Research Article

INVESTIGATIONS ON BIOACTIVITY OF ESSENTIAL OIL OF *AGERATUM CONYZOIDES* L., FROM BENIN AGAINST THE GROWTH OF FUNGI AND AFLATOXIN PRODUCTION

Euloge S. ADJOU*, Edwige DAHOUENON-AHOUSSI, René DEGNON, Mohamed M. SOUMANOU, Dominique C.K. SOHOUNHLOUE
Laboratoire d'Etude et de Recherche en Chimie Appliquée. Ecole Polytechnique d'Abomey-Calavi, Université d'Abomey-Calavi, Bénin, 01 BP : 2009 Cotonou, Republic of Benin.
***Corresponding author's E-mail:** ablawad2000@yahoo.fr

Accepted on: 26-12-2011; **Finalized on:** 30-02-2012.

ABSTRACT

Aflatoxins are highly toxic and carcinogenic metabolites produced by *Aspergillus parasiticus* on food and agricultural commodities. Natural products may control the production of aflatoxins. The aims of this study were to evaluate the Inhibition of *Aspergillus flavus (La3228)* and *A. parasiticus* (*Ab2242*) growth and their aflatoxin production exposed to the essential oil extracted from fresh leaves of *Ageratum conyzoides* from Bénin. Minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) of the oil were determined. The disc diffusion method was used to evaluate the zone of fungal growth inhibition compared to Nystatine fungicide. Essential oil was found to be strongly fungicidal and inhibitory to aflatoxin production. The oils analyzed by GC and GC/MS lead to identification of 7 components. The major components of *A.conyzoides* essential oil analyzed were precocene II (45.35%), precocene I (41.78%), cumarine (6.01%), and trans-caryophyllene (4.01%). Substitution of currently used antifungal and aflatoxin inhibiting chemicals by natural compounds such as essential oil of *Ageratum conyzoides* is recommended.

Keywords: Bioactivity, Ageratum Conyzoides, Essential Oil, Aflatoxin, Antifungal.

INTRODUCTION

Fungal deterioration of stored seeds and grains is a chronic problem in Benin storage system because of the tropical hot and humid climate. Harvested grains are colonized by various species of Aspergillus, under such conditions leading to deterioration and mycotoxin production. Aspergilli are the most common fungal species that can produce mycotoxins in food and feedstuffs. Mycotoxins are well known for their health–hazardous effects in human beings and animals.[1, 2] Among all the mycotoxins, particularly aflatoxin B1 (AFB1) is the most toxic form for mammals and presents hepatotoxic, teratogenic and mutagenic properties.[3] It has been classified as a class 1 human carcinogen by the International Agency for Research on Cancer.[4] The presence and growth of fungi may cause spoilage of food and its quality and quantity.[5] Chemical control remains the main measure to reduce the incidence of post harvest diseases in various foods. Antimicrobial chemicals belonging to the groups of benzimidazoles, aromatic hydrocarbons and sterol biosynthesis inhibitors are often used, as post harvest treatments. The application of higher concentrations of these synthetic chemicals in an attempt to control postharvest deterioration of food commodities increases the risk of toxic residues in the products. Due to the increasing public awareness of the pollutive, residual, carcinogenic and phytotoxic effects of many synthetic fungicides, the importance of alternative indigenous products to control phytopathogenic fungi is urgently needed.[6] Then, restriction imposed by the food industry and regulatory agencies on the use of some synthetic food additives have led to renewed interest in searching for alternatives, as natural antimicrobial compounds, particularly those from plants.[7] Essentials oils as well as compounds derived from them possess the wide range of activities of which the antimicrobial activity is most studied.[8,9] The use of essential oils, as antimicrobial agents present two main characters: the first is their natural origin which means more safety to the people and the environment and the second is that they have been considered at low risk for resistance development by pathogenic microorganisms.[10] *Ageratum conyzoides* L., Asteraceae, is an annual herbaceous plant with a long history of traditional medicinal uses in several countries of the world and also has bioactivity with insecticidal and nematocidal acitivity. This tropical species appears to be a valuable agricultural resource.[11] Its chemical composition is related to volatile compounds, including mono and sesquiterpenes, and non-volatile ones such as gallic, coumalic, protocatechuic, benzoic, sinapic, p-hydroxybenzoic and coumaric acid, and flavonoids such as kaempferol, quercetin and their glucosides.[12, 13] Ethnobotanic studies and preliminary surveys revealed that *Ageratum conyzoides* L., leaves are also used to preserve food items. For this, fresh leaves are introduced into grain bans to preserve stored cowpea and maize from insect and fungi damage.[14] The aim of this study was to test the effects of essential oil extracted from leaves of *Ageratum conyzoides* on the mycelial growth and Aflatoxin production by *Aspergillus parasiticus* (*Ab2242*) and *Aspergillus flavus (La3228).*

MATERIALS AND METHODS

Collection of plant leaves

Plant materials used for essential oil extraction were fresh leaves from *Ageratum conyzoides*. Plants were collected at *Bantè* (Center of Benin) and identified at the Benin

national herbarium, where voucher specimens are deposited.

Essential oil extraction

Essential oil tested was extracted by the hydro-distillation method using Clevenger-type apparatus. Oil recovered was dried over anhydrous sodium sulphate and stored at 4°C until it was used.[15]

Gas chromatography-mass spectrometry analysis

The EO was analyzed by gas chromatography (PerkinElmer Auto XL GC, Waltham, MA, USA) equipped with a flame ionisation detector, and the GC conditions were EQUITY-5 column (60 m x 0.32 mm x 0.25 µm); H_2 was the carrier gas; column head pressure 10 psi; oven temperature programme isotherm 2 min at 70°C, 3°C/ min gradient 250°C, isotherm 10 min; injection temperature, 250°C; detector temperature 280 °C. Gas chromatography-mass spectrometry (GC-MS) analysis was performed using PerkinElmer Turbomass GC-MS. The GC column was EQUITY-5 (60 m x 0.32 mm x 0.25 µm); fused silica capillary column. The GC conditions were injection temperature, 250°C; column temperature, isothermal at 70°C for 2 min, then programmed to 250°C at 37°C /min and held at this temperature for 10 min; ion source temperature, 250°C. Helium was the carrier gas. The effluent of the GC column was introduced directly into the source of MS and spectra obtained in the EI mode with 70 eV ionisation energy. The sector mass analyzer was set to scan from 40 to 500 amu for 2 s. The identification of individual compounds is based on their retention times relative to those of authentic samples.[16]

Preparation of media

Three different media were used in this study: Potato Dextrose Agar (PDA) for isolation of toxigenic fungi, Yeast Extract Sucrose Agar (YES) for testing antifungal potential of essential oil and the conventional Desiccated Coconut Agar medium (DCA) for the detection and visualization of aflatoxin production. PDA and YES was prepared as described by N'Guyen.[17] DCA was prepared by modification of the method of Davis *et al*.[18] as reported by Atanda *et al*.[19] as follows: two hundred grams of desiccated coconut were soaked in 1L of hot distillated water for 30min and filtered through four layers of cheese clothes. Two percent of bacteriological agar was added to the filtrate and heated to boiling. The media was then sterilized at 121°C for 15 min.

Fungal isolation

Strains of aflatoxigenic fungi: *Aspergillus parasiticus* (*Ab2242*) and *Aspergillus flavus (La3228)* were used for this study. They were obtained from the Plant Pathology Department of International Institute of Tropical Agriculture (IITA), (Benin). They were isolated and maintained on Potato Dextrose Agar (Oxoid Basingstoke) and renewed bimonthly.

Antifungal assay (Direct method)

Antifungal assay was performed by the agar medium assay.[15] Yeast Extract Sucrose (YES) medium with different concentrations of essential oil (1.5, 2.0 or 2.5 µL/ml) were prepared by adding appropriate quantity of essential oil to melted medium, followed by manual rotation of Erlenmeyer to disperse the oil in the medium. About 20 ml of the medium were poured into glass Petri-dishes (9 cm). Each Petri-dish was inoculated at the center with a mycelial disc (6 mm diameter) taken at the periphery of *A. parasiticus* (*Ab2242*) and *Aspergillus flavus (La3228)* colonies grown on PDA for 48 h. Control plates (without essential oil) were inoculated following the same procedure. Plates were incubated at 25°C for 8 days and the colony diameter was recorded each day. Minimal Inhibitory Concentration (MIC) was defined as the lowest concentration of essential oil in which no growth occurred. The inhibited fungal discs of the oil treated sets were re-inoculated into the fresh medium, and revival of their growth was observed. Minimal Fungicide Concentration (MFC) is the lowest concentration at which no growth occurred on the plates. Diameter of fungal colonies of treatment and control sets was measured, and percentage inhibition of fungal growth was calculated according to following formula.[20, 21]

$$PI = 1 - \frac{Dt}{Dc} \times 100$$

Dt: The diameter of growth zone in the test plate;

Dc: The diameter of growth zone in the control plate.

Antifungal assay (disk diffusion method)

Filter paper disks (6mm diameter) containing 5.0 µL of the essential oil of *A. conyzoides* was applied on the surface of Yeast Extract Sucrose (YES) medium plates previously inoculated with *A. parasiticus* (*Ab2242*) or *Aspergillus flavus (La3228)*. The inoculated plates were incubated at 25°C for 5 days. At the end of the period, antifungal activity was evaluated by measuring the zone of inhibition (mm) against tested fungi.[22] The fungicide Nystatine disc (Bio Merieux) was used as a positive control. All treatments consisted of three replicates, and the averages of the experimental results were determined.

Antiaflatoxin assay

Antiaflatoxin assay was performed using DCA medium according to the method described by Atanda and *et al*.[11] as followed: DCA medium with different concentrations of essential oil (1.0, 1.5, 2.0, 2.5 or 3µl/ml) were prepared by adding appropriate quantity of essential oil to melted medium, followed by manual rotation to disperse the oil in the medium. About 20 ml of the medium were poured into glass Petri-dishes. Care was taken to avoid trapping air bubbles in the media. Each Petri-dish was inoculated with single spores of *Aspergillus parasiticus* (*Ab2242)* or *Aspergillus flavus (La3228)* and incubated at 30°C for 48 hours. Control plates (without essential oil) were

inoculated following the same procedure. Thereafter, the plates were examined with some media characteristics. The reverse side of each plate, which consists of a single large colony, was observed under the long wave (365mn) UV light for blue / blue green fluorescence.[17, 23]

Statistical analysis

Experiments were performed in triplicate, and data analysed are mean ± SE subjected to one-way anova. Means are separated by the Tukey's multiple range test when anova was significant ($P < 0.05$) (SPSS 10.0; Chicago, IL, USA).

RESULTS

The yield of oils of *A. conyzoides,* was 0.5%, (v / w), during hydrodistillation and oils was yellow in colour. Chemical analysis by GC/MS of the components of the oil led to identification of 7 components (Table 1).

Table 1: Major components identified as constituents of essential oil of *A. conyzoides* drom Benin.

Constituents	RT	[%]
Bornyl acetate	19.494	0.20
Andro encecalinol	24.606	0.20
Trans-caryophyllene	25.458	4.01
Cumarine	25.621	6.01
α-humulene	26.892	0.18
Dimethoxi ageratocromene (Precocene I)	27.096	41.78
Ageratocromene (Precocene II)	35.056	45.35

The major components of the *A. conyzoides* oil were precocene II (45.35%), precocene I (41.78%), cumarine (6.01%), and trans-caryophyllene (4.01%). Essential oil of *A. conyzoides* exhibited pronounced antifungal activity against the growth of *Aspergillus flavus (La3228)* and *A. parasiticus* (*Ab2242*). MIC of essential oil of *A. conyzoides,* was found to be 2.0 µl/ml and 2.5 µl/ml respectively against *Aspergillus flavus (La3228)* and *Aspergillus parasiticus* (*Ab2242*). The MFC was recorded to be 2.5µl/ml and 3.0 µl/ml respectively against *Aspergillus flavus (La3228)* and *Aspergillus parasiticus* (*Ab2242*) (Table 2; Table 3). Essential oil of *A. conyzoides* exhibited important antifungal activity against the toxigenic strain *Aspergillus parasiticus* (*Ab2242*) at 2.5µl/ml and 3.0µl/ml. At concentration lower than these such as 1.5µl/ml and 2.0 µl/ml antifungal activity is less important with Percentage Inhibition of 12% and 83.44% respectively (Table 4). For *A. flavus (La3228)*, antifungal activity was very pronounced with 62% of inhibition at 1.5µl/ml. The influence of the essential oil on the inhibitory zone against toxigenic strains was measured at 1.8 mm and 3.0 mm (average n=3), and the fungicide was measure at 3.2 mm and 4.0mm respectively for *A. flavus (La3228)* and *A. parasiticus* (*Ab2242*). The results obtained by the disk diffusion method showed 56% and 75% of inhibition of the fungal growth for the essential oil respectively for *A. flavus (La3228)* and *A. parasiticus* (*Ab2242*) when compared with control.

Table 2: *Aspergillus parasiticus* colony diameters recorded

Days	*Aspergillus parasiticus* (Colony diameter in mm)			
	1.5µl/ml	2.0µl/ml	2.5µl/ml	3.0µl/ml
1	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
2	8.2 ± 0.05^{b}	6.2 ± 0.07^{b}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
3	10.7 ± 0.01^{c}	8.2 ± 0.04^{c}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
4	20.3 ± 0.04^{d}	12.0 ± 0.02^{d}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
5	25.7 ± 0.06^{e}	12.7 ± 0.03^{e}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
6	40.6 ± 0.03^{f}	14.6 ± 0.05^{f}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
7	65.4 ± 0.02^{g}	14.7 ± 0.02^{f}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
8	70.2 ± 0.03^{h}	14.9 ± 0.03^{f}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}

Values are mean (n = 3) ± SE. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests.

Table 3: *Aspergillus flavus* colony diameters recorded

Days	*Aspergillus flavus* (Colony diameter in mm)			
	1.5µl/ml	2.0µl/ml	2.5µl/ml	3.0µl/ml
1	4.2 ± 0.01^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
2	6.2 ± 0.08^{b}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
3	9.7 ± 0.03^{c}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
4	13.5 ± 0.07^{d}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
5	20.7 ± 0.01^{e}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
6	25.8 ± 0.03^{f}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
7	31.4 ± 0.06^{g}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
8	34.2 ± 0.07^{h}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}

Values are mean (n = 3) ± SE. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests.

Table 4: Percentage of mycelial growth inhibition (PI)

Concentration of EOs	Inhibition (%)	
	A. flavus	*A. parasiticus*
1.5µl/ml	62 ± 0.9	12 ± 1.1
2.0 µl/ml	100 ± 0.00	83.44 ± 0.3
2.5 µl/ml	100 ± 0.00	100 ± 0.00
3.0 µl/ml	100 ± 0.00	100 ± 0.00

The results of antiaflatoxinogenic assay (Table 5) show that EO of *A. conyzoides* has important aflatoxin inhibition potential on toxigenic strains *Aspergillus parasiticus* (*Ab2242*) at 2.0µl/ml and *A. flavus (La3228)* at 1.5µl/ml. However, at the lower concentration (1.5µl/ml), fluorescence was detected under UV (365nm) with *A. parasiticus* (*Ab2242*) indicated aflatoxin production. This aflatoxin production began at the third days of mycelia growth compared to control which began after five (5) days of mycelia growth. The fungal tested produced bluish white fluorescence which was stable up to eight (8) days of growth.

Table 5: Antiaflatoxinogenic assay

Days	Fluorescence intensity					
	A. flavus		*Aspergillus parasiticus*			Control
	1.5 µl/ml	2.0 µl/ml	1.5 µl/ml	2.0 µl/ml	2.5 µl/ml	
1	-	-	-	-	-	-
2	-	-	-	-	-	-
3	-	-	+	-	-	-
4	-	-	+++	-	-	-
5	-	-	+++	-	-	+
6	-	-	+++	-	-	+++
7	-	-	+++	-	-	+++
8	-	-	+++	-	-	+++

Bright fluorescence (+++); moderate fluorescence (++); weak fluorescence (+); No fluorescence (-)

DISCUSSION

The present study explores the efficacy of EO of *A. conyzoides* from Benin as the promising plant-based antimicrobials against fungal and aflatoxin contamination. EO of *A. conyzoides* was found to be effective against *A. flavus (La3228)* and *Aspergillus parasiticus* (*Ab2242)*. This may be because of the presence of some highly fungitoxic components in the oil. The antifungal activity was very pronounced on *A. flavus (La3228)* than *A. parasiticus* (*Ab2242*). A perusal of literature shows that the MIC of the oil was comparatively lower than the earlier reported antimicrobial oils such as *Lippia alba*[24], *Cymbopogon flexuosus*[21] and *Lantana indica*[25] tested against Aspergillus strains. The findings of the present investigation clearly showed that Aflatoxin production was significantly inhibited at concentrations lower than MIC of oil. Hence, the essential oil would be acting by two different modes of action as inhibitor of fungal growth and aflatoxin.[26] Based on such observation, it may be also concluded that the EO is more active as aflatoxin inhibitors than as fungal growth suppressors as emphasised by the earlier workers.[27-29] The GC–MS analysis of the oils was done for their proper standardisation and to know their chemical profile. The chemical profile of EO is reported to be influenced by the harvest period. Climatic, seasonal and geographical conditions and the amount and composition of active constituent can be significantly affected.[30] A number of chemotypes have been reported in the case of *Ageratum conyzoides* [31], because of variation in chemical components of it oil. However, in our study, GC–MS data, depicted remarkable variation with the earlier reports on the oils (Table 1). Ladeira *et al*.[32] in Brazil, reported three cumarinic compounds, including 1-2 benzopirone. Ekundayo *et al*.[33] identified 51 terpenoid compounds, including precocene I and precocene II. Gonzales et *al*.[34] found 11 cromenes in the essential oils, including a new cromene, 6-angeloyloky-7-methoxy-2, 2-dimethylcromen. Vera[35], in Reunion, found ageratocromene, other cromenes, and beta cariophylene in its essential oil. Mensah *et al*.[36] and Menut et *al*.[37] reported similar yields of precocene I in the essential oil of plants collected in Ghana. The species contains alkaloids, mainly the pirrolizidinic groups, which suggest that it may be a good candidate for pharmacological studies. Alkaloids also were found by Weindenfeld and Roder[38] in a hexane extract of *A. conyzoides* in Africa. The biologically active EO should be qualitatively standardized before their recommendation for practical exploitation as has been done in the present investigation. The antioxidant activity of the oils has been earlier reported[39], showing their promise in checking biodeterioration of food commodities because of lipid peroxidation. Prévious pharmacological studies showed that EO of *A. conyzoides* has important medicinal potential. In Cameroon and Congo, traditional use is to treat fever, rheumatism, headache, and colic.[37, 40] In Reunion, the whole plant is used as an antidysenteric.[35] The use of this species in traditional medicine is extensive in Brazil. Aqueous extracts of leaves or whole plants have been used to treat colic, colds and fevers, diarrhea, rheumatism, spasms, or as a tonic.[41] *A. conyzoides* has quick and effective action in burn wounds and is recommended by Brazilian Drugs Central as an antirheumatic.[11] Several pharmacological investigations have been conducted to determine efficacy. Almagboul *et al*.[42], using methanolic extract of the whole plant, verified inhibitory action in the development of *Staphylococus aureus*, *Bacillus subtilis*, *Eschericichia coli,* and *Pseudomonas aeruginosa*. Bioka *et al*.[40] reported effective analgesic action in rats using aqueous extract of *A. conyzoides* leaves (100 to 400 mg/kg). Assays realized in Kenya, with aqueous extract of the whole plant, demonstrated muscle relaxing activities, confirming its popular use as an antispasmotic.[43] Hence, the EO of *A. conyzoides* may be recommended as preservative of stored food commodities from fungal and aflatoxin contamination as well as lipid peroxidation.

CONCLUSION

This survey underlined the bioactivity of EO of fresh leaves of *Ageratum conyzoides* L., from Bénin as aflatoxin inhibitor and fungal growth suppressor. The major components of the EO were precocene II, precocene I, cumarine and trans-caryophyllene. Based on it antifungal and antioxidation potentials, the EO of *A. conyzoides* may be recommended as preservative of stored food commodities from fungal and aflatoxin contamination as well as lipid peroxidation.

Acknowledgement: The authors are grateful to the Department of Food Engineering of Polytechnic School of Abomey-Calavi University for their financial support. They would like to sincerely thank Professor Agbangla Clement (Laboratory of Genetic, Abomey-calavi University, Benin) for his technical assistance in the realization of this work.

REFERENCES

1. Probst, C., Njapau, H., & Cotty, P. J. Outbreak of an acute aflatoxicosis in Kenya in 2004: Identification of the causal agent. Applied and Environmental Microbiology, 73(8): 2007, 2762–2764.
2. Reddy, B. N., Raghavender, C. R. Outbreaks of aflatoxicoses in India. African Journal of Food, Agriculture, Nutrition and Development, 7(5): 2007, 1–15.
3. Santos C. C. M., Lopes, M. R. V., & Kosseki, S. Y. Ocorreˆncia de aflatoxinas em amendoime produtos de amendoim comercializados na regia~o de Sa~o Jose´ de Rio Preto/SP. Revista do Instituto Adolfo Lutz, 60(2): , 153–157.
4. IARC. Some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins. IARC monographs on the evaluation of carcinogenic risks to humans. 56 International Agency for Research on Cancer, 1993 pp. 489–521.
5. Candlish A. A. G., Pearson S. M., Aidoo, K. E., Smith, J. E., Kell, B., & Irvine, H. A survey of ethnic foods for microbial quality and aflatoxin content. Food Additives and Contaminants, 18: 2001, 129–136.
6. Bankole, S. A. Effect of essential oil from two Nigerian medicinal plants (Azadirachta indica and Morinda lucida) on growth and aflatoxin B1 production in maize grain by a toxigenic Aspergillus flavus. Letters in Applied Microbioliology, 24: 1997, 190–192.
7. Kumar G. D. S., Popat M. N. Knowledge and adoption of aflatoxin management practices in groundnut farming in Junagadh, Gujarat, India. SAT e- Journal, 3(1): 2007, 1–2.
8. Noudogbessi J.P., Kèkè M., Avlessi F., Dansou Kossou ; Sohounhloue C.K.D. Evaluation of the insecticidal, Larvidal and ovicidal effect on Callosobruchus maculatus of essential oils of Cymbopogon giganteus and of Xylopia aethiopica. Scientific study. Research. Vol 4: 2009, 182 – 186.
9. Yehouenou B., Noudogbessi J. P., Sessou P., Wotto V., Avlessi F., Sohounhloué C. K. D. Etude chimique et activités antimicrobiennes d'extraits volatils des feuilles et fruits de *Xylopia aethiopica* (Dunal) A. Rich. contre les pathogènes des denrées alimentaires. Journal de la Société Ouest-Africaine de Chimie, 2010, 029 : 19 - 27.
10. Tatsadjieu N., Jazet M., Ngassoum M.B., Etoa X., Mbofung C.M.F. Investigations on the essential oil of Lippia rugosa from Cameroon for its potential use as antifungal agent against Aspergillus flavus Link ex. Fries. *Food Control, 4(2),* 2008, *161–166*.
11. Lin Chau Ming. *Ageratum conyzoides*: A Tropical Source of Medicinal and Agricultural Products. Perspectives on new crops and new uses. J. Janick (ed.), ASHS Press, Alexandria, 1999, 473p.
12. Xuan, T.D., Shinkichi, T., Hong, N.H., Khanh, T.D., Min, C.I. Assessment of phytotoxic action of Ageratum conyzoides L. (billy goat weed) on weeds. Crop Protection 23, 2004, 915–922.
13. Kong, C., Hu, F., Xu, X., Liang, W., Zhang, C. Allelopathic plants. XV. Ageratum conyzoides L. Allelopathy Journal 14, 2004, 1–12.
14. Illiassa, N. (). Analyse de la gestion post-récolte de Vigna unguculata (WALP) et évaluation de l'importance insecticide des huiles essentielles de trois plantes aromatiques. Mémoire de maîtrise en Biologie Animale. Faculté des Sciences, Université de Ngaoundéré, 2004, 59p.
15. de Billerbeck, V. G., Roques, C. G., Bessière, J. M., Fonvieille, J. L., & Dargent, R. Effect of Cymbopogon nardus (L) W. Watson essential oil on the growth and morphogenesis of Aspergillus niger. Canadian Journal of Microbiology, 47, 2001, 9–17.
16. Adams R.P. Identification of Essential Oil Components by Gas Chromatography / Mass Spectrometry. Carol Stream, IL: Allured Publishing Corporation. 2007, 38p.
17. N'guyen M.T**.,** Identification des espèces de moisissures potentiellement productrices de mycotoxines dans le riz commercialisé dans cinq provinces de la région centrale du Vietman – Etude des conditions pouvant induire la production de mycotoxines. Institut National Polytechnique de Toulouse (INPT). Thèse de doctorat, 2007, p. 147.
18. Davis N. D., Iyer S. K., Diener U. L. Improved method of screening for aflatoxins with coconut agar medium. Applied and Environnemental Microbiology, 53: 1987, 1593-1595.
19. Attanda O.O., Developement of diagnostic medium for direct visual determination of aflatoxin and its control using traditional spices. PhD thesis. University of Agriculture, Department of microbiology, Abaokuta, Nigeria, 2005, 217p.
20. Kumar R., Mishra A.K., Dubey N.K., Tripathi Y.B. Evaluation of Chenopodium ambrosioides oil as a potential source of antifungal, antiaflatoxigenic and antioxidant activity. International Journal of Food Microbiology, 115, 2007, 159–164.
21. Kumar R., Dubey N.K., Tiwari O.P., Tripathi, Y.B. & Sinha, K.K. Evaluation of some essential oils as botanical fungi toxicants for the protection of stored food commodities from fungal infestation. Journal of the Science of Food and Agriculture, 87, 2007, 1737– 1742.
22. Yin M.C., Tsao S.M. Inhibitory effect of seven Allium plants upon three Aspergillus species. International Journal of Food Microbiology 49, 1999, 49–56.
23. Attanda O.O., Ogunrinu M. C., Olorunfemi F. M. A neutral red desiccated coconut agar for rapid detection of aflatoxigenic fungi and visual determination of aflatoxins. World Mycotoxin Journal 4 (2): 2011, 147-155.
24. Shukla, R., Kumar, A., Singh, P. & Dubey, N.K. Efficacy of Lippia alba (Mill.) N.E. Brown essential oil and its monoterpene aldehyde constituents against fungi isolated from some edible legume seeds and aflatoxin B1 production. International Journal of Food Microbiology, 135, 2009, 165–170.
25. Kumar, A., Shukla, R., Singh, P., Anuradha & Dubey, N.K. Efficacy of extract and essential oil of Lantana indica Roxb. Against food contaminating moulds and aflatoxin B1 production. International Journal of Food Science & Technology, 45, 2010, 179–185.

26. Rasooli I., Abyaneh, M.R. Inhibitory effects of Thyme oils on growth and aflatoxin production by A. parasiticus. Food Control, 15, 2004, 479–483.

27. Kumar A., Shukla, R., Singh, P. & Dubey, N.K. Biodeterioration of some herbal raw materials by storage fungi and aflatoxin and assessment of Cymbpogon flexuosus essential oil and its components as antifungal. International Biodeterioration & Biodegradation, 63, 2009, 712–716.

28. Prakash, B., Shukla, R., Singh, P., Kumar, A., Mishra, P.K. & Dubey, N.K. Efficacy of chemically characterized Piper betle L. essential oil against fungal and aflatoxin contamination of some edible commodities and its antioxidant activity. International Journal of Food Microbiology, 142, 2010, 114–119.

29. Prakash, B., Shukla, R., Singh, P., Mishra, P.K., Dubey, N.K., Kharwar, R.N. Efficacy of chemically characterized Ocimum gratissimum L. essential oil as an antioxidant and a safe plant based antimicrobial against fungal and aflatoxin B1 contamination of spices. Food Research International, doi: 10.1016/j. foodres2010 10.002.

30. Bakkali, F., Averbeck, S., Averbeck, D. & Idaomar, M. Biological effects of essential oils – a review. Food and Chemical Toxicology, 46, 2008, 446–475.

31. Sundufu, A.J., Shoushan, H. Chemical composition of the essential oils of Ageratum conyzoides L. occurring in south China. Flavour and Fragrance Journal, 19, 2004, 6–8.

32. Ladeira A.M, L.B.P. Zaidan, and R.C.L. Figueiredo-Ribeiro. *Ageratum conyzoides* L. (Compositae): Germinação, floração e ocorrência de derivados fenólicos em diferentes estádios de desenvolvimento. Hoehnea 15, 1987, 53–62

33. Ekundayo O., S. Sharma, and E.V. Rao. Essential oil of *Ageratum conyzoides*. Planta Med. 54, 1988, 55–57.

34. Gonzales A.G., Thomas G., and Ram P. Chromenes form *Ageratum conyzoides*. Phytochemistry 30, 1991, 1137–1139.

35. Vera, R. Chemical composition of the essential oil of *Ageratum conyzoides* L. (Asteraceae) from Reunion. Flavour Fragrance J. 8, 1993, 256–260.

36. Mensah, M., E.V. Rao, and S.P. Singh. The essential oil of *Ageratum conyzoides* L. from Ghana. J. Essent. Oil Res. 5(1), 1993, 113–115.

37. Menut, C., S. Sharma, and C. Luthra. Aromatic plants of tropical central Africa, Part X—Chemical composition of essential oils of *Ageratum houstonianum* Mill. and *Ageratum conyzoides* L. from Cameroon. Flavour Fragrance J. 8(1), 1993, 1–4.

38. Wiedenfeld, H. and E. Roder. Pyrrozidine alkaloids form *Ageratum conyzoides*. Planta Med. 57, 1991, 578–579.

39. Nantitanon, W., Chowwanapoonpohn, S., Okonogi, S. Antioxidant and antimicrobial activity of Hyptis suaveolens essential oil. Scientific Pharmaceuticals, 75, 2007, 35–46.

40. Bioka, D., Banyikwa, F.F. and Choudhuri M.A. Analgesic effects of a crude extract of *Ageratum conyzoides* in the rat. Acta Hort. 332, 1993, 171–176.

41. Oliveira, F., M.K. Akisue, and L.O. Garcia. Caracterização farmacognóstica da droga e do extrato fluído de mentrasto, *Ageratum conyzoides* L. Lecta 11(1), 1993, 63–100.

42. Almagboul, A.Z, Farroq, A.A. and Tyagi B.R. Antimicrobial activity of certain sudanese plants used in folkloric medicine: Screening for antibacterial activity, part II. Fitoterapia 56, 1985, 103–109.

43. Achola, K.J., Munenge, R.W. and Mwaura, A.M. Pharmacological properties of root and aerial parts extracts of *Ageratum conyzoides* on isolated ileum and heart. Fitoterapia 65, 1994, 322–325.

****