Euphorbia hirta, Vernonia amygdalina, Azadirachta indica*, and *Zingiber officinale,* with *B. nitida, V. amygdalina*, and *C. alata*, being the most effective. The activities of *B. nitida, A. indica, E. hirta, Z. officinale,* and *C. alata* were affected by autoclave treatment. The order of inhibition was *B. nitida* = *V. amygdalina* > *C. alata* > *A. indica* > *Z. officinale* > *A. cordifolia* = *E. hirta* > *C. prostrata* > control (Table 1). Comparing the unautoclaved extracts with the autoclaved, daily examination of the fungus showed that the growth rate was similar although linear mycelia growth was more on plates containing unautoclaved extracts. The autoclave heat treatment reduced the antifungal activity of *V. amygdalina*, and slightly affected the activities of *C. prostrata* and *E. hirta* (Table 1).

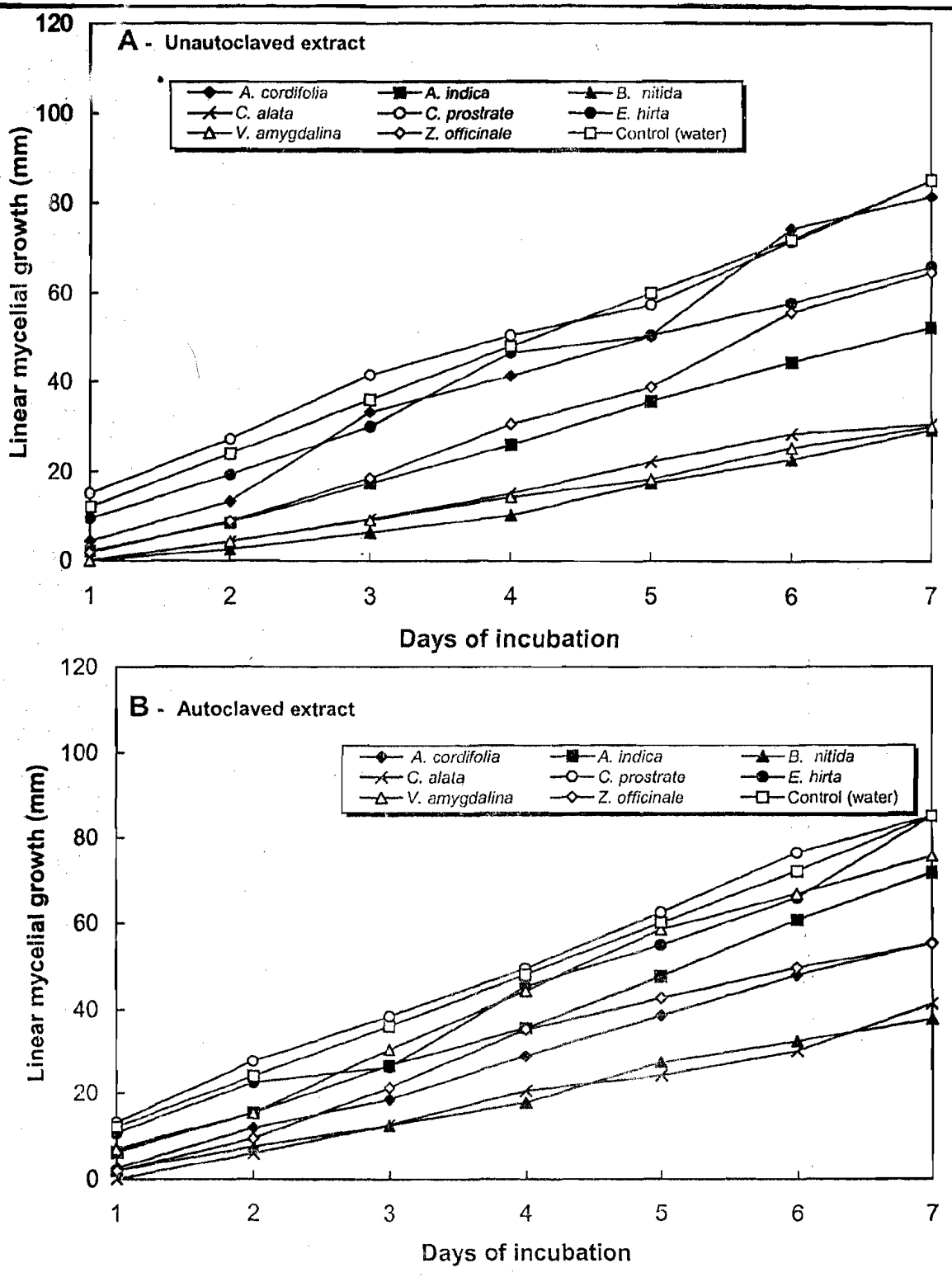

Figure 1. Effect of aqueous extracts of medicinal plants on linear mycelial growth of *Fusarium moniliforme var subglutinans* at different incubation periods.

Table 1. Influence of aqueous leaf extracts on mycelial growth, mycelial dry weight sporulation and spore germination of *Fusarium moniliforme* var *subglutinans in vitro*

Source of leaf extracts tested	Autoclaved extracts[a]			Unautoclaved extracts		
	Radial growth (mm) at 5 days[b]	Dry wt (mg) at 7 days[b]	Sporulation (conidia / ml)[c]	Radial growth (mm) at 5 days [b]	Dry wt (mg) [b]	Sporulation (conidia / ml) [c]
Alchornea cordifolia	38.50c[d]	60b	230×10^6	50.30e	80c	1.57×10^5
Azadirachta indica	47.50e	80c	2.30×10^5	35.48c	90cd	0
Baphia nitida	27.15b	50b	2.00×10^5	17.45a	10a	0
Cassia alata	24.10a	30a	4.12×10^6	22.15b	20a	0
Cyathula prostrata	62.40h	120e	1.90×10^6	57.25fg	130f	1.85×10^6
Euphorbia hirta	55.25fg	110de	4.60×10^5	50.35e	110e	4.60×10^5
Vernonia amygdalina	58.35g	80c	8.71×10^6	18.03a	40b	0
Zingiber officinale	42.50d	60b	1.11×10^8	38.75d	40b	0
Control (water)	60.00gh	100d	9.18×10^6	60.00g	100de	9.18×10^6

[a]Heat treatment was by autoclaving for 15 min. at 121°C, 1.03kg cm^{-2}.
[b]Chloramphenicol PDA (cPDA) amended with 20% plant extract. Values are means of 4 replicates.
[c]CPDA amended with 40% plant extract values are means of duplicate determinations from two replicates of leaf extracts.
[d]Values are means of 4 replicates. Means followed by different letters are significantly different according to Duncan's multiple range tests ($p \leq 0.05$).

With the exception of *E. hirta* and *C. prostrata*, whose dry weights were similar to, or above that of the control, unautoclaved extracts of all the test plants significantly reduced the dry weight of *F. moniliforme* var *subglutinans* (Table 1). *B. nitida* and *C. alata* were most inhibitory, followed by *V. amygdalina* and *Z. officinale*. The autoclave heat treatment affected the antifungal activity of *B. nitida, C. alata, V. amygdalina,* and *Z. officinale*, since the dry weight of the fungus grown in media amended with heat-treated extracts was more than the dry weight in media amended with similar but unheated extracts.

A higher concentration of the aqueous preparation of test plants was used to determine the effect of each medicinal plant extract on spore production and germination of the fungus. Five of the unautoclaved extracts, *A. indica, B. nitida, C. alata, V. amygdalina,* and *Z. officinale,* completely inhibited spore production after seven days of incubation in the dark (Table 1). But the unautoclaved extracts of *C. prostrata, E. hirta* and *A. cordifolia* supported spore production. Again the autoclave treatment affected the efficacy of the aqueous extracts. The ability of the fungus to produce conidia *in vitro* was not inhibited in the presence of heated extracts of the plants. Thus, all the autoclaved extracts supported conidia production, although the amount produced in the different extracts varied. Spore germination in full strength (w/v 1:1) was completely inhibited, although 90% of the spores germinated after 6 h in deionised water.

The inhibition of growth of *.F moniliforme* var *subglutinans* by aqueous leaf extracts of *B. nitida, C. alata, A. indica,* and *A. cordifolia*

suggests that extracts of some plants used in Nigerian ethnomedicine can control the growth of this pathogen. Although heated extracts reduced the growth of the fungus, their efficacy was less in comparison with the unheated extracts. The present study further confirm the observations of Singh *et al.* (1993) who found the extract of *A. indica* inhibitory to another species of *Fusarium*, *F. oxysporum* on infested banana fruits. In addition, Mishra and Dubey (1994) found the essential oil of lemon grass, *Cymbopogon citratus* inhibitory to seven species of *Fusarium*, including *F. moniliforme*.

The unautoclaved aqueous extracts of *A. indica*, *B. nitida*, *C. alata*, and *Z. officinale* completely inhibited conidia production while the heat-treated counterpart did not. These findings suggest that the leaf extracts may contain heat-labile fungitoxic compounds. The complete inhibition of conidia production of *F. moniliforme* var *subglutinans* by the extracts of the medicinal plants tested also suggests that the effective control of the reproductive stage of the fungus with Nigerian medicinal plants is possible. Bera and Saha (1983) noted that the aqueous extract of *Catharanthus roseus* appreciably reduced the growth and sporulation of pathogenic fungi, including *Fusarium* sp and suggested that the antifungal compounds present in the extracts may inhibit and inactivate the normal biochemical and/or cytogenetic activity during spore initiation. It is possible that the compounds that affect spore initiation may be volatile or heat sensitive since the present study showed that some of the heated extracts supported good sporulation while the comparable unheated extracts completely inhibited sporulation. Preformed antimicrobial compounds are present in plants and have been shown to play key role in resistance of plants to fungal pathogens (Keen 1980). Studies by Zeringue and Bhattnagar (1994) reveal the presence of sixty compounds including volatile alcohols, hydrocarbons, ketones, and aldehydes in fresh *A. indica* leaves, some of which were found to have significant effect on growth and aflatoxin production by *Aspergillus parasiticus.* Preliminary studies on the germination of conidia of *F. moniliforme* var *subglutinans* showed that full strength preparation of all the tested medicinal plants inhibited conidial germination after 6 h whereas 90% of the conidia germinated in water. The inhibition of the different stages of the pathogen by most of the medicinal plants tested, suggest the potential role of these plants as biocides.

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