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Inhibition of fungal development in maize grains under storage condition by essential oils

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Abstract

The ability of the essential oils from *Lippia rugosa* and *Plectranthus glandulosus* to inhibit the fungal infection in corn under storage condition was assessed. The mycelia growth inhibitory activity of *L. rugosa* and *P. glandulosus* essential oils against *Aspergillus flavus, Aspergillus niger, Aspergillus parasiticus* and *Fusarium moniliforme* was firstly determined on potato dextrose agar. Maize samples were distributed among boxes, inoculated with 2 ml (10^6 spores/ml) of conidia suspension of these strains, treated with essential oil and stored for 30 days. *L. rugosa* exhibited the lowest MIC value in liquid medium ranged from 0.2 to 0.5 mg/ml against all fungal strain, and *P. glandulosus* which was less active with value ranged from 0.8 to 1.6 mg/ml against all fungal strain. Under storage condition in corn, fungal contamination reductions from 63 to 97% by *L. rugosa* and from 27.3 to 70% by *P. glandulosus* against all fungal strain after 15 days of conservation were recorded with 6µL/g grain. These results suggest that the essential oils from *Lippia rugosa* and *Plectranthus glandulosus* may be used in grain storage against fungi responsible for biodeterioration.

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Introduction

Maize (*Zea mays* L.) constitutes one of the most important cereals cultivated in the world, serving as seed for growers, food for man and livestock as well as an industrial raw material (FAO, 1981). Huge amounts of the crop are stored mainly under subsistence conditions. Unfortunately, it is also a suitable substrate for growth, development and activity of spoilage fungi (Lacey, 1990) such as *Aspergillus flavus, Aspergillus parasiticus, Aspergillus niger* and *Fusarium moniliforme*. Fungal infections can discolour grain, change its chemical and nutritional characteristics, reduce germination and, most importantly, contaminate it with mycotoxins, such as aflatoxins, and fumonisins which are highly toxic to man and animals (Bennett and Klich, 2003).

Health and environmental problems associated with synthetic fungicides currently in use in agriculture have led to an intensification of efforts to find safe, effective and viable alternatives. In this regard plant-based fungicides can be less toxic to man, readily biodegradable, suitable for use by small scale farmers and yet capable of protecting crops. One of such alternatives is the use of natural plant preservatives such as essential oils, to prevent the proliferation of fungi or protect food from oxidation.

Plant products have for many generations been used by small scale farmers in parts of Africa to protect stored products from pest infestation (Baba Tierto, 1994; Parh et al., 1998). Protection of stored products generally involves mixing grains with protectants made up of plant materials. Many African plants are potential sources of pesticides and have been shown to exhibit biological activities, especially antimicrobial, since ancient time. With the growing interest of the use of either essential oils or plant extracts in the food and pharmaceutical industries, screening of plant extracts for these properties has become of increasing importance (Amvam *et al.,* 1998; Burt, 2004).

Lippia rugosa and *Plectranthus glandulosus* are a potential source of essential oils in Cameroon and other tropical areas (Ngassoum *et al.,* 2001; Ngassoum *et al.,* 2005). *L. rugosa* is a robust woody perennial plant of the Verbenacea family up to 12 feet high with large oblong-lanceolate bluish- green leaves, pleasant aromatic flowers (Oliver-Bever, 1982). *P. glandulosus* (Lamiacea) is plant whose leaves are commonly used to protect stored grains, as mosquito repellent and anthelminthic in Cameroon (Nukenine *et al.,* 2003). In the Adamaoua province, the plant is used in folk medicine for treatment of colds and sore throat (Ngassoum *et al.,* 2001). Ethnobotanic studies and preliminary surveys revealed that the fresh leaves of *Lippia rugosa* and *Plectranthus glandulosus* are also used to preserve food products. In this respect, fresh leaves are used to preserve stored cowpea and maize from insect and fungus damage (Illiassa, 2004). The present study was undertaken to screen some food-grade quality essential oils of leaves that can inhibit fungal infection and *in vivo* growth in post-harvest maize grains.

Materials and methods

Grains and organisms

Freshly harvested maize grains of local variety (Chaba) obtained from the Institute of Research and Development, Ngaoundéré-Cameroon were used in the present investigation. Moisture content was determined by heating of samples at 105°C until a constant weight was obtained (Marcia *et al.,* 1999).

The strains of *Aspergillus flavus, Aspergillus niger, Aspergillus parasiticus* and *Fusarium moniliforme,* isolated from maize collected in 2008 at Ngaoundere (Cameroon) according to the method used by (Foko and Sougnabé, 1991), maintained in the culture collection of Microbiology Laboratory of the National School of Agro-Industrial Sciences (The University of Ngaoundere, Cameroon) were used as test microorganisms. They were grown on Sabouraud dextrose agar (Difco, Detroit, MI) plate at 25°C for 5

days. Ten millilitres of 1% Tween 20 were added for spores' collection. The spore suspensions were further adjusted with sterile 1% Tween to give a final concentration of 10^6 spores/ml. Spore concentration was determined with a haemocytometer. The suspensions were stored at 4°C until used.

Plant material and extraction procedure

The essential oils tested were extracted by water steam distillation using a Clevenger apparatus from the leaves of *L. rugosa* and *P. glandulosus*. Fresh leaves of *L. rugosa* were collected in March 2008 at MBE (dry season) and leaves of *P. glandulosus* were collected in July 2008 at Ngaoundéré located in the Vina Division of the Adamawa region of Cameroon. The distilled essential oils were dried over anhydrous sodium sulphate and stored in a refrigerator at 4°C.

Minimum inhibitory concentration (MIC)

Antifungal assay was performed using the agar disc diffusion method (de Billerbeck et al., 2001). Potato dextrose agar (PDA) medium with different concentrations of essential oils (0.1, 0.2, 0.3, 0.4, 0.5, 0.8, 1 and 2 mg/ml) were prepared by adding the appropriate quantity of essential oil/compound to the melted medium, followed by manual rotation of the Erlenmeyer flask to disperse the oil in the medium. About 20 ml of the medium was poured into glass Petri-dishes (9 cm × 1.5 cm). Each Petri-dish was inoculated at the centre with a mycelia disc (6 mm diameter) taken at the periphery of a fungal strain colony grown on PDA for 48 h. Control plates (without essential oil) were inoculated following the same procedure. Plates were incubated at 30°C 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. For each concentration, three tests were carried out. The antifungal index (AI) was calculated as follows (Sheng-Yang *et al.*, 2005):

$$AI = 1 - \frac{G_0}{G_c} x100$$

Go = diameter of growth zone in the test plate

Gc = diameter of growth zone in the control plate.

Inoculation and oil-treatment

Grains, distributed in several lots of 200g quantities into tightly stopper jars glass and sterilized for 20 min at 121°C in autoclave, were inoculated with 2 ml (10^6 spores/ml) of conidia suspension. The final moisture content of the grains was reconstituted to 17% by adding sterile distilled water. The grains were incubated at ambient temperature (20.2°C) for 2 days to allow the fungal invasion and then treated separately with individual essential oil of *L. rugosa* and *P. glandulosus* at the level of 2, 4 and 6µl/g grain, tumbled thoroughly for 3 days after which the jars were opened and then stored in perforated (50-60 pores of 2 mm diameter/jar) under prevailing natural atmospheric conditions for 30 days and designated as test sample A. Inoculated but untreated grains in jars glass were termed as test sample B. Uninoculated and untreated grains in jars glass were designated as test sample C.

Evaluation of fungal infection

Grains samples, in triplicate, from test sample A and B were tested for grains infection by the inoculated fungi after 15 and 30 days of storage by the method used by Chatterjee (1990). Approximately 30 surface-sterilized (by 2% NaOCl) grains were plated aseptically on PDA supplemented with chloramphenicol. After 4 days incubation at 25 ± 2° C, the percentages of infected grains were recorded.

Grains samples, in triplicate, from test sample C were analysed in same conditions as control for new fungal invasion during storage in laboratory conditions.

Results and discussion

Minimal inhibitory concentration (MIC)

There are significant differences in the mycelia growth of oil-supplemented samples compared with the control (ANOVA and Duncan Multiple Range Test, $P < 0.05$). *L. rugosa* essential oil exhibited the lowest MIC values with 0.2 mg/ml against *F. moniliforme*; 0.3 mg/ml against *A. flavus*, *A. niger*; and 0.5 mg/ml against *A. parasiticus* (Table 1). Essential oil from *P. glandulosus* was less active with 0.8 mg/ml against *F. moniliforme*; 1 mg/ml against *A. flavus* and *A. niger* and 1.6 mg/ml against *A. parasiticus*. Growth inhibition was significantly ($P<0.05$) influenced by the incubation time and essential oil concentration. Mycelia growth was considerably reduced with increasing concentration of essential oil while their growth increased with incubation time.

Table 1. Minimal inhibitory concentration (mg/L) of essential oils.

Fungal species	Minimal Inhibitory Concentration (mg/L)	
	L. rugosa	*P. glandulosus*
A. flavus	0.3	1
A. niger	0.3	1
A. parasiticus	0.5	1.6
F. Moniliforme	0.2	0.8

The results obtained show that *L. rugosa* essential oil was more active in inhibiting mycelia growth of these fungi than *P. glandulosus*. The difference observed in activity between these essential oils can be explained by their chemical composition difference. Reports have indicated that biological activity of essential oil is depended on his chemical composition (Mirsha et Dubey, 1994; Amvam et al., 1998; Tagne et al., 2000). The antifungal activity of essential oil is related mainly to its proportion in oxygenated monoterpens (OMT). The antimicrobial activity has also been attributed to the presence of some active constituents in the essential oil such as geraniol, citral, thymol, eugenol and citronellol. Our GC-SM analysis revealed 50.5% and 47.9% of oxygenated monoterpens respectively in *L. rugosa* and *P. glandulosus* essential oils. Mahmoud (1999) found that geraniol was effective in suppressing A. flavus growth at 500m g/l. Viollon and Chaumont (1994) reported that citral, geraniol and citronellol showed antifungal activities among terpenoids. Earlier study found eugenol to be the active compound responsible for fungal inhibition produced by clove essential oil (Bullerman et al., 1977), but the authors raised the possibility that interactive effects of other compounds present in smaller quantities may also contribute. Although in minor percentages, these compounds together with the main compounds identified can be considered as the antifungal constituents of these essential oils.

Effect of essential oils on grains fungal infection

The uninoculated and untreated grains (test sample C) presented no fungal infection during storage. The untreated grains become infected by all the inoculated fungi within 10 days (test sample B). However, the treatment with essential oils of *L. rugosa* and *P. glandulosus*, at concentration of 6µl/g grains shows important inhibition of fungal infection. The reduction of fungal infection was dependent on the concentration of essential oil and storage time. There is significant difference ($p<0.05$) in the fungal infection of grain of treated sample compared with untreated sample. Essential oil of *L. rugosa* (Table 2) exhibited fungal infection inhibition percentage ranging from 63 to 97 % at the dose of 6µl/g grain after 15 days of storage. For the same dose after 30 days of storage, fungal infection inhibition percent were ranged from 20 to 83%. *P. glandulosus* essential oil exhibited lowest percentage of fungal infection inhibition with value ranging from 27.3 to 70% at the dose of 6µl/g grain after 15 days of storage (table 3). At 30 days after storage, for the same dose fungal infection percents observed were varied from 7.3 to 23%.

Table 2. Percentage of fungal infection inhibition in maize treated with *Lippia rugosa* essential oil and stored for 30 days

Fungi	Storage days	Inhibition % of corn infection*			
		Oil concentration (µL/g grain)			
		0	2	4	6
A. flavus	15	0^{a}	3.0 ± 0.6^{b}	50.0 ± 1.0^{b}	83.0 ± 0.6^{b}
	30	0^{a}	0^{c}	$6.7 \pm 0,6^{f}$	36.7 ± 0.6^{e}
A. niger	15	0^{a}	0^{c}	30.0 ± 1.0^{c}	63.0 ± 0.6^{d}
	30	0^{a}	0^{c}	0^{g}	23.0 ± 0.6^{f}
A. parasiticus	15	0^{a}	0^{c}	24.5 ± 0.6^{d}	77.3 ± 0.6^{c}
	30	0^{a}	0^{c}	3.0 ± 0.6^{d}	20.0 ± 0.0^{f}
F. moniliforme	15	0^{a}	23.0 ± 0.0^{a}	70.0 ± 1.0^{a}	97.0 ± 0.6^{a}
	30	0^{a}	0^{c}	20.0 ± 1.0^{e}	83.0 ± 0.6^{b}

*Mean of triplicates values with different letters are significantly different ($p < 0.05$) for each horizontal column.

Table 3. Percentage of fungal infection inhibition in maize treated with *Plectranthus glandulosus* essential oil and stored for 30 days **.**

Fungi	Storage days	Inhibition % of corn infection*			
		Oil concentration (µL/g grain)			
		0	2	4	6
A. flavus	15	0^{a}	0^{b}	33.0 ± 0.6^{b}	53.0 ± 0.6^{b}
	30	0^{a}	0^{b}	0^{e}	14.4 ± 0.6^{f}
A. niger	15	0^{a}	0^{b}	7.3 ± 0.6^{c}	43.0 ± 0.6^{c}
	30	0^{a}	0^{b}	0^{e}	7.3 ± 0.6^{b}
A. parasiticus	15	0^{a}	0^{b}	4.4 ± 0.6^{cd}	27.3 ± 0.6^{d}
	30	0^{a}	0^{b}	0^{e}	10.0 ± 0.0^{g}
F. moniliforme	15	0^{a}	2.0 ± 0.0a	50.0 ± 0.0^{a}	70.0 ± 1.0^{a}
	30	0^{a}	0^{b}	3.0 ± 0.6^{d}	23.0 ± 0.6^{e}

*Mean of triplicates values with different letters are significantly different ($p < 0.05$) for each horizontal column.

Essential oils MIC obtained in corn under storage condition were greater than those observed in liquid medium. *L. rugosa* exhibited MIC value in liquid medium ranged from 0.2 to 0.5 mg/ml for all fungal strains, but in corn under storage conditions, maximum inhibition of fungal infection was observed at 6 mg/ml for all fungal strains. The levels of antimicrobials required to inhibit microorganisms in foods have sometimes been found to be much higher than those determined using laboratory cultures (Farbood et al., 1976). This could be the result of an interaction between some essential oil compounds and certain proteins and lipids of maize grains which had as consequence the reduction in the essential oil activity. Reports have indicated *Agave asperrimma* extract MIC value from 0.5 to 1 mg/ml against *A. flavus* in liquid medium and 33 to 40 mg/ml under storage condition in corn, 66 and 40 times higher respectively (Sanchez et al., 2005). Certain former work in particular those of Belyagoubi (2006) showed that the vaporization of *Thymus capitatus* essential oil at the concentration of 52 µl/l (140µl/2.7L) approximately after 1 month of maize grains treatment revealed a reduction of 24% of the external mycological load. Fungal growth and the production of mycotoxins were prevented by 9µl of cinnamon oil directly added and mixed with 1 gram of rice grains (Patkar *et al.,* 1994). Many researchers have been conducted for antifungal activities of these essential oils. They depend on their chemical composition and on the presence of certain compounds which are known for their antifungal activities (Didry *et al.,* 1993; Periago *et al.,* 2002; Viollon & Chaumont, 1994). In fact, recent studies showed that the antimicrobial action of some

essential oils was related to the disruption of bacterial and fungal membranes (Bennis *et al.,* 2004; Cox *et al.,* 1998; Lambert *et al.,* 2001).

In this study the percentages of grains contamination are in all cases of application of these essential oils, higher after 30 days of conservation compared to those recorded after 15 days. This could be explained by the evaporation of the aromatic compounds responsible for the antifungal activity of the essential oils. According to the study of Suhr and Nielson (2003), the method used for the sifting of essential oils as antimicrobial potentials should correspond to the required application. Paster *et al.* (1995) suppose that the penetration of oils in the parts intern grain during the treatment could be improved in the presence of water, and thus the pathogenic microbes could more easily be controlled in the interior parts of the moist grains.

Conclusion

The important reduction of fungal development in maize grains during storage by these essential oils suggest their use as phytochemical compounds alone or in conjunction with other substances or processes to control fungal strains. These essential oils must be subject to further study to characterize the active compounds, define toxicity and evaluate feasibility.

References

Amvam Zollo PH, Biyiti L, Tchoumbougnang F, Menut C, Lamaty G, Bouchet P. 1998. Aromatic Plants of tropical central Africa. Part XXXII. Chemical composition and antifungal activity of thirteen essential oils from aromatic plants of Cameroon. Flavour and Flagrance Journal **13**, 107-114.

Baba Tierto N. 1994. The ability of powders and slurries from ten plants species to protect stored grain from attack *by Prostephanus truncatus* Horn and *Sitophilus oryzae* L. (Col., Curculionidae). Journal of Stored Products Research **30**, 297–301.

Belyagoubi L. (2006. Effet de quelques essences végétales sur la croissance des moisissures de détérioration des céréales. Mémoire en vue de l'obtention du diplôme de Magistère en Biologie. Faculté des Sciences, Université Abou Bekr Belkaid, Algérie, 110 pages.

Bennis S, Chami F, Chami N, Bouchikhi T, Remmal A. 2004. Surface alteration of *Saccharomyces cerevisiae* induced by thymol and eugenol. Letters in Applied Microbiology **38**, 454–458.

Bennett JW, Klich M. 2003. Mycotoxins. Clinical Microbiology Review **16**, 497-516.

Bullerman LB, Lieu FY, Seire AS. 1977. Inhibition of growth and aflatoxin production by cinnamon and clove oils, cinnamic aldehyde and eugenol. Journal of Food Science **42**, 1107-1116.

Burt S. 2004. Essential oils: Their antibacterial properties and potential applications in foods - A review. International Journal of Food Microbiology **94**, 223–253.

Chatterjee D. 1990. Inhibition of fungal growth and infection in maize by spice oils. Letters in Applied Microbiology **11**, 148-151

Cox SD, Gustafson JE, Mann CM, Markham JL, Liew YC, Hartland RP. 1998. Tea tree oil causes K+ leakage and inhibits respiration in *Escherichia coli*. Letters in Applied Microbiology **26**, 355–358.

De 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.

Didry N, Dubreuil, L, Pinkas M. 1993. Activité antibactérienne du thymol, du carvacrol, et de

l'aldhehyde cinnamique seuls ou associés. Pharmazie **48**, 301–304.

FAO (Food and Agriculture Organisation) 1981. *Production year book*. Food and Agriculture Organisation of the United Nations, Rome.

Foko J, Sougnabe 1991. Composition sites d'infection et Evolution de la spermoflore fongique de l'Arachide dans le bassin du mayo-kebbi (Tchad). Bioscience Procedures **2**, 314-317.

Illiassa N. 2004. 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, 59 p

Lacey J. 1990. Mycotoxins in UK Cereals and their control. Annals of Applied Biology **25**, 395-405.

Lambert RJW, Skandamis PN, Coote P J, Nychas GJE. 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. Journal of Applied Microbiology **91**, 453–462.

Mahmoud ALE. 1999. Inhibition of growth and aflatoxin biosynthesis of *Aspergillus flavus* by extract of some Egyptian plants. Letters in Applied Microbiology **29**, 334-336.

Marcia RF, Walderez G, Raul GC, Benedito C. 1999. Interaction between toxigenic Aspergillus flavus Link and mites (Tyrophagus putrescentiae Schrank) on maize grains: Effects on fungal growth and aflatoxin production. Journal of Stored Products Research **35**, 215–224.

Mirsha AK, Dubey NK. 1994. Evaluation of some essential oil for their toxicity against fungi causing deterioration of stored food comodities. Applied Environmental Microbiology **160**, 1101-1105.

Ngassoum MB, Jirovetz L, Buchbauer G, Fleischhacker W. 2001. Investigation of essential oil of *Plectranthus glandulosus* Hook f. (Lamiaceae) from Cameroon. Journal of oil Research **13**, 73-75.

Ngassoum MB, Tatsadjieu LN, Mapometsem PM, Jirovetz L, Buchbauer G, Shahabi M. 2005. Comparative aroma compound analysis of different essential oils of *Lippia rugosa* from Cameroon using GC-FID, GC-MS and olfactometry. Journal of Essential Oil Research **17**, 492-495.

Nukenine EN, Mebanga AS, Njan Nloga AN, Fontenille D, Nchoutpouen E, Tepamdi M. 2003. *Culicid diversity and plant materials used as mosquito repellents in northen Cameroon*. Paper presented at the 15th Conference of the African Association of Insect Scientists (AAIS) and the Entomological Society of Kenya, 9-13 June 2003, Nairobi, Kenya. Book of Abstract.

Oliver – Bever B. 1982. *Medicinal plant in West Africa*. Cambridge University Press 32-33.

Parh IA, Forbuzo BC, Matheney EL, Ayafor JF. 1998. Plants used for the control of insect pests on stored grains in parts of North West Highland Savana zone of Cameroon. Science Agronomique et Développement **1**, 54–60.

Paster N, Menasherov M, Ravid U, Juven B. 1995. Antifungal activity of Oregano and Thyme essential oils applied as fumigants against fungi attacking stored grain. Journal of Food Protection **58**, 81-85.

Patkar KL, Usha CM, Shetty HS, Paster N, Lacey J. 1995. Effects of spice oils treatment of rice

on moulding and mycotoxin contamination. Crop Protection **13**, 519-524.

Periago PM, Palop A, Fernandez PS. 2002. Combined effect of nisin, carvacrol and thymol on the viability of Bacillus cereus heat treated vegetative cells. Food Science and Technology International **7**, 487–492.

Sheng-Yang W, Pin-Fun C, Shang-Tzen C. 2005. Antifungal activities of essential oil and their constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) leaves against wood decay fungi. Bioresources Technology **96**, 813-818.

Suhr KI, Nielsen PV. 2003. Antifungal activity of essential oils evaluated by two different application techniques against rye bread spoilage fungi. Journal of Applied Microbiology **94**, 665-674.

Tagne A, Nguefack J, Nangmo R, Amvam Zollo PH. 2000. Natural control of fungi and mycotoxins in grains - a possible means of reducing human and animal contamination. Journal of Applied Science in Southern Africa **1**, 37-44.

Viollon C, Chaumont JP. 1994. Antifungal properties of essential oils and their main components upon *Cryptococcus neoformans*. Mycopathology **128**, 151-153.