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Bioactivity of essential oil from fresh leaves of *Lantana camara* against fungi isolated from stored cowpea in southern Benin

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Abstract

Cowpea is one of the most important foods in the diet of the population, especially in rural areas. Unfortunately, it is attacks by insects and a large number of microorganisms, especially fungi. In order to promote this legume, the present work aimed to develop an inexpensive and environmentally friendly method to reduce fungi contamination, by using essential oil of *Lantana camara* in the storage of cowpea. Microbiological analyzes were performed on cowpea seeds using the "Direct plating" method for the isolation of fungi. Antifungal tests were performed by the method of diffusion in a solid medium to determine the Minimal Inhibitory Concentration of essential oil. Results obtained indicated that *Aspergillus* and *Penicillium* were the most genera of fungi isolated from cowpea in post-harvest in southern Benin. The main species were, *Aspergillus flavus, Aspergillus tamarii, Aspergillus ustus* and *Penicillium roqueforti*. Antifungal tests showed that at a concentration of 20µL/mL, the essential oil was fongiostatique on four strains studied. From these results, the essential oil of *Lantana camara* could be used as preservation agent to protect cowpea in post-harvest in Benin

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Introduction

Cowpea (*Vigna unguiculata* (L.) Walp) is one of the most human foods and has probably been used as a crop plant since Neolithic times (Summerfield *et al.*, 1985). The world cowpea production was estimated at 3.319375 MT and 75% of that production is from Africa (FAOSTAT, 2011). The high protein content of cowpea and its use as a staple in the diets of sahelian and coastal populations make it also a crop with high potential for food security in these regions (Coulibaly and Lowenberg-DeBoer, 2002). Cowpea is particularly susceptible to infestation by several insects with devastating effects on plants in the field and seeds in storage. (IITA, 2012). However, the contamination of cowpea by fungi has also reported by Houssou *et al.*, 2011). Fungal contamination of food products is a chronic problem in developing countries and results in a decline in quality, quantity, nutrient content and monetary value (Sessou *et al.*, 2013). *Aspergillus* species are known to produce a broad spectrum of mycotoxins including aflatoxins, sterigmatocystin and ochratoxins, which are causative agents of several carcinogenic, hepatogenic, nephrogenic, and immunosuppressive effects (Dragan *et al.*, 2010; Gautam and Bhadauria, 2010b). Application of fungicide during the storage of cowpea in other to reduce the contamination by toxinogenic fungi, can reduce the rate of decay (Thayer, 1984). However, fungicides have various setbacks such as development of new resistance strains in the treated fungi, environmental toxic residues and eventual toxicity to consumers. Therefore, biodegradables alternatives should be developed for reducing postharvest losses. Plants and their essential oils have been evaluated as natural sources of compounds to reduce the attacks of fungi, insects and other storage pests (Tapondjou *et al.*, 2003). Medicinal plants, which form the backbone of traditional medicine, in the last few decades, have been the subject for very intense pharmacological studies. This has been brought about by the acknowledgement of the value of medicinal plants as potential sources of new compounds of therapeutics value and as sources of important compounds in the drug development. *Lantana camara* is an erect or subs cadent aromatic branching shrub with prickles. It belongs to the Verbenaceae family. It is a native of America and Africa and has been cultivated as an ornamental plant in other countries (Jawonisi and Adoga, 2013). In Benin, *Lantana camara* is found throughout all regions. Different parts of the plant, mainly the leaves, have been used in the treatment of scratching, stomachache, rheumatism, wound healing, biliary fever, toothache, bronquitis, antiseptic and other affections (Deena and Thoppil, 2000). This plant has been claimed to present activities antiprotozoal (Clarkson *et al.,* 2004), antibacterial (Mary, 2011), antifungal, antioxidant (Benitez *et al.,* 2009), insecticidal (Dua *et al.,* 2010), antiviral (Garcia *et al.,* 2002), allelopathic properties (Verdeguer *et al.*, 2009).

The essential oil of *Lantana camara* showed a wide spectrum of antibacterial, antimicrobial and antifungal activity (Kumar *et al.,* 2006). It has been used as natural insecticides before the discovery of synthetic organic insecticides (Kumar and Maneemegalai, 2008).

Ethnobotanic studies and preliminary surveys indicated that plant leaves are also used to preserve food. For this, fresh leaves are introduced into grain barns to preserve stored cowpea and maize from insect and fungal damage (Illiassa, 2004). The present work aims to evaluate the effects of EO extracted from fresh leaves of *L. camara* on the mycelial growth of strains of fungi isolated from cowpea at post-harvest in southern Benin.

Materials and methods

Collection of plant leaves

Plant materials used for EO extraction were fresh leaves from *Lantana camara* L. Plants were collected at Abomey-Calavi (south Benin) and identified at the Benin national herbarium, where voucher specimens are deposited.

Essential oil extraction

The EO used was extracted by the hydro distillation method using Clevenger-type apparatus. The oil

recovered was dried over anhydrous sodium sulfate and stored at 4 °C until it was used for assays.

Gas chromatography–mass spectrometry analysis

The EO were 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 a Perkin Elmer 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, retention indices relative to C5–C18 n-alkanes, and matching spectral peaks available in the published data (Adams, 2007).

Cowpea samples collection

Samples of Cowpea, (*Vigna unguiculata)* variety "Djombo" were obtained from different localities of Bonou (Southern Benin), which is one of the major areas of cowpea production in Benin.

Fungal isolation and identification

Samples were examined by the Direct Plating technique described by Pitt *et al.*, (1997). One hundred cowpea grains per sample were surface disinfected in 0.4 % active chlorine solution for one minute at room temperature. Then, they were placed directly on Dichloran 18% Glycerol Agar (DG18) and incubated at 25 ± 2°C, for 7 days and examined visually as well as under a compound light microscope daily for preliminary identification of fungal genera.

Pure cultures of fungi were examined macroscopically and microscopically, and their identification was carried out by using a taxonomic schemes primarily based on morphological characters using the methods given by Singh *et al.* (1991), Filtenborg *et al.* (1995), and Tabuc (2007).

Antifungal assay

Antifungal assay was performed by the agar medium assay (de Billerbeck *et al.* 2001). Yeast Extract Sucrose (YES) medium with different concentrations of EO were prepared by adding appropriate quantity of EO and Tween 20 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). The molds grown on YES for 48 h are transplanted (subcultured), using a disc of 6 mm in diameter which carries spores from the anamorph mold, on the surface of a Petri dish containing the former medium YES and EO at different concentrations. Control plates (without essential oil) were inoculated following the same procedure. Plates were incubated at 25 °C for 8 days and the mycelial growth was appreciated every day by measuring the average of two perpendicular diameters passing by the middle of the disc (Khallil 2001; Yehouenou *et al.* 2012). The percentage inhibition (PI) of fungal growth was evaluated by the following equation: PI 0 [1- (d/dc)] × 100 (Kumar *et al.* 2007), where d is the diameter of growth zone in the test plate, and dc the diameter of growth zone in the control plate (Petri dish without essential oil).

Statistical analysis

Experiments were performed in triplicate, and data analyzed are means ± 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 and discussion

Essential oil extraction and yield

Essential oil was obtained by hydrodistillation with a yield of 0.6% and was yellow in color. This yield is higher than that obtained by Alitonou *et al*, (2004) and Sonibare and Effiong (2008). This difference could be related to the zone of collection, the nature of the ground, the stage of development of the plant (Adjou and Soumanou, 2013) and the method used for essential oil extraction (Ogunsina *et al.,* 2010).

Table 1. Major components of essential oil of *Lantana camara* from Benin.

Components	RI	%
α- thujene	924	0,2
α-pinene	934	1,5
Camphene	946	0,7
octene-3-ol	965	0,6
Sabinene	968	13,1
β- pinene	975	1,6
Myrcene	983	1,5
α- phellandrene	994	1,0
δ- 3-carene	1004	1,6
α-terpinene	1012	0,2
p-cymene	1016	0,2
1,8-cineole	1020	9,0
(Z)-β- ocimene	1028	0,6
(E)-β- ocimene	1039	0,8
Hydrate of Sabinene (trans)	1062	0,7
Terpinolene	1082	0,4
Linalool	1086	0,7
Camphre	1129	1,3
Borneol	1151	0,6
terpinen-4-ol	1163	1,5
α-terpineol	1180	0,7
α-copaene	1376	0,5
β-elemene	1387	0,8
β- caryophyllene	1419	18,5
Aromadandrene	1434	0,7
α –humulene	1451	10
γ-murolene	1472	0,4
δ-guaiene	1495	5,0
germacrene D	1478	2,0
γ-cadinene	1506	0,6
δ-cadinene	1514	0,4
davadone (I)	1538	1,5
davadone (II)	1544	1,6
trans-Nerolidol	1549	4,0
davadone (III)	1562	0,8
Spathulenol	1565	1,0
oxyde of caryophyllene I(trans)	1570	0,8
oxyde of humulene	1594	1,2
Torreyol	1630	0,9
t-muurolol	1639	2,3
Total		91,9

Chemical composition of the essential oil

The essential oil, obtained by hydro-distillation of shade fresh leaves of *L. camara*, analyzed by GC and GC-MS led to the identification of 40 different constituents, representing 91.9% of the total oil (Table1).

The major components identified in this essential oil

were β-caryophyllene (18.5%), sabinene (13.1%), α-humulene (10%); 1.8-cinéol (9%). In the volatile extract, different groups of Monoterpene hydrocarbons (31.0%), Oxygenated monoterpenes (5.5%), Sesquiterpen hydrocarbons (42.2%), Oxygenated sesquiterpens (14.1%) and Aliphatic components (1.9%) were present. These results were similar to those of Alitonou *et al.* (2004). In our study, GC–MS data, depicted remarkable variation with the earlier reports on the oils (Tables 1). Tesch and *al.* (2011) in Venezuela, have identified 33 compounds (97.1%) of which the major components were germacrene D (31.0%). followed by β-caryophyllene (14.8%), α-phellandrene (6.7%), limonene (5.7%) and 1.8-cineole (5.2%).

Table 2. Occurrence of fungi isolated from cowpea samples.

Fungal strains	Occurrence (%)
Aspergillus flavus	100
Aspergillus tamarii	10
Aspergillus ustus	10
Penicillium spp.	100

The biologically active EOs should be qualitatively standardized before their recommendation for practical exploitation, as it has been done in the present investigation.

Table 3. Percentage of mycelial growth inhibition, of essential oil of *Lantana camara*.

Concentration of EO (µL/mL)	Mycelial growth inhibition (%)			
	A. flavus	*A. tamarii*	*A. ustus*	*A. roqueforti*
25	90,8±00a	93±00a	89±00a	97.2±00a
30	100±00b	100±00b	100±00b	100±00a
35	100±00b	100±00b	100±00b	100±00a

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.

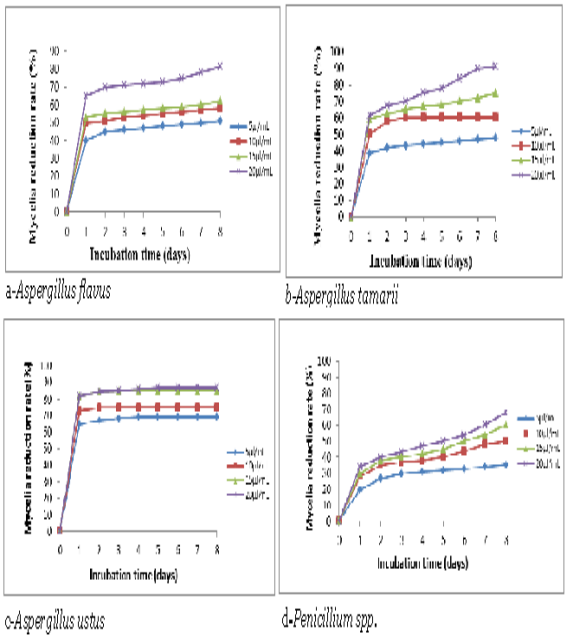

Fig. 1. Effect of *Lantana camara* essential oil on mycelial growth of strains isolated from cowpea

Fungi isolated from cowpea samples

The result of microbial analysis and isolation of fungi in pure culture revealed that cowpea samples collected were contaminated by fungi (Table 2).

Fungal isolates include *Aspergillus flavus, Aspergillus, ustus Aspergillus tamari* and *Penicillium spp.* These results were similar to those of (Houssou *et al.*, 2009 and 2011). The presence of these fungi in cowpea samples could be due to the storage conditions and could also constitute a serious threat to food safety due to the toxigenic potential of some of them, such as *Aspergillus flavus* strains.

Antifungal potential of the essential oil

Essential oil exhibited pronounced antifungal activity against the growth of fungi isolated from cowpea samples (Figures 1- 4). The Minimal Inhibitory Concentration (MIC) is 30µL/mL (Table 3). This EO was found to be effective against all fungi strains tested. The antifungal activity was very pronounced on *Aspergillus flavus, Aspergillus ustus, Aspergillus tamari* compared with *Penicillium spp.* This bioefficacy may be due to the presence of some highly

fungitoxic components in the oil, such as terpenoïds. Similar results were found by Adjou *et al.*,(2012) and several studies has revealed the antibacterial and antifungal (Kumar *et al.*, 2006), antioxidant (Basu *et al.*, 2006), insecticidal (Abdel-Hady *et al.*, 2005), nematicidal activities (Oamar *et al.*, 2006) and the used of this plant in folk remedies for cancers and tumours (Ghisalberti, 2000).

Conclusion

The present study underlined the efficacy of essential oil of fresh leaves of *Lantana camara* from Benin as an infecting cowpea fungal growth suppressor. Different major components such as β-caryophyllene (18.5%), sabinene (13.1%), α-humulene (10%) and 1.8-cinéol (9%) were present in the volatile extract. Based on its antifungal potentials, this natural plant product may successfully replace synthetic chemicals and provide an alternative method to protect cowpea during storage. . The results of this study also encourage further investigation on the effect of each of the major components of the EO of *L. camara* against plant pathogenic fungi.

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