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Effect of Essential Oils of Some Meliaceous Plants on Aflatoxin Production and Growth of *Aspergillus parasiticus*

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Abstract: Different concentrations of the essential oils obtained from hydrodistillation of five meliaceous plants were screened *in vitro* for effectiveness in inhibiting the growth and aflatoxin production by *Aspergillus parasiticus*. All the essences reduced the yield of fungal mycelia as well as the quantity of aflatoxin B_1 produced in liquid culture. However, percentage inhibition was linearly related to oil concentrations. Essences from *Khaya grandifoliola* C.DC was the most efficacious, irrespective of the concentration. This was followed in a decreasing order of magnitude by the essential oils of *Pseudocedrela kotschyi* Harms, *Entandrophragma utile* Linn., *Trichilia heudilotii* Planch and *Lovoa trichiliodes* Harms. The amounts of aflatoxin B1 isolated from broth (culture filtrate) extract were usually twice that of the mycelia extract.

Key words: Aflatoxin, meliaceae, essential oil, inhibition, *Aspergillus parasiticus*

INTRODUCTION

Mycotoxins are a group of highly poisonous metabolites produced by different fungi growing on crops and their products. They cause great economic loss by damaging about 25% of world's crop[1]. Amongst these, aflatoxins which are potentially carcinogenic metabolites of *Aspergillus flavus* and *A. parasiticus* have been isolated from a number of sources including foods and feeds. The increasing awareness to health hazards caused by aflatoxins Hawskworth[2] has stimulated the current move to control aflatoxin contamination of agricultural commodities. A number of chemicals have been found effective in the reduction of growth of toxigenic *Aspergillus* and aflatoxin levels in contaminated foodstuffs[3]. In addition, products of many spices are known to inhibit aflatoxin production in culture medium[4-6]. Some findings have also revealed that oils obtained from some angiospermic plant parts contain antitoxigenic substances[7-14].

The present study was undertaken to investigate the efficacy of essential oils from five meliaceous plants on mycelial growth and aflatoxin production by *Aspergillus parasiticus* in liquid medium. The family meliaceae is composed of trees and shrubs with alternate, pinnate and exstipulate, eglandular leaves. The wood usually emits a strong offensive odor and the small, usually whitish, flowers are borne on loose axillary panicles[15]. Some plants in this family are important timber species and have been found effective in folkloric remedies against a wide range of human infections[16].

MATERIALS AND METHODS

Plant materials and essential oils: The leaves of *Entandrophragma utile* Linn. *Khaya grandifoliola* C.DC. *Lovoa trichiliodes* Harms, *Pseudocedrela kotschyi* Harms and *Trichilia heudilotii* Planch were harvested from different parts of Southern Nigeria between March and July 2005 and authenticated by Dr A. Shahina Ghazanfar (Herbarium Royal Botanic Garden, Kew, U.K.). Voucher specimens have been deposited in the Department of Forestry and Wood Technology Herbarium, Federal University of Technology, Akure, Nigeria.

Fresh leaves of each plant (500 g) and water (1:3 w/v) in a 10-quart stainless StoveStill™ (Clevenger-type) apparatus (Essential Oil University, New Albany, IN, USA) were hydrodistilled for 3 h. The steam distillate was dried over anhydrous sodium sulphate[17].

Fungal culture: The toxigenic strain of *Aspergillus parasiticus* which originated from peanut was usually maintained by regular transfers unto fresh Sabouraud Dextrose Agar and incubated for 7 days at 28±2°C, repeated twice, immediately before the experiment. The spores were harvested by flushing the culture in sterile distilled water. Suspensions were adjusted to 3×10^6 spores per mL, as enumerated by a haemocytometer and 0.5 mL was used for inocula.

Inoculation: The basal medium used consisted of yeast extract (3.5 g), sucrose (100 g), KNO_3 (1.5 g), $MgSO_4.7H_2O$ (0.25 g) in 500 mL distilled water[18]. Each of the essential

oils was mixed with 50 mL medium in 250 mL conical flask to give concentrations of 50, 100, 150 and 200 μg mL^{-1}. The initial pH of the medium was adjusted to 5.5. Each flask was inoculated with a 0.5 mL spore suspension of *A. parasiticus* and incubated for 14 days at 28°C in a Gallenhamp rotary incubator (300 rpm). Each treatment was replicated five times.

Processing of fungal cultures: After incubation, the fungal culture was passed through Whatman No. 1 filter paper under vacuum to separate the mycelia from the culture broth. The mycelia were weighed, triturated in a domestic blender and extracted with chloroform (1:1w/v). The final pH value of the broth was noted, after which it was extracted with equal volume of chloroform. In each study, the organic layer was dried over anhydrous $NaSO_4$ and concentrated *in vacuo* in pre-weighed vials. The dried extracts obtained were reconstituted in methylene chloride for aflatoxin analysis[19].

Analysis of aflatoxin: Analytical thin-layer chromatography was performed on pre-coated silica-gel 60F 254 plates (0.2 mm thick, 20×20 cm, Merck). The re-dissolved sample was spotted against the standard solutions of aflatoxin B_1 (0.4 μg mL^{-1}; Romer Labs Inc., USA) with a mixture of toluene, isoamyl alcohol and methanol (45:16:1 v/v/v) as the solvent system[20]. For this, Romer^R Autospotter was used. Then the aflatoxin B_1 with R_f value of 0.5 was visually estimated under UV light (365 nm) relative to the standard spots[19]. Quantitative estimation of aflatoxin B_1 was done spectrophotometrically according to Nabney and Nesbitt[21].

RESULTS AND DISCUSSION

During hydrodistillation, the essential oils of the species of *Entandrophragma, Khaya, Lovoa*, *Pseudocedrela* and *Trichilia* were respectively isolated with 0.4, 0.24, 0.18 and 0.25% recovery. In general, the yield of aflatoxin B_1 recorded in the culture filtrate was considerably higher than that of fungal mycelia Table 1. All the essential oils inhibited the growth of *Aspergillus parasiticus* and caused a remarkable reduction in the amounts of aflatoxin B1 produced by the fungus. However, antifungal concentrations of oil were linearly proportional, but varied from one plant to another. The yields of aflatoxin B_1 and fungal mycelia reduced with a corresponding increase in oil concentrations. *Khaya grandifoliola* oil was the most efficacious even at the lowest concentration of 50 μg mL^{-1}. This was followed in a decreasing order of potency by *Pseudocedrela kotschyi*,

Table 1: Effect of essential oils on mycelial growth and aflatoxin production by *Aspergillus parasiticus*

Plant species	Oil conc μg mL)	Mycelial wet mass (g/flask)	Aflatoxin (μg mL^{-1}) Broth	Mycelia
E. utile	50	2.41±0.06	13.13±0.01	6.12±0.03
	100	2.28±0.01	12.62±0.01	5.61±0.01
	150	2.19±0.03	10.95±0.03	5.21±0.01
	200	2.13±0.02	9.93±0.02	4.55±0.02
K. grandifoliola	50	1.92±0.03	9.71±0.01	5.13±0.02
	100	1.86±0.01	8.84±0.01	4.11±0.01
	150	1.81±0.04	7.58±0.04	3.32±0.01
	200	1.73±0.02	6.73±0.02	2.87±0.03
L. trichiliodes	50	3.75±0.08	23.34±0.03	10.13±0.02
	100	3.68±0.01	22.11±0.06	9.81±0.01
	150	3.61±0.05	19.91±0.01	9.64±0.04
	200	3.47±0.03	19.15±0.02	8.83±0.02
P. kotschyi	50	2.15±0.01	10.82±0.01	5.57±0.02
	100	2.08±0.03	10.54±0.01	4.92±0.03
	150	1.980.020	9.93±0.03	4.14±0.02
	200	1.89±0.04	9.67±0.01	3.72±0.02
T. heudelotii	50	2.92±0.02	17.31±0.03	8.72±0.03
	100	2.86±0.06	16.81±0.01	7.94±0.01
	150	2.810.010	14.58±0.03	6.71±0.02
	200	2.73±0.03	13.93±0.02	6.17±0.01
Untreated control	-	3.83±0.04	24.62±0.08	11.22±0.03

Each value is a mean of five replicates±SE

Entandrophragma utile, *Trichilia heudilotii* and *Lovoa trichiliodes*. Apart from *K. grandifoliola* there has been, hitherto, a dearth of information on the antimicrobial efficacy of the plants investigated here. What is well known however is that the wood is resistant to insect attack and that the strong and characteristic odor of the parts is a useful taxonomic criterion[15].

From the present study, it was found that among all the Meliaceous plants tested, *Khaya grandifoliola* and *Pseudocedrela kotschyi* have essential oils that showed the greatest inhibition of mycelial growth and aflatoxin production by *A. parasiticus* at a very low concentration. This indicates that the oils of these plants could be further exploited in the protection of stored grains from the infestation of *A. parasiticus* and aflatoxin contamination. However, bioassay-directed fractionation of the biologically active principles inherent in the oils as well as their mechanism of action calls for further investigations.

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