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Antifungal activities of the essential oil extracted from the tea of savanna (*Lippia multiflora*) in Côte d'Ivoire

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ABSTRACT

The objective of this study was to evaluate the antifungal potency of the essential oil of tea of savanna (*Lippia multiflora*) on three fungal strains. The essential oil is extracted of *Lippia multiflora* by steam distillation and the antifungal activity *in vitro* was investigated on *Apergillus flavus, Asperguillus Niger* and *Fusarium sp* species. This activity was realized by incorporation of the plant extract in Sabouraud medium prepared by a double dilution. The study revealed a sensitivity of these three species to the essential oil extracted from *Lippia multiflora*. It has been observed, in a descending order of sensitivity, a minimum fungicidal concentration (MFC) of 2.08 ± 0.58 µl / ml with *Aspergillus flavus*; 4.16 ± 1.17 µl / ml with *Aspergillus Niger* and 8.33 ± 2.35 µl / ml with *Fusarium sp*. The antifungal potency of the essential oil extracted from *Lippia multiflora*, allows considering its use as a novel approach in the field of integrated management of cereal stocks in post-harvest.

Keywords: Essential oil, *Lippia multiflora*, Antifungal, *Aspergillus*, *Fusarium.*

INTRODUCTION

Plants and their derivatives play an important role in food systems particularly in Africa. Many vegetables are used as sources of protein in the human diet (Khelil, 1977). Dry vegetables grains deteriorate rapidly if they were stored in adverse conditions. Then, several phenomena contribute to their degradation and the action of insects and microorganisms is the most important (Terrain et Graallet, 2003). Among these microorganisms, fungi are the most diverse group that can cause significant losses by reducing the quality and / or quantity of the stored dry vegetables (Terrain et Graallet, 2003). The species of the genus *Aspergillus* and *Fusarium* are particularly known for their high potential of alteration and production of various mycotoxins in food. Ochratoxin A, aflatoxin, fumonisin, trichothecene are the best known and frequently mycotoxins encountered. They can be produced before, during and after vegetables harvest and are

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highly resistant to conditions of food preparation (Terrain et Graallet, 2003).

Because of their easy application and practical, the use of chemicals products is currently the only way to fight against harmful fungi (Magan et Olsen, 2004). However, intensive and indiscriminate use of these products has caused contamination of the biosphere and the food chain, an eradication of certain species of auxiliary fauna and the appearance of resistant microorganisms. Therefore, WHO prohibited the use of certain chemical fungicides (Khelil, 1977). The search for new drugs has become indispensable. Biological control through the use of natural antifungal substances may be an alternative to chemicals products. Among these natural substances, they found essential oils which are extracted from aromatic plants (Maihebiau 1994). Scientific investigations have revealed that the essential oil from the leaves of *Lippia multiflora* has antimicrobial properties. The studies of Mevy et al. (2006) showed that the oil strongly inhibits the growth of *Staphylococcus aureus* and *Enterococcus hirae*, and has a moderate effect on *Candida albicans* and *Saccharomyces cerevisiae*. Oladimeji et al. (2004) have shown that fungi were more sensitive to oil of *Lippia multiflora* than bacteria. The antimicrobial activity of carvacrol and thymol, which are the majority component of essential oil of genre Lippia has been reported (Kunle et al., 2003; Botelho et al., 2007).

In Côte d'Ivoire, few data are available regarding the evaluation of antifungal potency of the essential oil while some previous studies have shown, at plants in the Ivorian pharmacopoeia promising virtues (Oussou et al., 2004; Seri-Kouassi et al., 2004). The objective of the study was to evaluate the antifungal activity of the essential oil extracted from *Lippia multiflora*, plant of pharmacopoeia of Côte d'Ivoire.

MATERIALS AND METHODS

Context of the study and sampling

The plant material was collected in July and September 2013, in the region of Yamoussoukro in central of Côte d'Ivoire. The fresh leaves of *Lippia multiflora* (Photo 1) were harvested early in the morning before seven o'clock. Transferred to the laboratory, samples were dried for ten days in the shade at room temperature (25 ± 2 °C). The botanical identification was performed in the laboratory of the Department of Botany Agriculture and Animal Resources (ARA) of the National Polytechnic Institute Felix Houphouet-Boigny of Yamoussoukro (INP-HB).

Extraction of essential oil

The extraction of essential oil was produced by hydro-distillation in a Clevenger apparatus (Simard et al., 1988) at Laboratory of Industrial Processes, Synthesis, Environment and New Energy (LAPISEN) of the National Polytechnic Institute Felix Houphouet-Boigny (INP-HB) of Yamoussoukro (Côte d'Ivoire). Indeed, 300 g of dried leaves are introduced into a pressure cooker containing distilled water. The mixture is boiled using a heating mantle. The steam of water loaded with essential oil is condensed in the coil of the Clevenger, using a water flow. Two hours after the appearance of the first drop of the distillate, the essential oil is separated from the water and dried with magnesium sulfate (Merck, Germany). The oil was then stored at 4 °C, protected from light in a vial (opaque) sealed. Oil yield of the dried leaves of *Lippia multiflora* was determined by the ratio of the mass of the extracted oil and the mass of the treated plant (Caree, 1953) using the formula: R (%) = m / M x 100 ; R =

yield (%); M = mass of plant (g) and m = mass of the oil after 2 hours of distillation (g).

Antifungal activity of the essential oil

Fungal strains tested

Fungal strains studied are constituted of *Aspergillus Niger* (*Aspergillus Niger* n°: 13-096: isolated from cola sample), *Aspergillus flavus* (*Aspergillus flavus* n°: 13-147 isolated from wheat bran) and *Fusarium sp* (*Fusarium sp*. n°: 13-187 isolated from cola sample). The three fungal species were obtained from the laboratory of Mycology and Parasitology of the Pasteur Institute of Côte d'Ivoire (IPCI).

Determination of antifungal activity in a solid medium

Antifungal tests were performed on culture medium Sabouraud chloramphenicol (Bio-Rad) (France). The Incorporation of the plant extract on the agar was carried out according to the double dilution method (Kra, 2001).

The prepared agar is dispensed into test tubes numbered from 2 to 7 with 15 ml per tube. Two other tubes are also prepared. One as growth control tube contain 15 ml of Sabouraud agar and the other, number 1 as experimental tube contain 27 ml of the same agar. After sterilization, 0.30 ml of the essential oil to be tested is added to 29.7 ml of warm agar Sabouraud contained in the tube number 1. From this solution for 10% concentration, a serial dilution in geometric progression of ratio 2 in order to obtain a final concentration range of (0.07%, 0.15%, 0.31%, 0.62%, 1.25%, 2.5%, 5% and 10%) is made. The essential oil (EO) extract was obtained by dissolving the oil in Tween 80 according to proportions 1/9 (Tween / EO), as described by Opalchenova et al. (2003).

The tubes are properly stirred to homogenize the mixture. The content of each tube was poured into Petri dishes of 90 mm of diameter previously marked. After solidification of the medium, explants aged of 7 days with 5mm of diameter were inoculated by central puncture, using sterile platinum Anse. These explants were placed in a previously wells made with a sterile pipette Pasteur at the center of the Petri dish containing increasing doses of essential oil. A control Petri dish without essential oil is also prepared. Both Petri dishes (control and test) were incubated for 10 days at 25 ± 2 °C. Mycelial growth was recorded every 24 hours, by measuring the average of two perpendicular diameters passing through the center of the Petri dish (Mishra et Dubey, 1994; Khallil, 2001). The inhibitory rate (I' %) is calculated according to Kordali et al. (2003) method; or I '(%) = 100 x (dC-dE) / dC

I '(%) = rate expressed as percentage inhibition

dC = Diameter of colonies on both "positive control"

dE = Diameter of colonies in the dishes containing the essential oil.

The effectiveness of the essential oil on these strains is evaluated by expressing the proportions of these have shown an inhibition rate of greater or equal to 50%. The tests were conducted in three replicates.

Determination of antifungal activity in a liquid medium

The technique consisted to determinate the Minimum Inhibitory Concentration (MIC) and the nature of the antifungal activity of the essential oil. The Sabouraud agar prepared and sterilized at 121 °C for 15 minutes and has been cooled to reach 45 °C by supercooling. The essential oil has been incorporated to the agar and was performed according to the method of double dilution inclined tubes (Kra, 2001; Zihiri et al., 2003). A serial of ten test tubes was prepared with eight test tubes containing different

concentrations of the essential oil and two control tubes. Among the two control tubes, one was used for the medium sterility; it contained neither essential oil nor strains; the other one without plant extract but with strains was used for the control of microorganisms growth. In the tube, 1.200 µl/ml of essential oil are aseptically added to 19.8 mL of Sabouraud liquid medium. According to a geometric ratio binding ½ reason, the concentration in the eight test tubes varies from 10 to 0.15 µl/mL. After incorporation of the oil, the tubes are inclined to permit cooling and solidification of the agar, at room temperature (Kra, 2001; Zihiri et al., 2003).

After 48 hours, one colony of each species is taken and homogenized in 10 ml of sterilized distilled water. Thus, the initial suspension (10^0) is obtained. From this suspension, the suspension 10^{-1} was prepared. Strains cultures were made on the media previously prepared with 13 µL of the suspension 10^{-1}. The cultures were incubated at 25 ± 2 °C for 72 hours. After incubation, the tubes in which no mold growth are identified as IMC.

If any "spores" is found in the tubes, new tubes are identified and performed. The agar surface of experimental tubes is scraped and seeded on new agar. After 48 hours of incubation, the subcultures in which there is no recovery of growth were identified as the minimum fungicidal concentrations (MFC) (Maury, 1987; Guédé-Guina et al., 1997). Concentrations of the extracts which contained growth of mold were identified as fungistatic concentrations (CFS).

Statistical analysis

The results were analyzed by the variance method (ANOVA) using the STATISTICA software version 6.0 (treatment by ANOVA 1 factor). Comparison of the means was performed by the Tukey's test at 5%.

RESULTS

Extraction of essential oil

In our study, the ratio determined from *Lippia multiflora* leaves is 1.3%. The color of essential oil extracted is yellow.

Determination of antifungal activity in a solid medium

The results of antifungal tests show a progressive decrease in the mycelial diameter as the concentration of the extract increased in the experimental tubes, compared to controls. Both strains of *Aspergillus* tested are 100% sensitive to *L. multiflora* essential oil in concentrations of 2.5% for *A. Niger* and 1.25% for *A. flavus* (Figures 1 and 2). The high sensitivity of the genre *Aspergillus* strains, producing aflatoxin is due to their activity against aflatoxin of the essential oil. However, at 0.62%, the *Aspergillus* strains develop resistance to the essential oil from 48 hours for *Aspergillus Niger* and from 72 hours for *Apsergillus flavus*. However, for all concentrations of essential oil used in the study, the *Fusarium sp* strain is resistant. But at a concentration of 2.5%, there was a total inhibition for 24 hours (Figure 3). *Fusarium sp* strain develops a stronger resistance to the essential oil compared to *Aspergillus* strains (Table 1).

Determination of antifungal activity in a liquid medium

The results of the liquid medium in antifungal tests confirm the ability of the oil to inhibit mycelial growth of the three strains (Table 1).

Photo 1: *Lippia multiflora.*

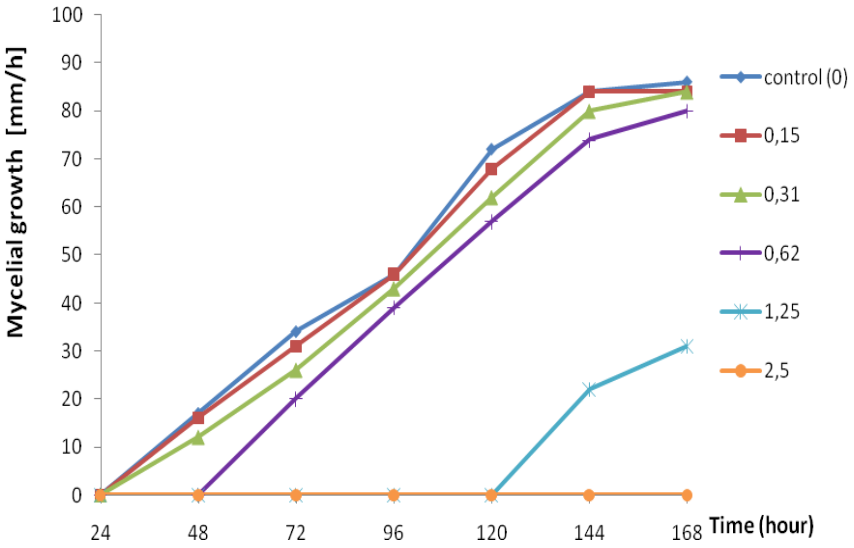


Figure 1: Evolution of the mycelial growth of *Aspergillus niger* according to time and concentrations of essential oil of *Lippia multiflora.*

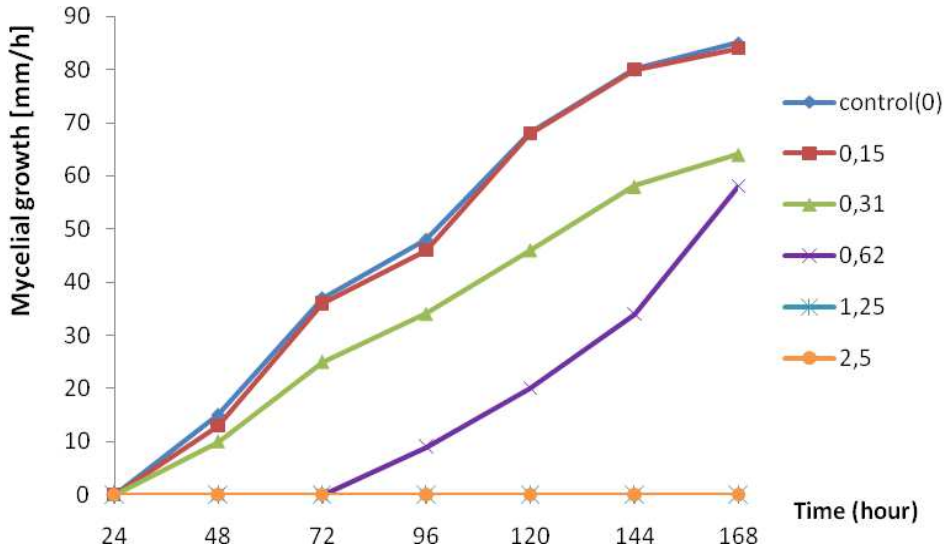


Figure 2: Evolution of the mycelial growth of *Aspergillus flavus* according to time and concentrations of essential oil of *Lippia multiflora*.

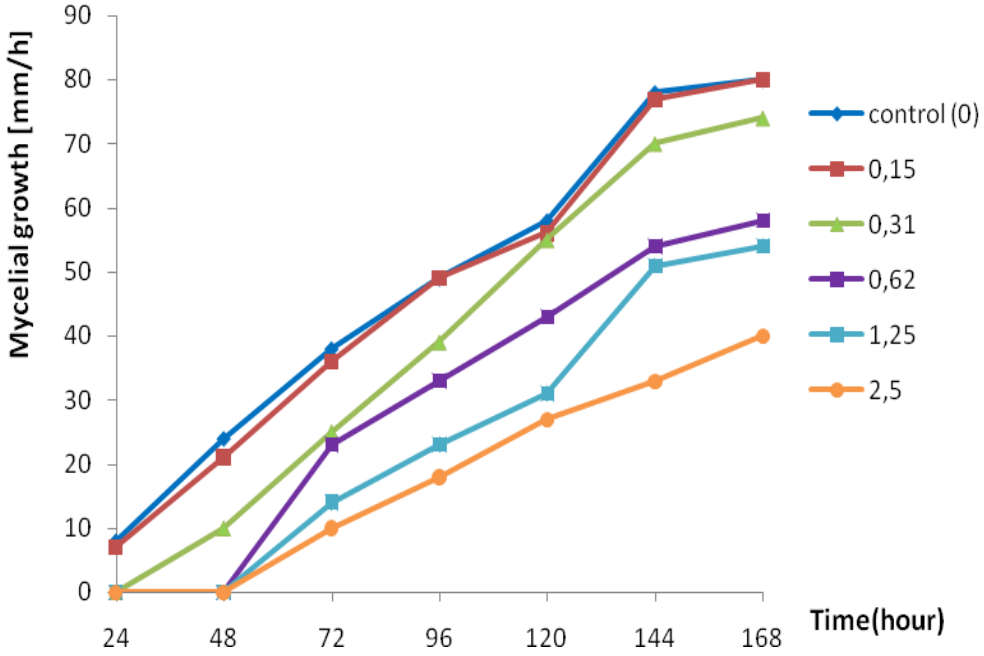
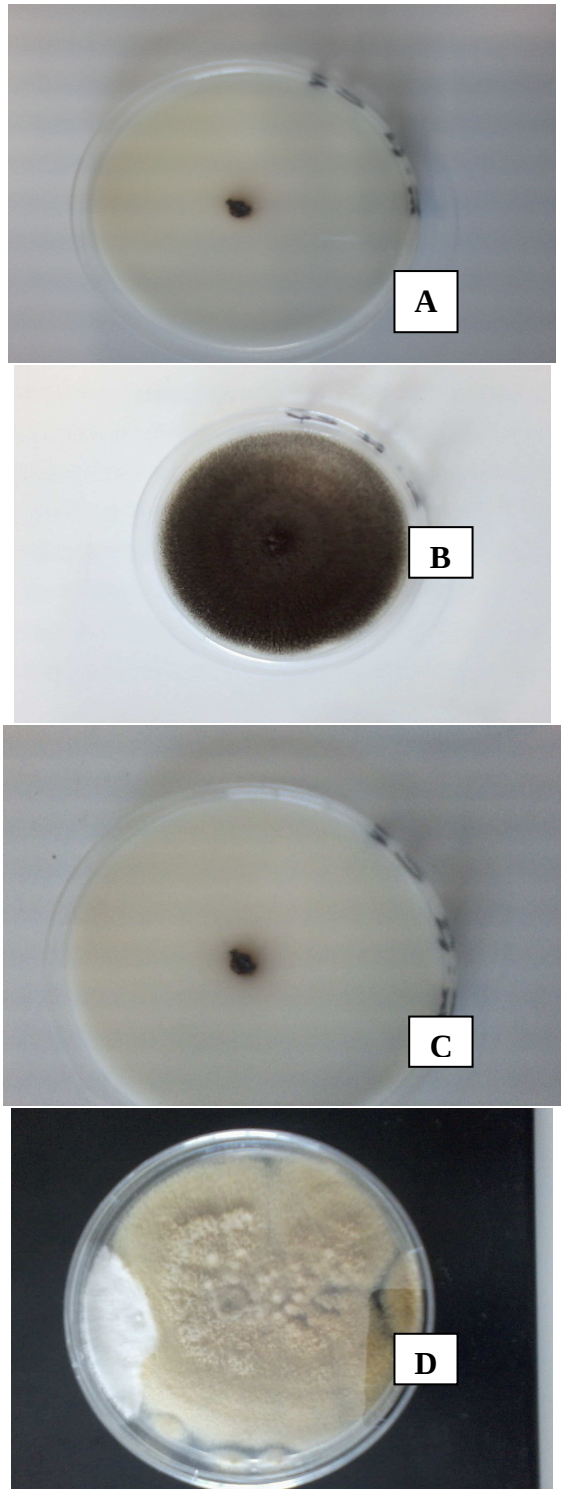


Figure 3: Evolution of the mycelial growth of *Fusarium sp* according to time and concentrations of essential oil of *Lippia multiflora*.


A
B
C
D

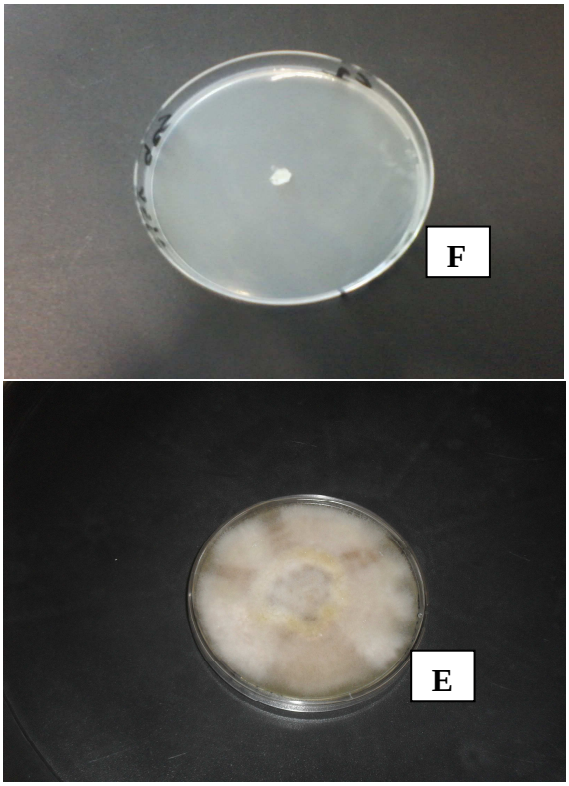


Figure 4: Mycelial growth aspect in minimal inhibitory concentration of essential oil of *Lippia multiflora* on *Aspergillus flavus*, *Aspergillus niger* and *Fusarium sp*. A: EO (2,5%) on *Apergillus niger* ; B: *Apergillus niger* (control); C: EO (1,25%) on *Apergillus flavus;* D:*Apergillus flavus* (control); E: EO (5%) on *Fusarium sp;* F: *Fusarium sp* (control).

Table 1: Minimal Inhibitory and Fongicidal Concentrations of essential oil of *Lippia multiflora.*

	Antifungal activities	
Fungal strains	**CIM (µl /ml)**	**CFM (µl /ml)**
Aspergillus flavus	1.66 ± 0.58	2.08 ± 0.58
Aspergillus niger	2.08 ± 0.58	4.16 ± 1.17
Fusarium sp	4.16 ±1.17	8.33 ± 2.35

MIC : Minimum Inhibitory Concentration; CFM: Minimum Fongicidal Concentration.

DISCUSSION

In our study, the ratio determined from *Lippia multiflora* leaves is 1.3%. This result although lower than that of Kunle (2000) which is 1.57%, is higher than that determined by Oussou et al. (2008), which is 1.2%. Both authors have worked on the same plant. The essential oil content can be influenced by several factors such as temperature, humidity,

soil composition, the growth cycle of the plant (Bruneton, 1999).

The results of antifungal tests show a progressive decrease in the mycelial diameter as the concentration of the extract increased in the experimental tubes, compared to controls. Both strains of Aspergillus tested are 100% sensitive to *L. multiflora* essential oil in concentrations of 2.5% for *A. Niger* and 1.25% for *A. flavus* (Figures 1 and 2). The high sensitivity of the Aspergillus strains, producing aflatoxin is due to their activity against aflatoxin of the essential oil. This activity against aflatoxin of essential oils has already been mentioned by Adjou et al. (2013). Indeed, the inhibition of the production of mycotoxins by the fungal strain in the presence of the essential oil, should promote this one to cross the plasma membrane. According to Conner and Beuchat (1984), the activity of essential oils would be in their ability to cross the cell wall, but also their ability to damage cellular enzyme system, including that relating to the production of energy. Billerbeck et al. (2001) also reported the thinning of A. Niger hyphae when it cultivate in the presence of the essential oil of *Cymbopogon nardus* (L.). The sensitivity of *Aspergillus* strains can also be explained by a synergistic effect between certain molecules contained in the essential oil (Didry et al., 1993).

However, at 0.62%, the *Aspergillus* strains develop resistance to the essential oil from 48 hours for *Aspergillus Niger* and from 72 hours for *Apsergillus flavus*. However, for all concentrations of essential oil used in the study, the *Fusarium sp* strain is resistant. But at a concentration of 2.5% there was a total inhibition for 24 hours (Figure 3). *Fusarium sp* strain develops a stronger resistance to the essential oil compared to *Aspergillus* strains (Table 1)

Resistance observed in *Aspergillus* strains, after 2 to 3 days may be explained by the fact that the molecules contained in the essential oil are volatile. The low content of essential oil in agar could promote evaporation of molecules. The synergism between bioactive molecules is thus inhibited. The resistance of *Fusarium sp* can be explained by the difference between the membrane structures. The antifungal effect of the essential oil of *Lippia multiflora* is justified by its chemical composition. Indeed, the essential oil of *Lippia multiflora* is very rich in monoterpenes such as carvacrol, linalool, terpinene and sabinene (Oussou et al., 2008). The high sensitivity of *Aspergillus flavus* has been demonstrated by the work of Nogueira et al. (2010). According to these authors, the essential oil induces morphological changes in the endomembrane system of *Aspergillus flavus*. The results of the liquid medium in antifungal tests confirm the ability of the oil to inhibit mycelial growth of the three strains (Table 1).

Conclusion

This survey underlined the bioactivity of essential oil of leaves of *Lippia multiflora* from Côte d'Ivoire as fungal growth suppressor. Based on it antifungal potential, the essential oil of *Lippia multiflora* may be recommended as preservative of stored food commodities from fungal and mycotoxin contamination.

REFERENCES

Adjou ES, Soumanou MM. 2013. Efficacité des extraits de plantes contre les moisissures toxinogènes isolées da l'arachide. *Journal of Applied Biosciences*, **70**: 5555–5566.

Billerbeck VG, Roques CG, Bessière JM, Fonvieille JL, Dargent R. 2001. Effect of *Cymbopogon nardus* (L) W. Watson essential oil on the growth and morphogenesis of Aspergillus niger. *Canadian Journal of Microbiology*, **47**: 9–17.

Botelho MA, Nogueira NAP, Bastos GM, Fonseca SGC, Lemos TLG, Matos FJA, Montenegro D, Heukelbach J, Rao VS, Brito GAC. Antimicrobial activity of the essential oil from *Lippia sidoides*, carvacrol and thymol against oral pathogens. *Braz. J. Med. Biol. Res*., **40**(3): 349-356.

Bruneton J. 1999. *Pharmacognosie, Phytochimie, Plantes Médicinales* (3ème). Edition Technique et Documentation, Edition Lavoisier: Paris; 1120.

Caree P. 1953. *Précis de Technologie et de Chimie Industrielle*. Ed. Ballière JB et fils; 238p.

Conner DE, Beuchat LM. 1984. Effect of essential oils from plants on growth of food spoilage yeasts. *J. Food. Sci.,* **49**: 429-434.

Didry N, Dubreuil L, Pinkas M. 1993. Antibacterial activity of thymol, carvacrol and cinnamaldehyde alone or in combination. *Pharmazie*, **48**(4): 301-304.

Guede-Guina F, Kra AKM, Vangah-Manda M, Bonga GM, De Souza C. 1997. Inhibition par MISCA-F_2 de la croissance de *Aspergillus fumigatus*; *Candida albicans* et *Cryptococcus neoformans*, 3 germes fongiques opportunistes du SIDA. *J. Afr. Biomed.*, **2**: 11 -16.

Khallil ARM. 2001. Phytofungitoxic properties in the aqueous extracts of some plants. *Pakistan Journal of Biological Science*, **4**(4): 392–394.

Khelil MA. 1977. Influence de la chaleur utilisée comme moyen de lutte contre le bruche du haricot Acanthoscelides obtectus Say (Coleopterae: Bruchidae) sur les différents états et stades de développement. Mémoire d'Ingénieur en Agronomie, INA El Harrach, 77p.

Kolai Naouel, Saiah Farida, Boudia Abdelkader, 2012. Effet inhibiteur in vitro de l'huile essentielle d'*Artimesia herba alba* sur deux souches de *Fusarium oxysporum* f. sp. radicis-lycopersici, *Algerian Journal of Arid Environment*, **2**(1): 71-76.

Kordali S, Cakir A, Zengin H, Duru ME. 2003. Antifungical activities of the leaves of three Pistacia species grown in Turkey. *Fitoterapia*, **74**: 164-167.

Kra AKM. 2001. Evaluation et amélioration par séquençage chromatographique d'une action fongique de MISCA contre *Aspergillus fimugatus*, Thèse, Pharma., Bioch. Univ. Abidjan, 126 p.

Kunle O, Okogun J, Egamana E, Emojevwe E, Shok M. 2003. Antimicrobial activity of various extracts and carvacrol from *Lippia multiflora* leaf extract. *J. Phytomedicine*, **10**: 59 - 61.

Kunle OF. 2000. Phytochemical and microbiological studies of the leaf of *Lippia mutiflora* Mold., Fam Verbenaceae. Unpublished Ph.D dissertation of the Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Magan N, Olsen M. 2004. Mycotoxines in food: Detection and control. Woodhead Publishing in *Food Science and Technology*, **2004**: 190-203.

Maihebiau P. 1994. La nouvelle aromathérapie: biochimie aromatique et influence psychosensorielle des odeurs. Lausanne, 635p.

Mevy JP, Bessiere JM, Dherbomez M, Millogo J, Viano J. 2007. Chemical composition and some biological activities of the volatile oils of a chemotype of *Lippia chevalieri* Moldenke. *Food Chem.*, **101**(2): 682-685.

Mishra AK, Dubey NK. 1994. Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities. *Applied and Environmental Microbiology*, **60**(4): 1101-1105.

Nogueira JHC, Gonçalez E, Galleti SR, Facanali R, Marques MOM, Felício JD, 2010. Ageratum conyzoides essential oil as aflatoxin suppressor of Aspergillus

flavus. *International Journal of Food Microbiology*, **137**: 55–60.

Oladimeji FA, Orafidiya LO, Okeke IN. 2004. Physical properties and antimicrobial activities of leaf essential oil of *Lippia multiflora* Moldenke. *Int. J. Aromatherapy*, **14**(4): 162-168.

Opalchenova G, Obreshkova D. 2003. Comparative studies on the activity of basil - an essential oil from *Ocimum basilicum L.* - against multidrug resistant clinical isolates of the genera Staphylococcus, Enterococcus and Pseudomonas by using different test methods. *Journal of Microbiological Methods*, **54**: 105– 110.

Oussou KR, Kanko C, Guessend N, Yolou S, Koukoua G, Dosso M, N'guessan YT, Figueredo G, CHALCHAT J-C. 2004. Activités antibactériennes des huiles essentielles de trois plantes de Côte d'Ivoire. *C.R. Chimie*, **7** : 1081-1086.

Oussou KR, Yolou S, Boti JB, Guessennd KN, Kanko C, Ahibo C, Casanovad J. 2008. Etude chimique et activité antidiarrhéique des huiles essentielles de deux plantes aromatiques de la pharmacopée ivoirienne. *European Journal of Scientific Research*, **24**(1): 94-103.

Seri-kouassi BP, Kanko C, Aboua LRN, Bekon KA, Glitho AI, Koukoua G, N'guessan YT. 2004. Action des huiles essentielles de deux plantes aromatiques de Côte d'Ivoire sur *Callosobruchus maculatures* F. du niébé. *C.R. Chimie*, **7** : 1043-1046.

Simard S, Hachey JM, Colin GJ. 1988. The variation of the essential oil composition with the extraction process, the case of *Thuya occidentalis* L and *Abies balsamea* L J. Mill. *Wood Techn.*, **8**: 561-573.

Terrain C, Graallet H. 2003. *Séchage des Grains en Organisme Stockeur: Guide Pratique*. Ed ARVALIS, Institut du végétal et FFCA ; 1-5.

Zihiri GN, Kra AM, Guédé-Guina F. 2003. Evaluation de l'activité antifongique de *Microglossa pyrifolia* (LAMARCK) O. KUNZE (Asteraceae) « PYMI» sur la croissance in vitro de *Candida albicans*. *Revue de Méd. et Pharma. Afr.*, **17**: 11-18.