International Research Journal of Microbiology (IRJM) (ISSN: 2141-5463) Vol. 3(12) pp. 406-415, December, 2012
Available online http://www.interesjournals.org/IRJM

Full Length Research Paper

Bioefficacy of *Cymbopogon citratus* essential oil against foodborne pathogens in culture medium and in traditional cheese wagashi produced in Benin

Philippe Sessou[1,2], Souaïbou Farougou[1*], Sandrine Kaneho[1] , Sébastien Djenontin[2], Guy Alain Alitonou[2], Paulin Azokpota[3], Issaka Youssao[1], Dominique Sohounhloué[2]

[1]Laboratory of Research in Applied Biology, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, 01 P.O. Box 2009 Cotonou, BENIN
[2]Laboratory of Study and Research in Applied Chemistry, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, 01 P.O. Box 2009 Cotonou, BENIN
[3]Laboratory of Food Microbiology and Biotechnology, Faculty of Agricultural Sciences 01 P.O. Box 526 Cotonou, BENIN

Abstract

The objective of this study was to evaluate the effectiveness of *Cymbopogon citratus* essential oil against spoilage and pathogens moulds isolated from wagashi. For this purpose, mycelial growth Inhibition, minimal inhibitory concentration and minimal fungicidal concentration of the oil on these strains were determined by agar diffusion method in culture medium. The antifungal activity in cheese wagashi of this oil based on the highest MIC value against the resistant moulds was also tested. The chemical composition of the essential oil hydrodistillated and analyzed by GC-FID and GC-MS showed geranial (44.5%) and neral (31.5%) as major compounds. The evaluation of antifungal activity revealed that lemongrass oil possessed fungicidal activity against *Scopulariopsis brevicaulis* at 400 mg/L, *Fusarium poae* at 800 mg/L, *Aspergillus* (*niger* and *terreus*) and *Fusarium verticillioides* at 1000 mg/L in culture medium. The addition of lemongrass oil to wagashi mixture with moulds' spores exerted high sporale reduction rate on *Penicillium citrinum* and *Aspergillus flavus* which are potential producers respectively of the most important and harmful mycotoxins, citrinin and aflatoxin M1 encountered in dairy products especially cheese. Results obtained indicate the possibility of exploiting lemongrass oil to preserve wagashi against moulds contamination and probably mycotoxins inhibition during wagashi production and storage.

Keywords: *Cymbopogon citratus*, essential oil, chemical composition, antifungal activity, wagashi, Benin.

INTRODUCTION

Cheese is considered as one of the most important foodstuffs consumed by human and it contains a source of high quality animal proteins having amino acids. It is also a rich source of calcium, phosphorus and many other micronutrients (Nasser, 2001). In Benin, traditional cheese locally called wagashi is the most popular type of cheese which could efficaciously contribute to the resolution of nutritional problems due to the deficiency of proteins (Kèkè et al., 2008; Sessou et al., 2012 a,b,c). However, wagashi is produced and preserved using rudimentary methods under unsanitary conditions which may lead to its contamination by toxinogenic or pathogenic microorganisms especially fungi (Aissi et al., 2009; Sessou et al., 2012 a,b,c). Fungal contamination of this product may result in a decline of its quality and quantity. According to an investigation, pathogenic fungi alone cause a nearly 20% reduction in the yield of major food and cash crops

*Corresponding Author E-mail: souaibou.farougou@uac.bj; farougou@gmail.com, Phone: (229) 90 90 14 84;
Fax: +229 21 36 11 19

(Tian et al., 2011). Wagashi exposed to deterioration by the fungi can have a decreasing in its sensory, nutritive and medical characteristics (Barkat and Bouguerra, 2012). Fungi can also produce mycotoxins which may penetrate wagashi and affect consumer's health (Sessou et al., 2012c,d). A better control to prevent spoilage of wagashi is necessary to avoid its contamination by mycoflora and minimize public health hazards. Chemical preservatives used long to control these pathogens are considered responsible for many carcinogenic and teratogenic attributes as well as residual toxicity (Barkat and Bouguerra, 2012). One of the major problems related to the use of these chemicals is that the fungi develop resistance. For these reasons, alternative methods to control cheese-borne fungi of wagashi are needed to be performed. Essential oils classified as Generally Recognized As Safe (GRAS) and considered at low risk for developing resistance to pathogenic microorganisms could be a credible alternative (Cardile et al., 2009; Murthy et al., 2009; Nguefack et al., 2007, 2012; Tian et al., 2012). Several studies have reported results on their preservative action (Nielsen and Rios, 2000; Burt, 2004). Essential oil of *Cymbopogon citratus* mainly composed of citral *(neral + geranial)* had both antimicrobial activity against many strains such as *Aspergillus flavus, Penicillium expansum, A. ochraceus, P. verrucosum, Listeria monocytogenes, S. aureus, E. coli, S. typhimurium* (Burt, 2004; Nguefack et al., 2004, 2009, 2012). Based on our knowledge, its use as cheese preservative has been few studied. The efficacy of lemongrass essential on moulds isolated from wagashi must be verified in order to measure its potential biopreservation for the valorization of this product. The aim of this research was to assess antifungal activity of essential oil of *Cymbopogon citratus* against twelve fungi (*Aspergillus aculeatus, A. flavus, A. niger, A. tamarii, A. terreus, A. ustus, Fusarium* (*poae* and *verticillioides*) and *Penicillium brevicompactum*, *P. citrinum*, *P. griseofulvum* and *Scopulariopsis brevicaulis*) isolated from a traditional cheese wagashi in culture medium and in this foodstuff for its potential use as biopreservative.

MATERIAL AND METHODS

Plants material and extraction of the essential oil

Fresh leaves of *Cymbopogon citratus* were collected at Abomey-Calavi in South of Republic of Benin at November 2011 and were identified by Doctor YEDOMOHAN of National Herbarium of Benin. They were hydrodistilled for about 3 hours, using a Clevenger apparatus. Oil recovered in a dark sterile glass was dried over anhydrous sodium sulfate and stored at +4 °C until it was used (Yehouenou et al., 2012).

Chemical analysis of *Cymbopogon citratus* essential oil

Quantitative and qualitative analyses of the essential oil of *Cymbopogon citratus* were carried out by gas chromatography/flame ionization detection (GC/FID) and gas chromatography/mass spectrometry (GC/MS).

GC/FID analyses were performed using a Varian CP-3380 GC equipped with a DB1 (100% dimethylpolysiloxane) fitted with a fused silica capillary column (30 m x 0.25 mm, film thickness 0.25 µm) and Supelcowax 10 (polyethylene glycol) fused capillary column (30 m x 0.25 mm, film thickness 0.25 µm); temperature program 50°-200°C at 5°C/min, injector temperature 220°C, detector temperature 250°C, carrier gas N_2 at a flow rate of 0.5 mL.min^{-1}. Diluted samples (10/100, v/v, in methylene chloride) of 2.0 µL were injected manually in a split mode (1/100). The percentage compositions were obtained from electronic integration measurements without taking into account relative response factors. The linear retention indices of the components were determined relatively to the retention times of a series of *n*-alkanes (C_9-C_{20}).

GC/MS analyses were performed using a Hewlett Packard apparatus equipped with a HP1 fused silica column (30 m x 0.25 mm, film thickness 0.25 µm) and interfaced with a quadruple detector (Model 5970). Column temperature was programmed from 70° to 200°C at 10°C/min; injector temperature was 220°C. Helium was used as carrier gas at a flow rat of 0.6 mL.min^{-1}, the mass spectrometer was operated at 70 eV. 2.0 µL of diluted samples (10/100, v/v, in methylene chloride) were injected manually in the split mode (1/100).

The identification of individual compounds was based on the comparison of their relative retention times with those of authentic samples on the DB1 column and by matching the linear retention indices and mass spectra of peaks with those obtained from authentic samples and/or the NBS75K.L and NIST98.L libraries and published data (Adams, 2007).

Strains of filamentous fungi tested

The fungi used in this study were: *Aspergillus aculeatus, A. flavus, A. niger, A. tamarii, A. terreus, A. ustus, Fusarium* (*poae* and *verticillioides*) and *Penicillium brevicompactum*, *P. citrinum*, *P. griseofulvum* and *Scopulariopsis brevicaulis*. They have been isolated and identifying from a traditional cheese wagashi collected near its vendors. Colonies of these moulds isolated from DBRC medium by dilution method (ISO 21527-1: 2008) were purified by streaking onto Malt Extract Agar and then three point inoculated onto MEA and Czapeck Yeast autolysate Agar before identify-

cation based both on macroscopic characters (colony growth, colony diameter) and microscopic characters using the identification schema of Samson et al. (1995) and Pitt and Hocking (2009).

Preparation of conidial suspension

The strains isolated from cheese wagashi were cultured on Potato Dextrose Agar medium for 10–14 days at 25 ± 1 °C. Conidia were harvested by adding 10 ml of 0.05% Tween 80 solution to culture and gently scraping the mycelia with a sterile inoculating loop to free spores. Conidial concentration was determined by a haemocytometer and the suspension was diluted with 0.05% Tween 80 solution to give a final concentration of 10^8 spores/mL approximatively (Gandomi et al., 2009).

Antifungal assay in culture medium

The test was performed by the agar medium assay (Tatsadjieu et al., 2009). Potato Dextrose Agar (PDA) medium with different concentrations of essential oil (200, 400, 600, 800 or 1000 $mg.L^{-1}$) were prepared by adding appropriate quantity of essential oil to melted medium, followed by addition of Tween 80 (100 µL to 100 mL of medium) to disperse the oil in the medium. About 20 ml of the medium were poured into glass Petri-dishes (9 cm x 1.5 cm). Each Petri-dish was inoculated at the centre with a mycelial disc (6 mm diameter) taken at the periphery of a fungus colony grown on PDA for 48 h. Positive Control (without essential oil) plates 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 MGI (Mycelia Growth Inhibition) percentage was calculated according to the equation:

MGI = (dc-dt)/dc x 100 where dc = mean diameter for control – 6 mm and dt = mean diameter for treated mycelium – 6 mm.

The Minimal Fungicidal Concentration (MFC) values were determined by the method described by Angelini et al. (2006). This was done by subculturing the inhibited fungal discs at MICs on PDA medium without essential oil. Observations were recorded after 7 days of incubation at 25 °C. Fungal growth on the seventh day was indicative of a fungistatic nature, while the absence of fungal growth denoted a fungicidal action of the oil.

Antifungal assay in cheese wagashi

The procedure was based on that of Smith-Palmer et al. (2001). 10 g of cheese wagashi was added to 90ml of 0.1% Peptone (CM0009 Oxoid, LTD Basingstoke, Hampshire, England) in stomacher bags and homogenized for 2 min in a stomacher. Plant essential oil of *Cymbopogon citratus* were added to the cheese mixture to achieve final concentrations wished. The controls contained Peptone but no plant essential oil. The bags were homogenized for a further 30 s to ensure even mixing of the plant essential oil with the cheese. The cheese mixture was inoculated with 100 µl of sporale suspension culture, which had been prepared previously. The inoculum was mixed thoroughly with the cheese mixture by gently squeezing the bags by hand and the concentration of mould in the cheese determined at 0 hours and 1, 2, 3, 4, 7, 10 and 14 days using the serial dilution and spread plate technique.

Statistical analysis

Data from three independent replicate trials were subjected to statistical analysis using Statistica version 6.0. Differences between means were tested using Z-test. Results were considered statistically significant when $p<0.05$.

RESULTS AND DISCUSSION

Chemical composition of lemongrass essential oil

The chemical composition of *Cymbopogon citratus* essential oil with yield equal to 1.5 ± 0.25 % is presented in Table 1. Twenty components which represented 97.5 % of the total oil were identified in the essential oil. The oil was constituted mainly of geranial (44.5%) and neral (31.5%). The minor compounds presented in significant percents in lemongrass oil investigated were myrcene (9.9%), geraniol (7.9%) and geranyle acetate (2.2%). Based on literature data, it appears that geranial, neral, geraniol, limonene and β-myrcene have been found as major compounds in many other *Cymbopogon citratus* species with citral as the main chemical component of lemongrass oil (Huynh, 2008). Citral or 3,7-dimethyl-2,6-octadienal, an important raw material used in the pharmaceutical, perfumery and cosmetic industries, especially for the synthesis of Vitamin A and ionone (Mirghani et al., 2012), is the name given to a natural mixture of two isomeric acyclic monoterpene aldehydes: geranial (*trans*-citral, citral A) and neral (*cis*-citral, citral B) (Huynh, 2008). The major components found in our essential are quite similar compared with data reported by Mirghani et al. (2012) for Malaysia specimen; they have found a prominent composition in geranial (29.64%), neral (21.73%), geraniol (7.75%), limonene (5.92%) and β-myrcene (2.28%). This composition is also similar compared with data provided by Koba et al. (2009) where geranial (45.2%), neral (32.4%) and myrcene

Table 1. Chemical composition of essential oil of *Cymbopogon citratus*

Components	KI	%
Myrcene	991	9.6
(Z)-β-ocimene	1035	0.1
(E)-β-ocimene	1048	0.1
6,7-epoxymyrcene	1091	0.1
Linalool	1101	0.3
2,2-octa-3,4-dienal	1105	0.1
menth-3-en-9-ol	1149	0.1
Citronellal	1154	0.7
cis-chrysanthenol	1162	0.2
epoxy rose furane	1171	0.1
Nerol	1231	0.2
Neral	1245	31.2
Geraniol	1255	7.9
Geranial	1277	44.5
geranyl formate	1298	-
géranyl acetate	1377	2.2
β-caryophyllene	1418	-
trans-α-bergamotene	1435	-
Caryophyllene oxide	1586	0,1
Hydrogenated monoterpens		9.9
Oxygenated monoterpens		85.3
Hydrogenated Sesquiterpens		2.2
Oxygenated Sesquiterpens		0.1
Total		97.5

t <0.05

(10.2%) were the main components of essential oil of lemongrass specie from Togo. Matasyoh et al. (2011) found in lemongrass essential oil of Kenya, geranial (39.53%), neral (33.31%), and myrcene (11.41%) as major compounds. Geranial (40.82-44.44%), neral (33.56-33.54%), and myrcene (13.60-14.78%) were the major compounds isolated from essential oil of lemongrass cultivated at Brazil by Rocha et al. (2011). Loumouamou et al. (2010) analyzed essential oil of lemongrass of Congo-Brazzaville and found geranial (48.88%) and neral (36.24%) in prominent concentration. Lemongrass essential oil from Malaysia studied by Tajidin et al. (2012) contained geranial (37.58%-45.95%) and neral (29.4%-31.13 %) as main components. The same data was reported by Bassole et al. (2011) who found geranial (48.1%), neral (34.6%) and myrcene (11.0%) as major constituents of *Cymbopogon citratus* essential oil from Burkina-Faso.

Biological activity of essential oil of *Cymbopogon citratus*

In recent years, consumer demand for effective, safe natural products to control food spoilage without chemical residues has increased. Essential oils, aromatic volatile products of plant secondary metabolism, have formed the basis of many applications in food flavoring and preservation industries (Rahman and Kang, 2009; Tsigarida et al., 2009; Tian et al., 2011). Our study had assessed the antifungal activity of essential oil of *Cymbopogon citratus* against moulds by agar diffusion method in culture medium and in cheese wagashi. The results obtained from this work showed that the percentages of mycelial growth inhibition are significantly ($p < 0.05$) influenced by incubation time and essential oil concentrations. Mycelia growth was reduced with increasing concentration of essential oil while their growth increased with incubation time (Figures 1-12). The MGI, fungistatic and fungicidal activities values of the essential oil of lemongrass in culture medium against the tested fungi are reported in Table 2. It revealed that essential oil of lemongrass had significant fungicidal activity against *Scopulariopsis brevicaulis* at 400 mg/L, *Fusarium poae* at 800 mg/L, *Aspergillus* (*niger* and *terreus*) and *Fusarium verticillioides* at 1000 mg/L. Essential oil of lemongrass possessed therefore a fungistatic activity against *Aspergillus aculeatus*, *A. flavus*, *A. tamarii*, *Penicillium*

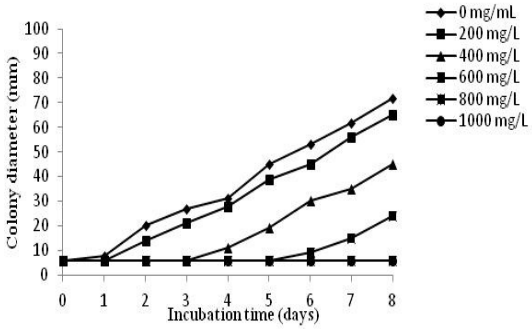

Figure 1: Effect of different concentration of *Cymbopogon citratus* essential oil on *Fusarium poae* growth

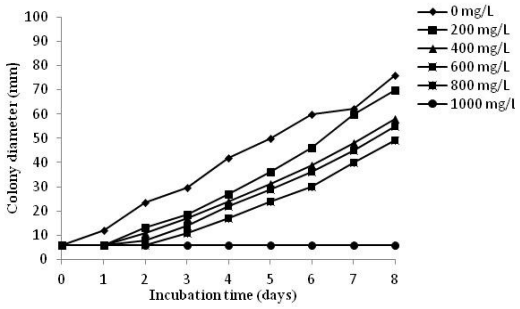

Figure 2: Effect of different concentration of *Cymbopogon citratus* essential oil on *Fusarium verticillioides* growth

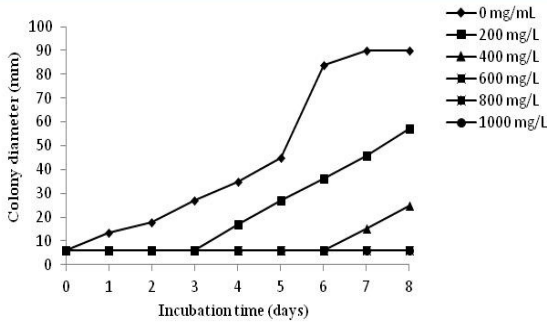

Figure 3: Effect of different concentration of *Cymbopogon citratus* essential oil on *Aspergillus terreus* growth

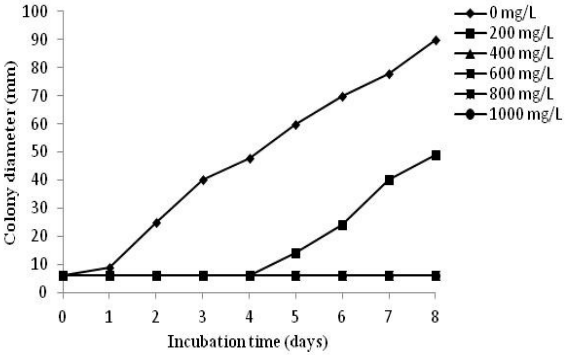

Figure 4: Effect of different concentration of *Cymbopogon citratus* essential oil on *Scopulariospis brevicaulis* growth

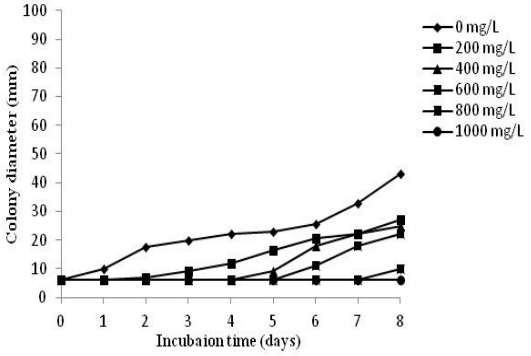

Figure 5: Effect of different concentration of *Cymbopogon citratus* essential oil on *Penicillium citrinum* growth

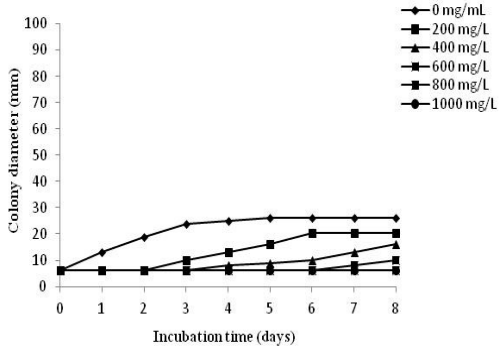

Figure 6 : Effect of different concentration of *Cymbopogon citratus* essential oil on *Penicillium brevicompactum* growth

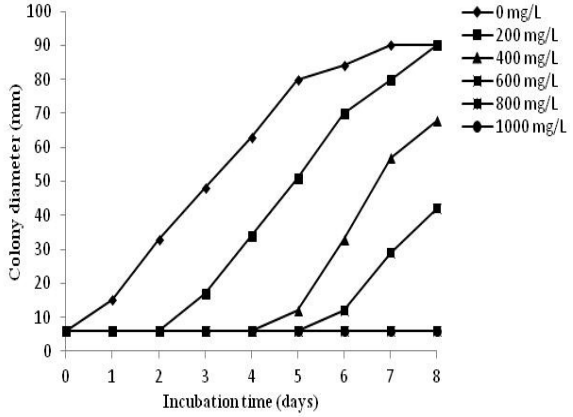

Figure 7: Effect of different concentration of *Cymbopogon citratus* essential oil on *Aspergillus niger* growth

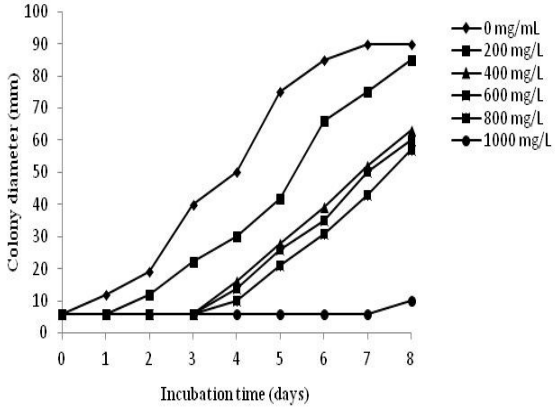

Figure 8: Effect of different concentration of *Cymbopogon citratus* essential oil on *Aspergillus ustus* growth

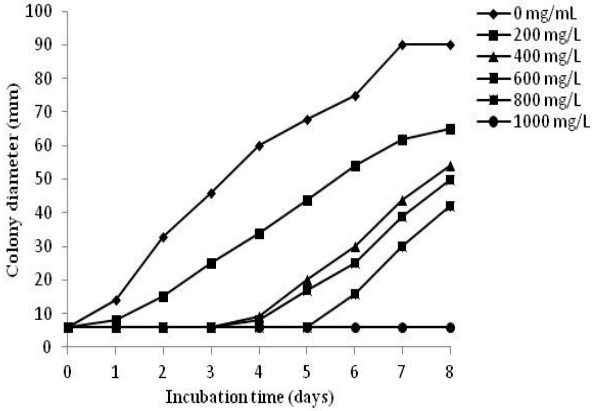

Figure 9: Effect of different concentration of *Cymbopogon citratus* essential oil on *Aspergillus tamarii* growth

Figure 10: Effect of different concentration of *Cymbopogon citratus* essential oil on *Aspergillus aculeatus* growth

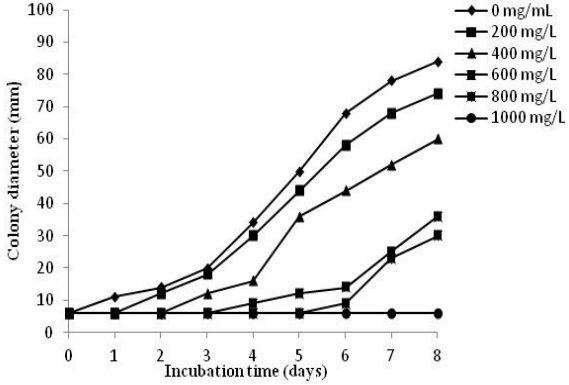

Figure 11: Effect of different concentration of *Cymbopogon citratus* essential oil on *Aspergillus flavus* growth

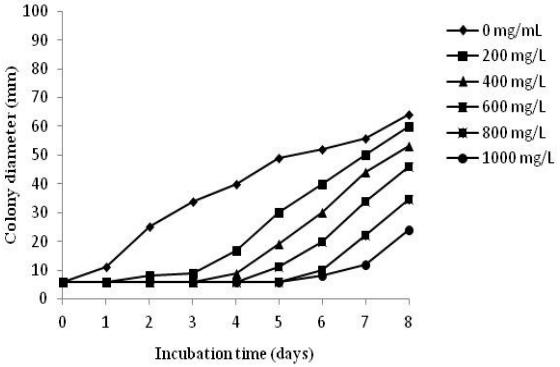

Figure 12: Effect of different concentration of *Cymbopogon citratus* essential oil on *Penicillium griseofulvum* growth

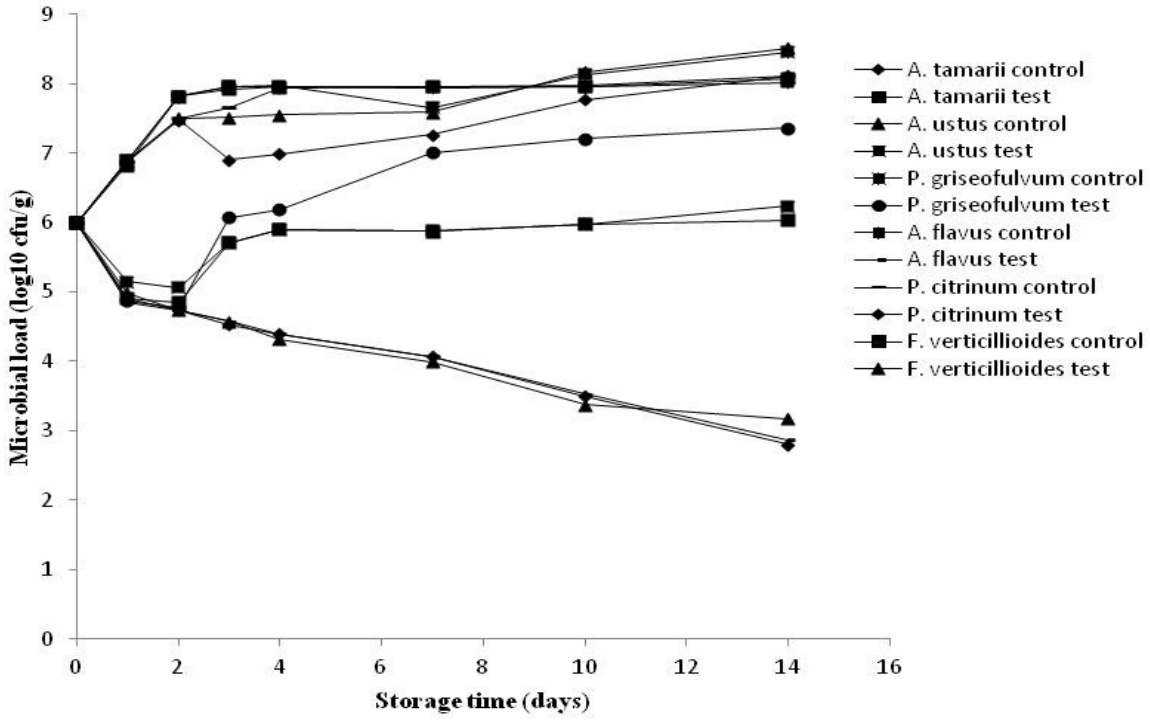

Figure 13: Inhibition of moulds investigated in traditional cheese wagashi by *Cymbopogon citratus* essential oil at concentration of 1000 mg/L.

brevicompactum and *Penicillium citrinum*. Species of *Aspergillus ustus* and *P. griseofulvum* were most resistant to this oil. Indeed, these last moulds were partially inhibited in the range of the essential oil concentrations tested with MGI inferior to 100%. Lemongrass essential oil efficacy against these species isolated from wagashi is thought to depend on specific toxicity of its single main active constituents or by its synergic effect (Sessou et al., 2012a). Furthermore, the biological activity of this oil is probably due to its prominent concentration in citral. In fact, citral which is a natural mixture of two isomeric acyclic monoterpens aldehydes, geranial and neral, has been reported to have high antifungal activity (Lee et al., 2008; Matasyoh et al., 2011). Previous studies reported that the sporulation of *Aspergillus flavus* was completely inhibited by *C. citratus* (2800 ppm) when used as fumigant whereas aflatoxin production inhibited at 100 ppm of *C. citratus* treatments (Paranagama et al., 2003).

Action of lemongrass oil on moulds in cheese wagashi

The data presented in this section shows the potential effectiveness of *Cymbopogon citratus* essential oil as natural cheese preservative against pathogenic and adulterated moulds *Aspergillus ustus*, *A. tamarii, A. flavus, Penicillium griseofulvum*, *P. citrinum* in sterile traditional cheese wagashi. These moulds were selected for the fact that they were resistant to essential oil of *Cymbopogon citratus* in culture medium assay. It should be taken into consideration that the inhibition reported here was for cheese diluted 1 in 10 with peptone water and therefore may not be a true reflection of inhibition achieved in cheese, but was necessary due to practical considerations. Despite this, the research has shown the potential application of lemongrass oil as wagashi preservative. In fact, antifungal activities of studied essential oil have been observed in cheese wagashi (Figure 13). As seen from this figure, after addition of 1000 ppm of lemongrass into cheese, *Penicillium citrinum*, *Aspergillus flavus* and *Fusarium verticillioides* were reduced to 2.80 $\log_{10}$ cfu/g, 2.86 $\log_{10}$, cfu/g and 3.16 $\log_{10}$ cfu/g respectively at the day 14 of storage. However, in control samples without essential oil, an increasing colony count was observed: 8.07 $\log_{10}$ cfu/g, 8.02 $\log_{10}$ cfu/g and 8.11 $\log_{10}$ cfu/g for *Fusarium verticillioides*, *Aspergillus flavus* and *Penicillium citrinum* respectively at the day 14. Concerning *Penicillium griseofulvum*, *Aspergillus tamarii* and *Aspergillus ustus*, we noticed that the essential oil was less active. In fact, *Penicillium griseofulvum* test (with essential oil) load's decreased to 4.87 $\log_{10}$ cfu/g at the day 2 and then increased to 6.08 $\log_{10}$ cfu/g and 7.36 $\log_{10}$ cfu/g at days 6

Table 2: Mycelial growth inhibition, fungistatic and fungicidal activities of essential oil of *Cymbopogon citratus* on tested fungi

Essential oil (mg/L)	*Mycelial growth inhibition (%)*											
	A. aculeatus	*A. flavus*	*A. niger*	*A. tamarii*	*A. terreus*	*A. ustus*	*F poae*	*F verticillioides*	*P. brevicompactum*	*P. citrinum*	*P. griseofulvum*	*S. brevicaulis*
200	64.1 ± 2.5a	12.8 ± 2.0f	0.0 ± 0.0	29.8 ± 0.3d	39.3 ± 1.7c	5.9 ± 2.9h	10.6 ± 0.6fg	8.6 ± 1.6gh	27.5 ± 0.9e	43.2 ±3.2 bc	6.9 ± 1.3h	48.8 ± 2.7b
400	78.2 ± 1.2b	30.8 ± 0.9e	26.2 ± 0.8f	30.9 ± 1.1e	77.4 ± 1.2b	32.1 ± 0.4e	40.9 ± 0.9d	25.7 ± 1.3f	50.0 ± 1.3c	48.6 ± 0.8c	18,9 ± 0.7g	100a (FS, FC)
600	96.1 ± 0.8b	61.5 ± 0.5e	57.1 ± 2.8f	47.6 ± 1.5g	100a (FS)	35.7 ± 1.4h	72.7 ± 2.7d	30.0 ± 0.1i	80.0 ± 2.2c	56.7 ± 1.5f	31.0 ± 1.1i	100a (FS, FC)
800	100a (FS)	69.2 ± 0.7c	100a FS	57.1 ± 0.7d	100a (FS)	39.9 ± 2.3f	100a (FS, FC)	38.6 ± 1.1f	100a (FS)	89.2 ± 0.3b	50,9 ± 2.1e	100a (FS, FC)
1000	100a (FS)	100a (FS)	100a (FS, FC)	100a (FS)	100a (FS, FC)	95.2 ± 0.5b	100a (FS, FC)	100a (FS,FC)	100a (FS)	100a(FS)	68.9 ± 0.5c	100a (FS,FC)

EO: Essential oil; FS: fungistatic activity; FC: fungicidal activity; Data in the line followed by different letters are significantly different ($p < 0.05$). The values are means of three repetitions ± standard deviation

and 14 respectively. Its control without essential oil increased to 8.49 $\log_{10}$ cfu/g at the day 14. As regards of *Aspergillus tamarii* and *Aspergillus ustus*, their loads decreased until to 5.980 $\log_{10}$ cfu/g at day 12 and increased to 6.03 $\log_{10}$ cfu/g and 6.23 $\log_{10}$ cfu/g at the day 14 respectively. In the same time, their controls had increased to 8.11 $\log_{10}$ cfu/g and 8.52 $\log_{10}$ cfu/g respectively. These results showed that *Penicillium citrinum*, *Aspergillus flavus* and *Fusarium verticillioides* were the most sensible strains to this oil in cheese wagashi whereas the rest were resistant. As well as in *in vitro* experiment than in food system, *Penicillium griseofulvum* was the most resistant mould to this oil. As *in vitro* experiments showed, 1000 ppm of lemongrass essential oil could inhibit the growth of *A. flavus, Penicillium citrinum, Fusarium verticillioides* and *Aspergillus tamarii* completely (100%) but percentages of inhibition of these strains in cheese were less than 100%. The percentages of inhibition of the moulds in cheese were then lower than in culture medium. This fact may be related to the more complex matrix (fat and proteins) of cheese wagashi than culture medium. In general, the levels of essential oils and their compounds necessary to inhibit microbial growth are higher in foods than in culture media. This is due to interactions between compounds and the food matrix (Nuchas and Tassou, 2000). Farbood (1976) suggested that the fat in food could form a protective coat around microorganism, thereby protecting them from antimicrobial agents. These researchers also suggested that the lipid fraction of the food absorbs the antimicrobial agent, thus decreasing the concentration in the aqueous phase and hence it's fungicidal action. The protein content of the food may also have been an influencing factor in the effectiveness of the essential oils (Smith-Palmer et al., 2001). Juven et al. (1994) reported bovine serum albumin neutralized the antimicrobial action of thymol suggesting complex formation between phenolic compounds in the oil and proteins in the food (Omidbeygi et al., 2007). In sum, the addition of lemongrass oil to wagashi mixture with moulds' spores exerted high sporale reduction rate on *Penicillium citrinum* and *Aspergillus flavus* which are potential producers respectively of the most important and harmful mycotoxins, citrinin and aflatoxin M1 encountered in dairy products especially cheese (Sengun et al., 2008). This study indicated that essential oil of *Cymbopogon citratus* possessed antifungal activity and can be exploited as an ideal treatment for cheese eliminating fungal spread.

CONCLUSION

Volatile extracted from fresh leaves of *Cymbopogon citratus* with geranial (44.5%) and neral (31.5%) as major compounds had high effect on the radial growth of *Scopulariopsis brevicaulis*, *Fusarium poae*, *Aspergillus* (*niger* and *terreus*) and *Fusarium verticillioides* in culture medium on the one hand and a high sporale reduction rate of *Penicillium citrinum, Aspergillus flavus and Fusarium verticillioides* in cheese wagashi in the other hand. Moreover, this oil exhibited a weak antifungal activity mainly against *Aspergillus tamarii, Aspergillus ustus and Penicillium griseofulvum*. Lemongrass essential oil could be used as natural antimicrobial agent in the fight against *Penicillium citrinum, Aspergillus flavus* and *Fusarium verticillioides* strains and as wagashi preservative. For the practical use of this oil as wagashi preservative, further research is needed on safety issues for human health and acceptability by consumers.

ACKNOWLEDGMENTS

The authors are grateful to University Council of Development (CUD) for its financial support.

They are thankful to Doctor YEDOMONHAN Paul of National Herbarium of Abomey-Calavi for identification of plant, Mrs. Fanou Brice, Agniwo Bienvenu, Chabi-Egba Mispar, Dossa François and Ahounou Serge for their technical assistance.

REFERENCES

Adams RP (2007). Identification of essential oil components by Gas Chromatography/Mass Spectrometry, 4th edition; Allured Publishing Corporation Carol Stream. IL.

Aïssi VM, Soumanou MM, Bankolé H, Toukourou F, de Souza CA (2009). Evaluation of Hygienic and Mycological Quality of Local Cheese Marketed in Benin. AJBAS. 3: 2397-2404

Angelini P, Pagiotti R, Menghini A, Vianello B (2006). Antimicrobial activities of various essential oils against foodborne pathogenic or spoilage moulds. Ann. Microbiol. 56: 65-69

Barkat M, Bouguerra A (2012). Study of the antifungal activity of essential oil extracted from seeds of Foeniculum vulgare Mill. for its use as food conservative. Afr. J. Food Sci. 6: 239-244

Bassolé IH, Lamien-Meda A, Bayala BB, Obame LC, Ilboudo AJ, Franz C, Novak J, Nebié RC, Dicko MH (2011). Chemical composition and antimicrobial activity of Cymbopogon citratus and Cymbopogon giganteus essential oils alone and in combination. Phytomedicine 18: 1070-1074.

Burt S (2004). Essential oils: their antibacterial properties and potential applications in food-areview. Int. J. Food Microbiol. 94: 223-253.

Cardile V, Russo A, Formisano C, Rigano D, Senatore F, Arnold NA (2009). Essential oils of Salvia bracteata and Salvia rubifolia from Lebanon: Chemical composition, antimicrobial activity and inhibitory effect on human melanoma cells. J. Ethnopharmacol. 126: 265–272.

Farbood MI, MacNeil JH, Ostovar K (1976). Effect of rosemary spice extractive on growth of microorganisms in meat. J. Milk Food Technol. 39: 675–679.

Gandomi H, Misaghi A, Basti AA, Bokaei S, Khosravi A, Abbasifa A, Javan AJ (2009). Effect of Zataria multiflora Boiss. Essential oil on growth and aflatoxin formation by Aspergillus flavus in culture media and cheese. Food Chem. Toxicol. 47: 2397–2400.

Huynh KPH, Maridable J, Gaspillo P, Hasika M, Malaluan R, Kawasaki J (2008). Essential Oil from Lemongrass Extracted by Supercritical Carbon Dioxide and Steam Distillation. Philipp. Agric. Sci. 91: 36 -41..

ISO 21527-1 : 2008 (F). Microbiologie des aliments-Méthode horizontale pour le dénombrement des levures et moisissures, Partie 1 : Technique par comptage des colonies dans les produits à activité d'eau supérieure à 0,95, 1ère édition 2008-07-01.

Juven BJ, Kanner J, Schved F, Weiss- lowicz H (1994). Factors that interact with the antibacterial action of thyme essential oil and its active constituents. J. Appl. Bacteriol. 76: 626–631.

Kèkè M, Yèhouénou B, Dahouénon E, Dossou J and Sohounhloué DCK (2008). Contribution à l'amélioration de la technologie de fabrication et de conservation du fromage peulh waragashi par injection de Lactobacillus plantarum. Ann. Sci. Agron. Benin 10: 73-86

Koba K, Sanda K, Guyon C, Raynaud C, Chaumont JC, Nicod L (2009). In vitro cytotoxic activity of Cymbopogon citratus L. and Cymbopogon nardus L. essential oils from Togo. Bangladesh J. Pharmacol. 4: 29-34.

Lee Y, Kim J, Shin S, Lee S, Park I (2008). Antifungal activity of Myrtaceae essential oils and their components against three phytopathogenic fungi. Flavour Fragr. J. 23(1): 23-28.

Loumouamou AN, Biassala E, Silou Th, Ntondele-Nsansi P, Diamouangana MJ, Nzikou J, Chalchat JC, Figuérédo G (2010). Characterization of a Giant Lemon Grass Acclimatised in the Congo-Brazzaville. Adv. J. Food Sci. Technol. 2: 312-317.

Matasyoh JC, Wagara IN, Nakavuma JL, Kiburai AM (2011). Chemical composition of Cymbopogon citratus essential oil and its effect on mycotoxigenic Aspergillus species. Afr. J.Food Sci. 5: 138-142.

Mirghani MES, Liyana Y, Parveen J (2012). Bioactivity analysis of lemongrass (Cymbopogan citratus) essential oil. Int. Food Res. J. 19: 569-575.

Murthy PS, Ramalakshmi K, Srinivas P (2009). Fungitoxic activity of Indian borage (Plectranthus amboinicus) volatiles. Food Chem. 114: 1014–1018.

Nasser AL (2001). Fungal contamination of white cheese at the stage of consumption in Saudi Arabia. Pak. J. Biol. Sci. 4: 733-735.

Nguefack J, Lekagne Dongmo JB, Dakole CD, Leth V, Vismer HF, Torp J, Guemdjom EFN, Mbeffo M, Tamgue O, Fotio D, Amvam Zollo PH, Nkengfack AE. 2009. Food preservative potential of essential oils and fractions from Cymbopogon citratus, Ocimum gratissimum and Thymus vulgaris against mycotoxigenic fungi. Int. J. Food Microbiol. 131: 151-156.

Nguefack J, Leth V, Amvam Zollo PH, Mathur SB (2004). Evaluation of five essential oils from aromatic plants of Cameroon for controlling food spoilage and mycotoxin producing fungi. Int. J. Food Microbiol. 94: 329– 334.

Nguefack J, Nguikwie SK, Fotio D, Dongmo B, Leth V, Nkengfack AE (2007). Fungicidal potential of essential oils and fractions from Cymbopogon citratus, Ocimum gratissimum and Thymus vulgaris to control Alternaria padwickii and Bipolaris oryzae, two seed-borne fungi of rice (Oryza sativa L.). J.Essent. Oil Res. 19: 581-587.

Nguefack J, Tamgue O, Lekagne JB, Dongmo CD, Dakole V, Leth HF, Vismer PH, Amvam Z, Nkengfack AE (2012). Synergistic action between fractions of essential oils from Cymbopogon citratus, Ocimum gratissimum and Thymus vulgaris against Penicillium expansum. Food Control 23: 377-383.

Nielsen PV, Rios R (2000). Inhibition of fungal growth on bread by volatile components from spices and herbs, and the possible application in active packaging, with special emphasis on mustard essential oil. Int. J. Food Microbiol. 60: 219–229.

Nuchas GE, Tassou CC (2000). Traditional preservatives-oils and spices. In R. K. Robinson, C. A. Batt, & P. D. Patel (Eds.), Encyclopedia of food microbiology (pp. 1717–1722). London, UK: Academic Press.

Omidbeygi M, Barzegar M, Hamidi Z, Naghdibadi H (2007). Antifungal activity of thyme, summer savory and clove essential oils against Aspergillus flavus in liquid medium and tomato paste. Food Control 18: 1518–1523

Paranagama PA, Abeysekera KHT, Abeywickrama K, Nugaliyadd L (2003). Fungicidal and anti-aflatoxigenic effects of the essential oil of Cymbopogon citratus (DC.) Stapf. (lemongrass) against Aspergillus flavus Link. isolated from stored rice. Lett. Appl. Microbiol. 37: 86–90.

Pitt JI, Hocking AD (2009). Fungi and Food spoilage, Springer Dordrecht Heidelberg London New York, USA, 518p.

Rahman A, Kang SC (2009). In vitro control of food-borne and food spoilage bacteria by essential oil and ethanol extracts of Lonicera japonica Thunb. Food Chem. 116: 670–675

Rocha RP, de Castro Melo E, Demuner JA, Radünz LL, José Bon Corbín BJ (2011). Influence of drying air velocity on the chemical composition of essential oil from lemongrass. Afr. J. Food Sci. Technol. 2: 132-139.

Samson A, Hoekstra E, Frisvad JC, Filtenborg O (1995). Introduction to food-borne fungi. Copyright 1995 Centraalbureau voor Schimmelcultures ISBN 90-70351-27-7, p 322.

Sengun IY, Yaman DB, Gonul SA (2008). Mycotoxins and moulds contamination in cheese: a review. World Mycotoxin J. 1: 291-298

Sessou P, Farougou S, Alitonou G, Djenontin TS, Yèhouénou B, Azokpota P, Youssao I, Sohounhloué D (2012a). Chemical Composition and Antifungal activity of Essential oil of Fresh leaves of Ocimum gratissimum from Benin against six Mycotoxigenic Fungi isolated from traditional cheese wagashi. Int. Res. J. Biological Sci. 1: 22-27.

Sessou P, Farougou S, Azokpota P, Youssao I, Sohounhloué D (2012b). In vitro Antifungal activities of Essential oils extracted from Fresh Leaves of Cinnamomum zeylanicum and Ocimum gratissimum against Foodborne pathogens for their use as Traditional Cheese Wagashi conservatives. Res. J. Recent Sci. 1: 67-73.

Sessou P, Farougou S, Noudogbessi JP, Fanou B, Azokpota P, Youssao I, Sohounhloué D (2012c). Chemical composition and in vitro antifungal activity of Zingiber officinale essential oil against foodborne pathogens isolated from a traditional cheese wagashi produced in Benin. Int. J. Biosci. 2: 20-28.

Sessou P, Farougou S, Noudogbessi JP, Yèhouenou B, Azokpota P, Youssao I, Menut C, Sohounhloue D (2012d). Chemical composition and antifungal activity of essential oil of fresh leaves of Cinnamomum zeylanicum from Benin against six mycotoxigenic fungi isolated from a traditional cheese wagashi. Elixir Appl. Biol. 49: 9787-9791.

Sessou P, Farougou S, Sohounhloué D (2012e). Major component and potential applications of plant essentials oils as natural food preservatives: a short review research results. Int. J. Biosci. 2: 45-57.

Smith-Palmer A, Stewart J, Fyfe L (2001). The potential application of plant essential oils as natural food preservatives in soft cheese. Food Microbiol. 18: 463-470.

Tajidin EN, Ahmad SH, Rosenani AB, Azimah H, Munirah M (2012). Chemical composition and citral content in lemongrass (Cymbopogon citratus) essential oil at three maturity stages. Afr. J.Biotechnol. 11: 2685-2693.

Tatsadjieu NL, Dongmo Jazet PM, Ngassoum M B, Etoa F-X, Mbofung CMF (2009). Investigations on the essential oil of Lippia rugosa from Cameroun for its potential use as antifungal agent against Aspergillus flavus Link ex Fries. Food Control 20: 161-166.

Tian J, Ban X, Zeng H, He J, Huang B, Wang O (2011). Chemical composition and antifungal activity of essential oil from Cicuta virosa L. var. latisecta Celak. Int. J. Food Microbiol. 145: 464–470.

Tian J, Huang B, Luo X, Zeng H, Ban X, He J, Wang Y (2012). The control of Aspergillus flavus with Cinnamomum jensenianum Hand.-Mazz essential oil and its potential use as a food preservative. Food Chem. 130: 520–527.

Tsigarida E, Hugas M, Robinson T (2009). The EFSA Scientific Panel on Biological Hazards first mandate: May 2003–May 2006. Insight into scientific advice on food hygiene and microbiology. Trends Food Sci. Technol. 20: 587–594.

Yèhouenou B, Ahoussi E, Sessou P, Toukourou F, Sohounhloué D (2012). Chemical composition and antimicrobial activities of essential oils (EO) extracted from leaves of Lippia rugosa A. Chev against foods pathogenic and adulterated microorganisms. Afr. J. Microbiol. Res. 6: 5496-5505.