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Antifungal properties of essential oils and some constituents to reduce foodborne pathogen

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Investigations were conducted to evaluate the antifungal activities of the essential oils of *Lippia rugosa, Plectranthus glandulosus*, *Clausena anisata* and *Vepris heterophylla* and some essential oils compounds as citral, geraniol, nerol, citronellol, fenchone, linalool, 1,8 cineol, nerolidol, terpen 4-ol and α-terpinolene on mycelia growth of different strains of *Aspergillus*, *Penicillium* and *Fusarium* genus, common fungi causing spoilage of stored food product. The disc diffusion method was used to evaluate fungal growth inhibition at various concentrations. The strains of fungi exhibited similar susceptibilities (90 mm) to the action of *L. rugosa* essential oil and different susceptibilities (34 - 90 mm) to *P. glandulosus*, 0 to 44 mm to *C. anisata* and 0 to 30 mm to *V. heterophylla* essential oils. These inhibition halos varied from 0 to 84 mm according to each compound activity. The mycelial growth of fungal species tested was totally inhibited by MIC values ranged from 0.2 to 0.6 mg/ml for *L. rugosa*, 0.8 to 2 mg/ml for *P. glandulosus* and 0.5 to 1.2 mg/ml for citral, geraniol, nerol and citronellol which are the more active among the ten components tested. Results obtained indicate the possibility of exploiting *L. rugosa*, *P. glandulosus* essential oils and citral, geraniol, nerol, and citronellol to fight these strains responsible for biodeterioration of stored food.

Key words: Sanitizing agents, essential oils, antifungal activity, essential oil constituents.

INTRODUCTION

Spoilage and poisoning of foods by fungi is a major problem, especially in developing countries. *Aspergillus*, *Fusarium* and *Penicillium* species are the most important fungi causing spoilage of foodstuffs. Their growth in food crops are also responsible for off-flavour formation and production of allergenic compounds and mycotoxins, which lead to qualitative losses (Nielsen and Rios, 2000; Bennett and Klich, 2003). And because of their toxicity, Aflatoxin B_1, Ochratoxin A and fumonisin B_1 produced by these fungi display carcinogenic properties in humans and in laboratory animals, leading to the appearance of hepatocarcinoma (IARC, 1993; Pfohl-Leszkowicz and Manderville, 2007).

To manage post harvest losses caused by these fungi, producers usually rely on a release of chemical fungicides (group of benzimidazoles, aromatic hydrocarbons). Currently, there is a strong debate about the safety aspects of chemical preservatives since they are considered responsible for many carcinogenic and teratogenic attributes as well as residual toxicity. For these reasons, consumers tend to be suspicious of chemical additives and thus the demand for natural and socially more acceptable preservatives has been intensified. The increase of fungal resistance to classical drugs, the treatment costs, and the fact that most available antifungal drugs have only fungistatic activity, justify the search for new strategies (Rapp, 2004).The exploration of naturally occurring antimicrobials for food preservation receives increasing attention due to awareness of natural food products and a growing concern of microbial resistance towards con-

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ventional preservatives (Schuenzel and Harrison, 2002). Plant essential oils and their components have been known to exhibit biological activities, especially anti-microbial, 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). Biological, because of their natural origin, are biodegradable and they do not leave toxic residues or by-products to contaminate the environment.
Lippia rugosa A. Chev*, Plectranthus glandulosus* Hook*, Clausena anisata* (Willd.) Hook. F. ex Benth (family Rutaceae) and *Vepris heterophylla* (Engl.) Letouzey are a potential source of essential oils in Cameroon and other tropical areas (Hawthorne, 1998; Ngassoum et al., 2001; IBKAS, 2001; Ngassoum et al., 2005). *P. glandulosus* (Lamiacea) is plant whose leaves are commonly used to protect stored grains, as mosquitoes repellent and anthelminthic in Cameroon (Nukenine et al., 2003). In the Adamawa region, the plant is used in folk medicine for treatment of colds and sore throat (Ngassoum et al., 2001). The traditional medicinal values of different parts of *Clausena anisata* have been reported to be useful as effective remedies against parasitic infections, especially flatworm infections, hepatic diseases causing bad breath, malaria and fevers. Its uses include the burning of the dried plant to repel mosquitoes (Adewole and Olaiga, 1987).

Previously works conducted in our laboratory on some plants used traditionally for several purposes, have demonstrated antifungal activities of *L. rugosa* and *P. glandulosus* essential oils against *Aspergillus flavus* strain (Tatsadjieu et al., 2007) and this antifungal activity has been attributed to some main compounds of these oils. Their GC analysis revealed that main components of the essential oil of *L. rugosa* are geraniol (51.6%), nerol (18.2%) and geranial (10.8%), for *P. glandulosus*: fenchone (29.28%), α-terpinolene (28.29%) and piperitenone oxide (11.08%), for *C. anisata*: E-ocimenone (15.1%), Z-ocimenone (11.5%), γ-terpinene (11.4%) and germacrene D (10.9%) and those of *V. heterophylla* are Elemol (19.6%), guaiol (19.1%) and Sabinene (15.49%). In order to get more information on the inhibition potential of these essential oils, their effect on the growth of others fungal genus and other *Aspergillus* species were investigated. In addition, the antifungal activities of some of their main components were subsequently examined to help to understand the antifungal activities of these essential oils.

MATERIALS AND METHODS

Fungal strains and production of conidia

The strains of *A. flavus, Aspergillus niger*, *Aspergillus parasiticus*, *Aspergillus* spp, *Fusarium moniliforme*, *Fusarium* spp1, *Fusarium* spp2, *Penicillium roqueferti* and *Penicillium* spp2 isolated from maize collected in 2008 at Ngaoundéré (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 6 x 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

Fresh leaves of *L. rugosa* were collected in March 2008 at MBE (dry season), leaves of *P. glandulosus* and *C. anisata* were collected in July 2008 at Ngaoundéré located in the Vina Division of the Adamawa region of Cameroon and *V. heterophylla* leaves were collected at Mokolo located in the far North region of Cameroon. The leaves were hydro-distilled for about 5 hours using a Clevenger apparatus. Oils recovered were dried over anhydrous sodium sulfate and stored at 4 °C until they used.

The 10 essential oils constituents (citral, geraniol, nerol, citronellol, fenchone, linalool, 1.8cineol, nerolidol, terpen 4-ol and α-terpinolene) were kindly supplied by the Institute of Bimolecular Max Mousseron (University of Montpellier II).

Determination of sensitivity

The sensitivity of fungi to the essential oils was determined by the agar diffusion method (Maruzella and Liguori, 1958). Sterile paper discs (Whatman No. 1; 6.0 mm in diameter) were soaked with 10 µl of pure essential oils/compounds and placed on the surface of the inoculated agar plates. The dishes were incubated at 30°C for 48 h and the zones of inhibition were measured. The sensitivity to the different oils was classified by the diameter of the inhibition. Each assay was performed by duplication on two separate experimental runs.

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 center 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/compound) 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):

$$AI = 1 - \frac{Go}{Gc} x\ 100$$

Go = diameter of growth zone in the test plate
Gc = diameter of growth zone in the control plate.

Table 1. Inhibition zones diameter of fungal growth by essential oils.

Fungal species	Mean of inhibition zone diameter (mm)[a *]				
	L. rogusa	***P. glandulosus***	***C. anisata***	***V. heterophylla***	**Control**
Aspergillus flavus	90.0 ± 0.0$^{a\ A}$	85.7 ± 4.0$^{a\ A}$	50.0 ± 5.0$^{a\ B}$	17.3 ± 0.56$^{b\ C}$	-
Aspergillus niger	90.0 ± 0.0$^{a\ A}$	68.0 ± 2.6$^{b\ B}$	35.3 ± 5.3$^{c\ C}$	17.0 ± 1.0$^{b\ D}$	-
Aspergillus parasiticus	90.0 ± 0.0$^{a\ A}$	41.0 ± 1.0$^{c\ B}$	0.0 ± 0.0$^{f\ D}$	15.0 ± 1.0$^{bc\ C}$	-
Aspergillus spp	90.0 ± 0.0$^{a\ A}$	69.0 ± 3.6$^{b\ B}$	10.0 ± 1.0$^{e\ C}$	10.0 ± 2.0$^{de\ C}$	-
Fusarium moniliforme	90.0 ± 0.0$^{a\ A}$	90.0 ± 0.0$^{a\ A}$	30.7 ± 0.6 B	13.0 ± 1.0$^{cd\ C}$	-
Fusarium spp1	90.0 ± 0.0$^{a\ A}$	41.3 ± 2.3$^{c\ B}$	19.3 ± 1.5$^{d\ C}$	11.7 ± 0.6$^{de\ D}$	-
Fusarium spp2	90.0 ± 0.0$^{a\ A}$	34.0 ± 1.0$^{d\ B}$	11.0 ± 1.0$^{e\ C}$	10.3 ± 1.5$^{de\ D}$	-
Penicillium roqueferti	90.0 ± 0.0$^{a\ A}$	87.3 ± 2.5$^{a\ A}$	44.0 ± 5.3$^{b\ B}$	30.0 ± 5.0$^{a\ C}$	-
Penicilium spp	90.0 ± 0.0$^{a\ A}$	65.3 ± 4.5$^{b\ B}$	11.0 ± 1.7$^{e\ C}$	0.0 ± 0.0$^{f\ C}$	-

[a] The diameter of the filter paper discs (6mm) is included. *Values followed by a same alphabetic letter are not meaningfully different.

Nature of mycelia growth inhibition

Fungicidal effect was determined while transferring disks coming from Petri-dish where the mycelia growth inhibition by the essential oil/compound was total during incubation period, in a PDA medium without essential oil/compound. The effect was fungistatic if there was resumption of growth and fungicidal in the contrary case.

Statistical analysis

Data from three independent replicate trials for MIC and those for sensitivity were subjected to statistical analysis using Statistica .06, Statistical package (Statsoft, 1995). Differences between means were tested using Duncan Multiple Range Test.

RESULTS AND DISCUSSION

Antifungal inhibition zones

Antifungal inhibition zones for essential oils against fungi are showed in Table 1. Fungi susceptibility to these essential oils, as determined by the agar diffusion method, showed that *L. rugosa* produced 90 mm in diameter inhibition halos and presented the highest inhibitory effects. This oil completely inhibited the growth of fungi tested. Otherwise *P. glandulosus* essential oil produced inhibition zones ranging from 34 (*Fusarium* spp2) to 90 mm (*Fusarium moniliforme*). These Fungi are more susceptible to *L. rugosa* and *P. glandulosus* essential oils than the two others. Inhibition zones produced by *C. anisata* and *V. heterophylla* are ranged from 0 (*A. parasiticus*) to 44 mm (*P. roqueferti*) and 0 (*Penicillium* spp) to 30 mm (*P. roqueferti*) respectively. Diameter inhibition halos produced by *V. heterophylla* essential oil varied from 0 to 30.0 mm (*P. roqueferti*).

These results for *A. flavus* were not in agreement with those reported by Tatsadjieu (2003). This author indicated that essential oils of *L. rugosa* and *P. glandulosus* showed inhibition zones respectively 58.00 ± 2.64 and 76.33 ± 1.52 mm on *A. flavus*. The differences that we have found with this author can be attributed to the fact that essential oils are a heterogeneous group of complete mixtures' of organic substances the quality and quantity of which vary with growth stages, ecological conditions, and other plant factors based on which the essential oil is extracted (Ozcan and Erkmen, 2001; Elgayyar et al., 2001).

Inhibition zone diameters for compounds against fungi tested are represented in Table 2. It was observed that diameters inhibition zones produced by citral varied from 15.05 to 84.0 mm; from 31.7 to 50 mm for geraniol; from 39.7 to 53.0 mm for nerol and from 27.0 to 50.7 mm for citronellol. Fungi tested are all more susceptible to geraniol, citral, nerol and citronellol than others essential oils constituents. It was too observed that some fungal species tested did not present any sensitivity facing certain compounds as 1.8cineol, α-terpinolen and nerolidol. It is the case of *A. niger, A. parasiticus, Aspergillus* spp, *Fusarium* spp1 and *Fusarium* spp2 that did not present any halo of inhibition. Previous study reported the effect of some essential oils components on the growth of *A. niger*. Dharmendra et al. (2001) reported that citral produced 7 mm of inhibition zone; geraniol and citronellol produced each 15 mm against *A. niger*. The differences noted can be attributed to the difference of compound quantity used in the test. In fact in their test the authors used 5 µl of component whereas 10 µl were used in this work.

Following the results of the sensitivity test, *L. rugosa* and *P. glandulosus* essential oils and components as citral, geraniol, nerol and citronellol showed strong antifungal activities through high inhibition zones. The results revealed their potential as natural preservatives in food technology. To contribute to the successful of technological application of such natural preservatives in food it is necessary to determine the MIC of these essential oils and components.

Minimum inhibitory concentration

The MIC and fungicidal concentration values for different

Table 2. Inhibition zones of fungal growth by essential oils compounds.

Fungal species	Means of inhibition zone diameter ± SE in mm [a] *									
	Compounds									
	Geraniol	Citral	Nerol	Citronellol	Linalol	Fenchone	Terpen 4-ol	1,8 Cineol	α-terpinolene	Nerolidol
Aspergillus flavus	34.3 ± 0.6	45.7 ± 0.6	53.0 ± 1.0	50.7 ± 1.1	13.7 ± 0.6	20.3 ± 0.6	20.0 ± 0.0	10.6 ± 1.1	0.0 ± 0.0	0.0 ± 0.0
Aspergillus niger	35.3 ± 0.6	68.3 ± 1.5	46.7 ± 0.6	27.0 ± 1.0	33.0 ± 3.0	6.7 ± 0.6	21.3 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	11.7 ± 0.6
Aspergillus parasiticus	35.7 ± 0.6	15.7 ± 0.6	41.0 ± 1.0	39.3 ± 1.1	17.3 ± 2.1	0.0 ± 0.0	25.7 ± 1.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Aspergillus spp	32.0 ± 1.7	65.3 ± 1.5	40.7 ± 1.1	50.3 ± 0.6	11.7 ± 0.6	20.0 ± 1.0	40.3 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Fusarium moniliforme	37.3 ± 2.3	84.0 ± 3.6	45.0 ± 2.0	33.3 ± 1.1	27.3 ± 2.5	13.7 ± 1.5	15.7 ± 0.6	7.3 ± 0.6	7.7 ± 0.6	0.0 ± 0.0
Fusarium spp1	31.7 ± 1.5	78.7 ± 1.5	40.3 ± 0.6	32.7 ± 0.6	27.3 ± 3.2	10.3 ± 0.6	13.3 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Fusarium spp2	39.0 ± 1.0	80.3 ± 1.5	39.7 ± 0.6	35.7 ± 2.1	21.3 ± 1.5	10.7 ± 1.1	10.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Penicillium roqueferti	50.0 ± 1.0	56.3 ± 1.5	46.3 ± 1.1	37.0 ± 2.0	19.0 ± 1.0	14.7 ± 0.6	17.3 ± 0.6	24.7 ± 0.6	12.7 ± 3.4	0.0 ± 0.0
Penicillium spp	44.7 ± 0.6	69.7 ± 2.0	40.0 ± 0.0	42.3 ± 2.3	15.0 ± 0.0	7.7 ± 0.6	15.3 ± 1.1	11.3 ± 0.6	0.0 ± 0.0	0.0 ± 0.0

[a] The diameter of the filter paper discs (6 mm) is included.

Table 3. MIC and MIF values for different oils, obtained by the agar dilution method.

Fungal species	MIC (mg/ml)		Fungicidal concentration (mg/ml)	
	L. rogusa	*P. glandulosus*	*L. rogusa*	*P. glandulosus*
Aspergillus flavus	0.3	1.0	0.8	2.4
Aspergillus niger	0.3	1.0	0.8	2.4
Aspergillus parasiticus	0.5	1.6	1.5	3.0
Aspergillus spp	0.5	1.5	1.5	3.0
Fusarium moniliforme	0.2	0.8	0.8	2.0
Fusarium spp1	0.3	1.2	1.0	2.2
Fusarium spp2	0.3	1.2	1.0	2.2
Penicillium roqueferti	0.6	1.6	1.0	2.0
Penicilium spp	0.6	2.0	1.0	3.0

oils are presented in Table 3. There were significant differences in the mycelia growth of oil-supplemented samples compared to the control which was not supplemented with essential oil (ANOVA and Duncan Multiple Range Test, $P < 0.05$). 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 (Figures 1 to 4).

The essential oil with the lowest MIC and fungicidal concentration was *L. rugosa* with the values which vary from 0.2 to 0.6 mg/ml and from 0.8 to 1.5 mg/ml respectively. This fact was in agreement with the agar diffusion method results mentioned above (Table 1). The essential oil of *P. glandulosus* with the MIC values from 0.8 to 2

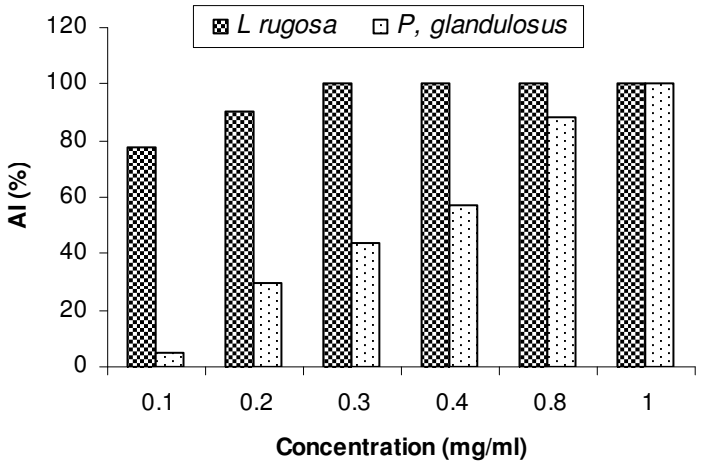

Figure 1. Effect of *L. rugosa* and *P. glandulosus* essential oils on the growth of *A. flavus*.

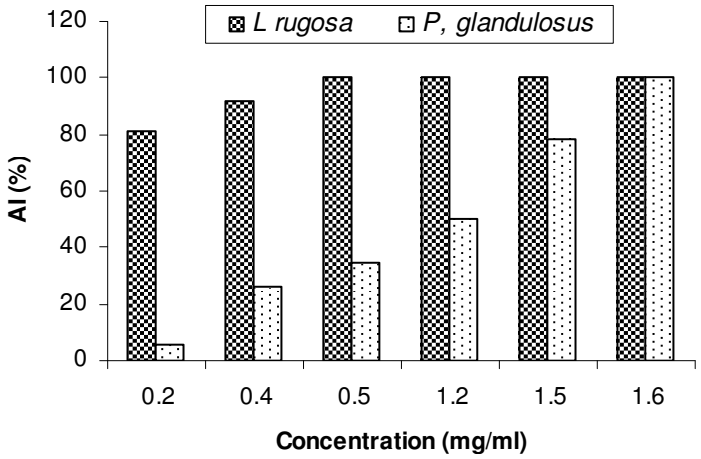

Figure 2. Effect of *L. rugosa* and *P. glandulosus* essential oils on the growth of *A. parasiticus*.

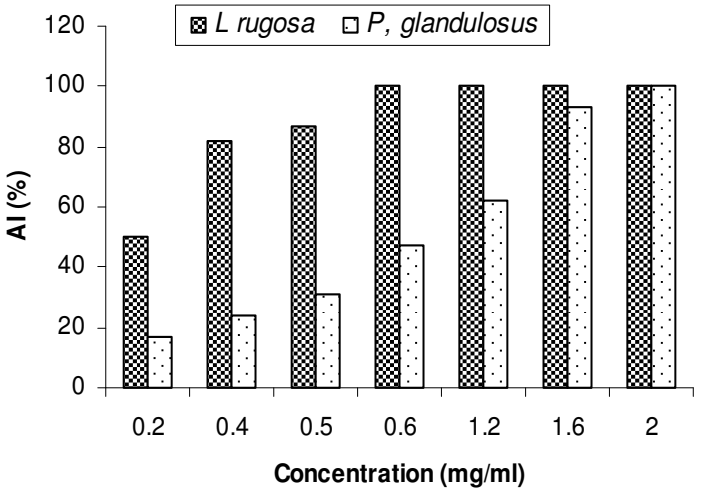

Figure 3. Effect of *L. rugosa* and *P. glandulosus* essential oils on colony diameter of *Penicillium* spp.

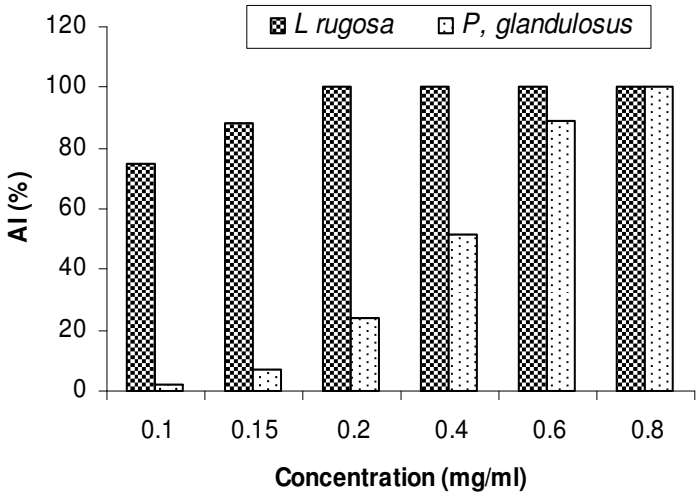

Figure 4. Effect of *L. rugosa* and *P. glandulosus* essential oils on the growth of *F. moniliforme*.

mg/ml was lest toxic than *L. rugosa*. Tatsadjieu et al. (2007) reported similar MIC value (1 mg/ml) for *P. glandulosus* and different MIC value against *A. flavus* for *L. rugosa*. The differences found could be attributed to the fact that in our study *L. rugosa* leaves were collected in the locality of MBE, situated 70 km north of the capital city of the region, while they had collected their *L. rugosa* leaves at Dang situated 15 km of the capital city of the region, Ngaoundere. *Penicillium* species were more resistant to these essential oils and present MIC values ranged from 1.6 to 2 mg/ml for *P. glandulosus* and at 0.6 mg/ml for *L. rugosa*.

The transfer of mycelial discs where growth inhibition was complete by *L. rugosa* and *P. glandulosus* into PDA medium without essential oil, showed mycelial growth after 2 to 10 days of incubation, indicating a no lethal effect of these concentrations. By increasing the concentration of essential oils, doses where there was not continuation of mycelial growth were obtained (Table 3). So *L. rugosa* essential oil at 0.8 mg/ml was fungicidal against *A. flavus, A. niger* and *F. moniliforme.* For the others fungal species, fungicidal effect appears at 1 mg/ml against *Fusarium* spp1*, Fusarium* spp2*, P. roqueferti* and *Penicillium* spp and at 1.5 mg/ml for the rest. Fungicidal action of *P. glandulosus* appears at a concentration higher than 2 mg/ml. In general, fungicidal values are more elevated in relation to the CMI values.

The fungistatic effects of essential oils previously indicated by many authors showed that some constituents in essential oil play an important role in its antifungal action. The antifungal index (AI) of four essential oils constituents on fungal species growth are presented in Table 4. It was observed that nerol completely suppressed the growth of *A. flavus, A. niger* at 0.8 mg/ml; those of *A. parasiticus, Aspergillus* spp and *Penicillium* spp at 1 mg/ml. *Fusarium* species which are more susceptible were totally inhibited at 0.5 and 0.6 mg/ml. Geraniol completely inhibited the growth of *A. flavus, A. niger* at 0.8 mg/ml; *A. parasiticus, Aspergillus* spp and *Penicillium* spp at 1.2 mg/ml and those of *Fusarium* species tested at 0.5 mg/ml (*F. moniliforme* and *Fusarium* spp1) and 0.6 mg/ml (*Fusarium* spp2). The mycelial growth of *Fusarium* species tested was completely inhibited at 0.5 mg/ml by citral. That of *A. niger* was at 0.6 mg/ml, *A. flavus* at 0.8 mg/ml and *Aspergillus* spp at 1.2 mg/ml.

The MIC values of geraniol, nerol and citronellol obtained against *A. flavus* were higher than those of Mamoud (1994), who reported that these essential oils constituents were effective at 500 ppm. Viollon and Chaumont (1994) reported that citral, geraniol and citronellol showed antifungal activities among terpenoids. Some compounds have not been tested in this study but however some previous works showed their antifungal activities. Hitokoto et al. (1980) reported that thymol extracted from thyme, at < 4 mg/ml, caused complete inhibition of growth of *A. flavus* and *A. versicolor*. Buchanan and Shepard (1981) found that 500 ppm of thymol completely prevented growth and aflatoxin production by *A. parasiticus*. It has been reported by Vazquez et al. (2001) that total inhibition of *Penicillium citrinum* was achieved by adding 2000 ppm of eugenol and thymol to the liquid medium. Authors raised the possibility that interactive effects of other compounds present in smaller quantities may also contribute (Bullerman et al., 1977). Although in minor percentages, these compounds together with the main compounds identified can be considered as the antifungal constituents of the active essential oils. The absence of the compounds, known for their antifungal activities as showed in the present study, in the essential oils of *C. anisata* and *V. heterophylla* would be to the origin of the weak antifungal activities of these essential oils.

Table 4. Effect of essential oil compounds on the growth of fungi tested.

Compounds	Concentration (mg/ml)	Mean antifungal index (AI) ± SE in %							
		Fungal species							
		A. flavus	***A. niger***	***A. parasiticus***	***A.* spp**	***F. moniliforme***	***F.* spp1**	***F.* spp2**	***P.* spp**
Nerol	0.4	57.9 ± 1.8	54.3 ± 0.7	23.4 ± 1.8	11.1 ± 1.4	83.7 ± 0.7	80.1 ± 1.4	51.9 ± 0.7	45.6 ± 2.5
	0.5	76.6 ± 0.7	68.6 ± 0.7	39.7 ± 1.4	24.6 1.4	100.0 ± 0.0	100.0 ± 0.0	79.7 ± 1.2	62.7 ± 1.4
	0.6	82.1 ± 1.2	86.1 ± 0.7	57.9 ± 1.4	52.4 ± 1.2			100.0 ± 0.0	76.2 ± 1.2
	0.8	100.0 ± 0.0	100.0 ± 0.0	65.8 ± 1.4	63.9 ± 0.7				84.5 ± 1.2
	1			100.0 ± 0.0	100.0 ± 0.0				100.0 ± 0.0
Citronellol	0.4	44.0 ± 1.2	53.6 ± 1.2	15.5 ± 1.2	31.7 ± 1.4	81.3 ± 0.7	76.6 ± 0.7	87.7 ± 0.7	21.4 ± 1.2
	0.5	49.2 ± 0.7	62.3 ± 1.8	21.0 ± 1.8	36.5 ± 1.4	100.0 ± 0.0	88.5 ± 0.7	91.3 ± 0.7	36.5 ± 1.4
	0.6	56.3 ± 1.4	75.8 ± 0.7	34.9 ± 0.7	40.9 ± 1.8		100.0 ± 0.0	100.0 ± 0.0	48.4 ± 1.8
	0.8	64.7 ± 0.7	85.7 ± 1.2	39.7 ± 1.4	44.4 ± 1.4				65.1 ± 1.4
	1	100.0 ± 0.0	100.0 ± 0.0	68.6 ± 1.8	66.2 ± 2.4				100.0 ± 0.0
	1.2			100.0 ± 0.0	100.0 ± 0.0				
Citral	0.4	57.9 ± 1.4	54.4 ± 0.7	23.4 ± 1.8	9.9 ± 1.8	94.3 ± 1.4	82.1 ± 1.2	74.2 ± 0.7	67.5 ± 1.4
	0.5	74.2 ± 1.8	80.1 ± 1.4	36.1 ± 1.8	11.1 ± 0.7	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	71.0 ±0.7
	0.6	84.1 ± 1.4	100.0 ± 0.0	63.9 ± 0.7	15.1 ± 1.4				85.3 ± 0.7
	0.8	100.0 ± 0.0		68.2 ± 1.4	17.4 ± 1.4				100.0 ± 0.0
	1			100.0 ± 0.0	63.1 ± 1.2				
	1.2				100.0 ± 0.0				
Geraniol	0.4	47.6 ± 2.4	29.7 ± 1.2	28.2 ± 1.8	24.2 ± 0.7	90.1 ± 0.7	89.3 ± 1.2	94.8 ± 0.7	52.7 ± 0.7
	0.5	70.2 ± 1.2	45.6 ± 1.4	32.5 ± 1.4	34.1 ± 0.7	100.0 ± 0.0	94.2 ± 0.7	96.8 ± 0.7	63.9 ± 0.7
	0.6	83.3 ± 1.2	89.7 ± 0.7	35.7 ± 1.2	36.5 ± 0.7		100.0 ± 0.0	100.0 ± 0.0	76.6 ± 0.6
	0.8	100.0 ± 0.0	100.0 ± 0.0	39.3 ± 1.2	38.9 ± 1.8				92.1 ± 0.7
	1			91.7 ± 1.2	94.4 ±1.4				96.0 ± 0.7
	1.2			100.0 ± 0.0	100.0 ± 0.0				100.0 ± 0.0
Control	0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

For the sensitivity test, fungal strains exhibited similar susceptibilities to *L. rugosa* essential oil effect which has completely inhibited the growth of the fungi tested, different susceptibilities to *P. glandulosus, C. anisata V. heterophylla* and essential oil constituents were tested. The results demonstrated that *L. rugosa* essential oil exerted a significant antifungal activity followed by *P. glandulosus*. Essential oil constituents as citral, geraniol, nerol and citronellol were more effective than other components and would be responsible to antifungal activity. This work has been conducted in culture media; consequently little is understood about their effectiveness in the food substrates. Further studies are needed to investigate the oils incorporation into appropriate food formulations, and evaluate flavour, chemical changes and antifungal effect in the whole food system.

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